Asian seabass: validation of commercial grow-out feeds containing optimal levels of SBM and SPC and impacts of feed attractants

Final Report Submitted to the United Soybean Board (USB)
New Uses Committee USB Project FY2011 #1463
2011

M.A. Booth, G.L. Allan, I. Russell & M. Elkins
NSW Department of Primary Industries
Port Stephens Fisheries Institute (PSFI)
Locked Bag 1, Nelson Bay, NSW 2315, Australia

Acknowledgments

The authors would like to thank Mr Nick Arena and his technical staff (Tailor Made Fish Farm, Bob's Farm, NSW, Australia) and the staff at Infinity Fisheries Pty Ltd (Bargo, NSW, Australia) for supplying the barramundi used in the experiments. Special thanks to Dr Richard Smullen (Ridley Aquafeed Pty Ltd) for advice on feed attractants and for supplying the majority of ingredients used in the digestibility experiment and to Dr Sagiv Kolkovski (WA Fisheries, Australia) for supplying the Artemia hydrolysate. We also acknowledge the support and advice received from Lukas Manomaitis (ASA-IM South East Asia), particularly in regard to ingredient selection for the digestibility trial.

We extend our thanks to Scott Rick (Smith-Bucklin Corporation Accounting Manager), Lisa Childs (Project Manager USB/Smith-Bucklin Management Team) and John Campen (New Uses Program Manager, United Soybean Board) for assistance with our research proposal, project management and technical advice. We also thank the USB, USSEC and ASA IM for providing additional funds for Dr Mark Booth to attend the 2011 US Soybean Export Council Global Soy in Aquaculture Stakeholders Meeting in Kona, Hawaii. Thanks also to Dr Rick Barrows for conducting proximate tests on samples of SBM and SPC sent from Australia and providing the results of comparative data on other soy products from related USB projects.

We acknowledge Dr Peter Martin, Adam Pytko and staff at the Animal Science & Industry Services Laboratory (Queensland Primary Industries and Fisheries Department) for conducting the chemical analyses related to the attractant study. Thanks to Michael Nielsen (EcoTeam) for determination of chromium and to Richard Meyer and staff (NSW DPI) for chemical analysis related to the digestibility trial. Amino acid analysis was undertaken by the Australian Proteome Analysis Facility (APAF; Macquarie University, Sydney Australia) and facilitated using infrastructure provided by the Australian Government through the National Collaborative Research Infrastructure Strategy (NCRIS).

Lastly, and in no particular order we thank Dr Wayne O'Connor for editorial review of this report and Messrs Luke Cheviot, Luke Vandenberg, Lachlan Bassett and Justin Kelly for assistance in transporting fish and running the experiments. Special thanks are reserved for Ms Michelle O'Leary for financial assistance and project management support.

Table of contents

List of tables

List of figures

Executive summary

This report details the results of several research activities conducted for the United Soybean Board by NSW DPI Fisheries in 2010 related to USB Project 1463 ***Asian seabass: validation of commercial grow-out feeds containing optimal levels of SBM and SPC and impacts of feed attractants***.

Two major research experiments were conducted with Asian seabass. The first experiment was designed to investigate the efficacy of three commercially available feed attractants when top-coated onto aquafeeds containing high dietary concentrations of soybean meal and or soy protein concentrate. The attractants were all hydrolysates and based on poultry raw material, fish raw material or Artemia raw material, respectively. The second experiment was designed to determine the apparent digestibility of protein, energy and amino acids in raw materials typically used to formulate aquafeeds for Asian seabass in South East Asia. Ingredients included poultry meal, blood meal, corn gluten meal, prime fishmeal, tuna meal, raw wheat and pregelatinized wheat starch. Faecal material was collected by stripping and indirect methods were used to calculated digestibility of ingredients.

None of the feed attractants had a significant influence on relative feed intake or growth of Asian seabass. The lack of efficacy in the tested hydrolysates may be related to the chemical composition of the individual hydrolysates, the inclusion rates we selected for the experiment or possibly the method of incorporation. Further research with Asian seabass in this field should include screening of additional feeding stimulants or mixtures and dose response studies aimed at determining suitable inclusion rates.

Protein digestibility of fishmeal, poultry meal, corn gluten meal and raw wheat were all greater then 82%. Energy from poultry meal, fishmeal and corn gluten was also well digested, but the protein and energy digestibility of blood meal was low (55 and 60%, respectively). Both dry matter and energy from wheat and pregelled wheat starch were very poorly digested by Asian seabass, acting as dietary diluents. This new data has been used to expand the existing formulation data base of feed ingredients for Asian seabass which already includes similar information on the digestibility of soybean meal, soy protein concentrate and South American fishmeal.

In addition to the major experiments, two desk-top studies were undertaken. One study aimed to review new scientific data on digestibility of feed ingredients by Asian seabass and the other to review scientific literature on the impacts of SBM and SPC on extruded aquafeeds, particularly with reference to the quality of extruded aquafeeds produced in earlier trials with Asian seabass for the USB.

Little new information on the digestibility of feed ingredients for Asian seabass was found in the peer reviewed literature. Only two recent papers were dedicated to the determination of apparent digestibility coefficients for Asian seabass. One paper investigated the digestibility of different cereal grains and the other investigated the digestibility of various ingredients such as lupin meal, lupin protein concentrate, soybean meal, canola meal, poultry meal and feather meal. None of the publications presented data on the availability of amino acids for any of the ingredients. Relevant data was used to update the basic or proximate feed ingredient data base for Asian seabass.

Review of the scientific literature in light of the pellet characteristics observed in our earlier formulations has indicated there is a need for further research into the interactions between SBM, SPC and other common feed ingredients subjected to extrusion processing. In addition, more research is required to better understand how the selection of adjustable extrusion parameters such as moisture level, barrel temperature, feeding rate, screw speed and retention time affect dependent variables such as motor torque, die pressure and specific mechanical energy (SME) in aquafeed formulations containing SBM and SPC. Ideally these factors should be examined under controlled conditions where most extrusion parameters are held constant and ingredient-ingredient interactions are minimised. This will lead to a far better understanding of ingredient-ingredient interactions and knowledge of the functional value of SBM and SPC in the presence of other important feed proteins.

Recommendations

NSW Department of Primary Industries (NSW DPI) recommends conducting the following nutritional research and activities on the use of soybean meal (SBM) and soy protein concentrate (SPC) in aquafeeds for Asian Seabass (*Lates calcarifer*) in FY 2012;

- Conduct a laboratory based growth study with Asian seabass aimed at reducing or eliminating fishmeal in grow-out diets for this species by using elevated levels of SBM, SPC and other feed ingredients available in ASA-IM target markets of South East Asia (SEA).
- Conduct a longer term grow-out verification trial of promising formulation/s for Asian seabass at a commercial saltwater facility in SEA or Australia (ideally in SEA).
- Conduct an economic feasibility analysis of alternative soy based diets compared to typical commercial Asian seabass diet/s (least-cost analysis).
- A visit by NSW DPI researcher/s conducting USB trials on Asian seabass to SEA, ideally at the inception and conclusion of FY2012 activities to allow NSW DPI researchers to see what challenges the SEA industry is facing and to disseminate past and current USB research on Asian seabass to specifically targeted audiences in SEA partner countries.

These recommendations are designed to build on the successful research conducted by NSW DPI on Asian seabass for the USB in FY2009 (project 9463), FY2010 (project 0463) and FY2011 (project 1463). If adopted, these recommendations will assist development of new commercial aquafeed formulations for Asian seabass that are specific to the SEA region. In addition, completion of the aforementioned research will allow the economics of feeding new USB formulations to be compared to the economics of feeding Asian seabass on current commercial formulations. Together, this information will increase the flexibility of formulating aquafeeds for Asian seabass and the acceptance of using SBM and SPC in commercial feeds for this species. The opportunity for NSW DPI researchers to travel to SEA and liaise with the SEA International Marketing Team and present seminars and exchange information on USB research pertinent to the nutrition and feeding of Asian seabass should further enhance the uptake of soy products and the reduction of fishmeal in diets for this species.

Introduction

Asian seabass: validation of commercial grow-out feeds containing optimal levels of SBM and SPC and impacts of feed attractants

The New Uses Committee of the United Soybean Board (USB) contracted NSW Department of Primary Industries (NSW DPI) (formerly Industry & Investment NSW) to conduct research to investigate the utilisation of solvent extracted soybean meal (SBM) and soy protein concentrate (SPC) in aqua-feeds for Asian Sea Bass *Lates calcarifer.*

The agreed research objectives for FY2011 were:

- Expand the current formulation data base developed for this species in USB projects 9463 and 0463 by identifying potential ingredients available for feed manufacturing in key target markets of South East Asia (SEA).
- Evaluate the digestibility and & utilisation of key feed ingredients identified from SEA.
- Understand the impacts of high dietary inclusion levels of SBM and/or SPC on the production and quality of modern extruded aqua-feeds for Asian seabass
- Improve the understanding of formulation flexibility for feeds with high contents of SBM/SPC.
- Investigate the use of feed attractants to stimulate feed intake in diets containing high levels of SBM or SPC (particularly for SPC).

These objectives were designed to build on successful research conducted on Asian seabass for the USB by NSW DPI in FY 2009 (Project # 9463) and FY 2010 (Project # 0463). The FY 2011 objectives were broken into the following activities:

Activity 1) Desk-top research designed to expand the current feed ingredient formulation data base for Asian seabass and improve knowledge of the impacts of high inclusion levels of SBM and SPC on the manufacture of extruded pellets.

Activity 2) Determination of the apparent digestibility of key feed ingredients from ASA International Marketing (IM) identified target markets in SEA such as Thailand, Philippines, Indonesia, Vietnam or Malaysia.

Activity 3) Identification and evaluation of suitable feed attractants to improve feed intake of diets with high levels of SBM or SPC by Asian seabass.

Background to 2011 research objectives

The New Uses Committee of the United Soybean Board (USB) contracted NSW DPI and its collaborators to conduct applied research to investigate the utilization of solvent extracted soybean meal (SBM) and a soy protein concentrate (SPC; Soycomil-K®) in aqua-feeds for Asian seabass *Lates calcarifer*.

The original research objectives were to:

1. Determine the maximum tolerance level of SBM and/or SPC that can be utilized in the diet of Asian seabass

2. Formulate a manufactured feed for Asian seabass that optimizes the combination of SBM and SPC for field testing and evaluation at a later date

In order to address the stated objectives, NSW DPI developed a two stage research strategy. The first stage determined the apparent digestibility (ADC) of gross nutrients, amino acids and energy from SBM and SPC at two dietary inclusion levels (e.g. 30% and 50%). The second stage used the experimentally determined ADC's for SBM and SPC in combination with digestibility data obtained from peer reviewed literature to formulate and then manufacture a series of semi-commercial feeds for Asian seabass designed to test the upper inclusion levels of SBM, SPC or a combination of both ingredients against a formulated reference diet.

Stage 1 was successfully completed in FY2009 and NSW DPI presented USB with a comprehensive final report which included data on the digestibility of SBM and SPC and a data base of other ingredients and ADC's that allowed accurate formulation of feeds for

Asian seabass. Stage 2 was successfully completed in FY2010 and NSW DPI presented the USB with data on the utilisation of extruded diets containing high dietary levels of SBM (i.e. 30, 35 or 38%) and SPC (i.e. 40, 50 or 60%) and data on diets containing a combination of both products. The FY2010 final report also explored the effect of feeding high levels of SBM and SPC on the intestinal morphology of Asian seabass as well as the impacts of the aforementioned feeds on proximate carcass composition.

The results of the FY2010 study confirmed that digestibility coefficients for SBM, SPC, fishmeal and other ingredients can be used to successfully formulate feeds for Asian seabass. Similarities in feed utilisation (FCR), carcass composition and protein retention efficiency (PRE) of all dietary treatments demonstrated that the NSW DPI strategy of formulating feeds on a digestible protein and energy basis to match a specific stage of growth was successful and underpins the premise that once the digestibility of ingredients is known, the risk of undersupplying basic nutrient requirements in formulated feeds is reduced. Consequently NSW DPI was able to show that diets for Asian seabass could contain as much as 35% SBM before feed intake and performance were adversely affected. In contrast, diets very low in fishmeal but high in SPC or combinations of SBM and SPC achieved acceptable FCR, but the relative feed intake and concomitant weight gain of fish reared on these diets was decreased. The results for SPC appeared to be driven by the apparent decrease in palatability of diets containing extreme levels of this product rather than gross nutritional deficiencies or anti-nutrient effects *per se*. Impacts of SBM or SPC on the distal intestinal integrity of fish were inconclusive and could not be related to diet type. Some histological variations were noted in all dietary groups and these tended to be consistent, but we could not ascertain whether these changes were representative of normal or abnormal histological structures in well fed fish. Further detailed research is required to enable potential effects of SBM and SPC on gut histology to be elucidated.

Based on the outcomes of FY2009 and FY2010 activities NSW DPI recommended several new research activities that they believed were needed to ensure the most effective utilisation of SBM and SPC in aqua-feeds for Asian seabass, particularly for formulation and manufacture of aqua-feeds in the South East Asian (SEA) industry. These FY2011 recommendations included the determination of additional digestibility coefficients for common feed ingredients available to SEA feed manufacturers and evaluation of the potential of several commercially available feed attractants to increase feed intake of aqua-feeds containing elevated levels of SBM and SPC. A third recommendation for FY2011 was to review the most recent nutritional literature on Asian seabass and capture relevant data on the digestibility of other feed ingredients in order that they may be incorporated into the formulation data base for Asian seabass developed in earlier work. In addition, as incorporation of high dietary levels of SPC into aqua-feeds was problematic for the manufacturers of the semi-commercial feeds used in FY2010, NSW DPI recommended undertaking a brief literature review to investigate the impacts of high SBM and SPC inclusion on pellet quality of aqua-feeds and to help understand how these ingredients work and interact with other feed ingredients.

This report details the results of three activities undertaken by NSW DPI to address the research objectives of the USB in FY2011. The activities were to;

- Evaluation of feed attractants to improve feed intake of diets with high levels of SBM or SPC by Asian seabass.
- Determination of the apparent digestibility of key feed ingredients from ASA International Marketing (ASA-IM) identified target markets in SEA such as Thailand, Philippines, Indonesia, Vietnam or Malaysia.
- Desk-top research designed to update the current feed ingredient formulation data base for Asian seabass and improve knowledge of the impacts of high inclusion levels of SBM and or SPC on the manufacture of extruded pellets.

Major research activities completed in FY 2011

Evaluation of feed attractants for improving feed intake of diets with high levels of SBM or SPC by Asian seabass.

Introduction

The switch from fishmeal and fish oil based diets to aqua-feeds containing higher levels of less palatable plant proteins has prompted researchers and feed manufacturers to explore the potential of feed attractants or stimulants (Floreto et al., 2001; Nunes et al., 2006). Feeding stimulants that tend to cause the greatest behavioural responses in fish are a chemically heterogeneous group of compounds (chemo-attractants) and mixtures that include various amino acids, phospholipids, nucleotides and nucleosides, quaternary ammonium bases and animal extracts or emulsions (Koskela et al., 1993; De la Higuera, 2001; Smith et al., 2005). The most common physicochemical properties of attractants are; non volatile, low molecular weight, nitrogen containing, amphoteric, water soluble and stable to heat treatment (De la Higuera, 2001). Various extracts, by-products and purified substances have been trialled for their feed attractant properties in fish such as krill meal (Gaber, 2005), krill hydrolysate (Oikawa & March, 1997; Kolkovski et al., 2000) and squid hydrolysate (Lian et al., 2008). However, the addition of feed attractants does not always promote increases in feed intake and growth and appears to be heavily dependant on the ingredient composition of the diet (Trushenski et al., 2011) or whether the attractant is coated on the surface of the pellet or incorporated into the mash prior to pelleting (Oikawa & March, 1997). Feeding stimulants appear to be species specific and there tends to be differentiation between the type and range of stimulants which affect carnivorous and herbivorous fish (De la Higuera, 2001; Gatlin et al., 2007).

Feed stimulants act on fish in different ways. They may have arrestant, attractant, repellent, incitant, suppressant, stimulant or deterrent effects (De la Higuera, 2001). The terms incitant, suppressant, stimulant or deterrent apply to positive or negative responses relating to the initiation or continuation of feeding and require direct contact with the feed to stimulate the particular response. Many chemicals fit into more than one definition and different concentrations of a chemical may promote a different level of response (De la Higuera, 2001). In terms of the feeding response, direct contact with the feed can result in rejection of the feed due to the presence of deterrents or initiation and continuation of feeding due to presence of stimulants (De la Higuera, 2001).

In previous studies for the USB we found that utilisation of feeds (FCR) containing high levels of soy protein concentrate (SPC) (e.g. 30, 40 or 50%) by Asian seabass were similar to test diets containing soybean meal (SBM; 30, 35 or 38%), a fishmeal control diet or a commercial Asian seabass feed (Ridley Aquafeed Pty Ltd). However, weight gain of Asian seabass fed diets high in SPC was reduced due to concomitant decreases in feed intake (Booth et al., 2010a). This indicated a reduction in the palatability of these feeds, most likely due to low levels of fishmeal (10%) and the extreme dietary levels of SPC (e.g. possible role of concentrated oligosaccharides and other anti-nutritional factors). Similar responses have been seen in Californian yellowtail *Seriola lalandi* (Jirsa et al., 2011) and hybrid striped bass (Blaufuss & Trushenski, 2011) fed experimental diets in which fishmeal is replaced with SPC. At the conclusion of the 2010 study we hypothesized that the use of feed attractants / stimulants might overcome problems with palatability and induce increased feed intake of experimental feeds high in SPC.

Therefore, the aim of this experiment was to test whether the external top-coating of semi-commercial aqua-feeds with different feed stimulants would increase feed intake and promote additional weight gain in Asian seabass fed diets containing elevated dietary levels of SBM and SPC.

Methods

Experimental design

Three liquid feeding stimulants were selected to determine if feed intake of aqua-feeds containing high levels of SBM and SPC could be increased in Asian seabass. The selected stimulants were hydrolysates based on fish raw material, poultry raw material or a crustacean raw material. The experiment was designed for evaluation using factorial ANOVA. Two fixed factors each consisting of 4 levels were employed; diet type (i.e. FM, SBM38, SPC60 or SBM/SPC) and feeding stimulant (i.e. none, Actipal HC1, Actipal ML8 or Artemia). Each of the 16 treatments was replicated 3 times (i.e. 48 experiment tanks).

Aqua-feeds for this experiment were from the same batch of extruded test feeds used in a previous growth study with Asian seabass (Booth et al., 2010a). These feeds were selected because previous growth and feed intake data were available for Asian seabass fed these diets and the upper levels of SBM and SPC content in the feeds was known to be high enough to stimulate a negative feeding response (Booth et al., 2010a). The diets selected contained the highest levels of SBM (38%) and SPC (60%) investigated in previous research, respectively. In addition, we selected the diet containing 30% SBM and 30% SPC as this diet contained the same dietary content of soy products to the diet containing 60% SPC. A reference diet containing high levels of fishmeal and no soy products was included in the experimental design as a control. The formulation and measured composition of the un-coated aqua-feeds used in the trial are presented in Table 1.

The recommended inclusion level given by manufacturers for each of the hydrolysates was between 2-5% w/w. Therefore, in order to standardise the addition of each hydrolysate we selected a top-coating addition rate of 20 g attractant kg^{-1} diet (i.e. 2% weight / weight). Each of the hydrolysates was top-coated onto separate batches of pellets using a plastic pressure-pack spray bottle. One spray bottle was used for each hydrolysate to ensure cross-contamination of feeding stimulants did not occur. Following manufacturers recommendations all hydrolysates were warmed in a water bath (<45°C) in order to reduce the viscosity of the individual solutions prior to spraying onto pellets; this greatly aided top-coating procedures. Following top-coating procedures all pellets were dried in a convection drier (<45°C) to return the moisture content of pellets to < 10%. Feeds were stored frozen (<-17°C) prior to use in the feeding experiment.

The contact details for the manufacturers of each hydrolysate are given below and proprietary brochures are included at the end of this report.

Actipal HC1 Aquativ

Fish raw material (tuna co-products) hydrolysate
Batch No. 201100909
Aquativ SPF Diana (Thailand) Co. Ltd.
68/5 M00 8 Rama 2 Road, Bangkrachao
Muangsamutsakorn, Samutsakorn, 74000 Thailand
Contact: R Smullen
(email) rsmullen@ridley.com.au

Actipal Fish ML8 039027

Poultry raw material hydrolysate
Batch No. 110214A
SPF Diana (Australia)
Aquativ Australia
Newcastle Plant
P.O. Box 191 Beresfield, NSW, 2311
Contact: Florence Motte, Director, SPF Diana Australia
(email) fmotte@spfaus.com

Artemia hydrolysate

Artemia raw material hydrolysate
The Western Australian Fisheries and Marine Research Laboratories
Department Fisheries WA, Hillarys Western Australia
Contact: Dr Sagiv Kolkovski
(email) Sagiv.Kolkovski@fish.wa.gov.au

Each of the hydrolysates was submitted for chemical analysis in order to characterize its proximate, amino acid and fatty acid composition (Tables 2 and 3). Dry matter content of each hydrolysate varied greatly and reflected the viscosity of each product. As a consequence the actual amount of dry matter (i.e. amino acids, fatty acids etc) added to the test feeds on a 2% w/w "as is basis" was very different. Actipal HC1 (fish hydrolysate) was relatively high in crude protein and amino acids compared to Actipal ML8 (poultry hydrolysate) and considerably different to the protein and amino acid composition of the Artemia hydrolysate. Actipal ML8 (poultry hydrolysate) contained the highest level of total lipid on a dry weight basis (Table 2).

The fatty acid composition of the 3 hydrolysates reflected the origin of the raw material used. For example, on a dry matter basis Actipal HC1 (fish hydrolysate) was rich in saturated and n-3 polyunsaturated fatty acids (PUFA) whereas the Actipal ML8 (poultry hydrolysate) was rich in saturated and monounsaturated fatty acids (MUFA). The Artemia hydrolysate was proportionally higher in MUFA, followed by saturated fatty acids then n-3 PUFA, but was relatively low in n-6 PUFA (Table 3). Total fatty acids represented 53.2, 83.1 and 22.0% of total lipids on a dry weight basis of the Actipal HC1, Actipal ML8 and Artemia samples, respectively. The fatty acid composition of the Artemia hydrolysate reflected values cited for starved adult Artemia (Fujita et al., 1980; Dhont & Van Stappen, 2003).

Fish stocks and handling protocols

Five hundred size graded Asian seabass (≈ 120 g body weight) were collected from Infinity Fisheries Pty Ltd (105 Silica Rd. Bargo NSW Australia 2574; www.infinityfisheries.com) and transported to PSFI in a single 1800 L rectangular fish transporter containing freshwater. During the 5 h journey the transport tank was supplied with medical grade oxygen (BOC Australia) via regulated flow meters (Ezi-Flow; Comweld Medical Cigweld Pty Ltd Preston, Victoria, Australia) feeding submerged ceramic diffusers. This ensured the dissolved oxygen concentration in the tank did not fall below 6 mg L^{-1}. Fish were collected, transported and removed from the transport tank according to bio-security protocols for Asian seabass established by NSW DPI (please refer to USB FY2009 Final Report). On arrival at PSFI fish were lightly anaesthetized (10ppm Aquis-S®) in the transport tank, captured and placed into a 3000 L holding tank inside the laboratory. Fish were held in the holding tank for approximately 1 week during which time the fish were acclimated to salt water conditions (salinity > 34 g L^{-1}) and sparingly fed a commercial Asian seabass feed (Ridley Aquafeed Pty Ltd). Following the acclimation period the whole group of fish was lightly anaesthetized before individual fish were hand selected for dispersion to 48 experiment tanks. Selected fish were carefully graded to ensure all stocked fish were of similar starting weight and length. Fish were systematically distributed to tanks in groups of 5 until each tank contained 10 fish.

PSFI fish transporter

Laboratory

The experiment was performed in a laboratory that housed 48 x 200 L white polyethylene aquaria supplied by two interlinked recirculating bio-filtration units of 1,700 L capacity. Water flow to each experimental tank was controlled by small PVC taps that provided a weak centripetal current (i.e. flow rate ≈ 7-8 L min^{-1}) within each tank that assisted the removal of waste through a central, vertical PVC standpipe (32 mm diameter) fixed approximately 10 mm from the base. Water temperature was controlled in each of the bio-filtration units using an immersion heater operating antagonistically against a water chilling unit to allow precise temperature control. Each unit was enriched with medical grade oxygen which was injected directly into each of the main supply manifolds. Each of the 48 experimental aquaria contained a central air-stone to provide additional aeration. Black plastic was wrapped around each of the 48 aquaria and a black plastic lid, which covered half the tank opening, was fitted to minimize external disturbance of fish. Black 'bird-mesh' was installed over each experiment tank to prevent fish escaping. Solid and dissolved matter was removed using in-line cartridge filters and foam fractionation.

Experiment tank system at PSFI

Feeding protocols and water management

After stocking (7th March 2011), each of the 16 dietary treatments was randomly assigned to 3 experiment tanks. Groups of Asian seabass were not fed the day of stocking and were introduced to their respective test diets the following day. Fish were hand fed to apparent satiation at 0830 h and 1430 h Monday through Friday and fed once at 0900 h on Saturdays and Sundays. To ensure feed intake among individual tanks of fish was not confounded by the leaching of different hydrolysates into the water column the flow of water to all tanks in each system was ceased immediately prior to feeding. After feeding, all water was purged from individual tanks and the sumps for a period of 30 min to ensure residual concentrations of hydrolysates were absent from the water column. Replacement water was pumped from a pre-heated reservoir to ensure nominal water temperatures did not fall. Following complete water exchange the systems were closed and returned to normal operation. Activated carbon filters were installed in each of the systems to further ensure residual concentrations of hydrolysates were absent. The carbon filters were exchanged on a daily basis.

Husbandry

Feed intake and water quality data were recorded on a daily basis. Fish were anaesthetized and bulk weighed 4 and 6 weeks (i.e. harvest; 18th April 2011) after stocking to assess the impacts of coating the test feeds with and without the feeding stimulants. At the conclusion of the experiment fish were returned to larger holding tanks. No fish died during the experiment.

Water quality was monitored on a daily basis during the experiment using a Horiba water quality analyser (Model U10). Mean ± SD for water temperature, dissolved oxygen, salinity and pH were 29.0±1.6˚C, 5.9±1.5 mg L^{-1}, 36.6±0.8 g L^{-1} and 7.9±0.2 pH units, respectively.

Performance calculations

Performance criteria were first calculated using measurements of individual fish housed in each replicate tank. The average tank value for specific performance criterion was used in statistical analysis. Raw growth and feed intake data is presented in Table 4. To examine relative differences in feed intake and weight gain, raw data was referred to allometric weight exponents of 0.8 in order to remove the influence of fish size on the selected response variables. Raw feed intake, relative feed intake and apparent FCR was corrected for the moisture content of the different dietary treatments and is presented on a dry matter basis (Table 4).

Weight gain (g $fish^{-1}$) = harvest weight (g) – stocking weight (g) Eq.1

Geometric mean body weight (GMBW) = (initial weight (g) x final weight $(g))^{0.5}$ Eq.2

FCR = feed consumption (g) / wet weight gain (g) Eq.3

Rel. feed intake (g $kgBW^{-0.8}day^{-1}$) = ind. feed intake (g) / $((GMBW/1000)^{0.8})$/days Eq.4

Rel. weight gain (g $kgBW^{-0.8}day^{-1}$) = ind. weight gain (g) / $((GMBW/1000)^{0.8})$/days Eq.5

Chemical analysis

Proximate, amino acid and fatty acid analysis (FAME) of hydrolysates was done by DEEDI (QLD Government, Australia). All analyses were conducted according to specific in-house laboratory methods or recent AOAC methods (AOAC, 2005). The crude protein content of samples was estimated by multiplying the measured nitrogen content of each sample by a factor 6.25.

Statistics

Two-way analysis of variance (ANOVA) was used to examine the interaction between diet type (4 levels) and hydrolysate (4 levels) on weight gain, feed intake and FCR of Asian seabass. Statistical tests were based on n=3 replicates per treatment and alpha was set to 0.05. If ANOVA was significant the Tukey-Kramer multiple comparison procedure was used to separate treatment means at the 95% confidence interval. Cochran's C-test was used to determine if the standard deviations (SD) among treatments were similar prior to ANOVA. If treatment SD were found to be heterogeneous, appropriate transformations were applied to the raw data before ANOVA was conducted. Data for FCR was log transformed to meet the assumptions of ANOVA. All statistical analysis was performed using Statgraphics Plus Version 4 software (Manugistics Inc. 1998).

Results

All fish survived and remained healthy during the feeding experiment. Weight gain was rapid during the experiment, generally exceeding 4 g day^{-1} for fish reared on the fish meal diet (FM). In relative terms this was approximately 14.5 g kgBW$^{-0.8}$ day^{-1}. These growth rates were similar to the growth rates of Asian seabass reared in previous USB trials at PSFI (Booth et al., 2010a) and are in line with growth rates predicted for this species using factorial models (Glencross, 2008). The results of two-way ANOVAs indicated that there was no significant interaction of main effects or of attractant type (i.e. hydrolysate) on relative feed intake, relative weight gain, FCR, individual weight gain or individual feed intake (Table 5). However, two-way ANOVA revealed that there was a highly significant effect of diet type on all the aforementioned performance criteria (Table 5). These results indicated that top-coating the four feeds selected for this trial with 2% w/w of different hydrolysates was not effective at promoting additional feed intake or weight gain.

Multiple comparisons of the pooled means for diet type (n=12) indicated that individual feed intake and individual weight gain were significantly higher in Asian seabass fed the fishmeal and SBM 38 feeds compared to fish fed the SPC 60 and SBMSPC 30 feeds.

These trends were also reflected in multiple comparisons of relative feed intake and relative weight gain (Figure 1). Food conversion ratio was significantly better (i.e. lowest) in fish fed the fishmeal diet than fish fed any of the other test feeds (Figure 1). FCR of fish fed the SBM 38 diet was significantly higher (i.e. worse) than those fed fishmeal diets, but only by a small margin. FCR was poorest in fish fed the SBM/SPC 30 and the SPC 60 diets; however the average FCR for these diets was still below 1.16:1 (Figure 1).

Discussion

None of the feed attractants used in this experiment had a significant influence on relative feed intake or growth of Asian seabass. Instead, the overwhelming driver of feed intake and subsequent weight gain was diet type. The lack of significant interaction between attractant type and diet type further highlighted the lack of efficacy in the selected attractants; at least at the concentrations we investigated (i.e. 2% w/w). As a result, the average feed intake of diets high in SPC and low in fish meal (i.e. SPC60 and SBM/SPC30) was lower than that of the other test feeds and the growth potential of fish was reduced.

The performance of Asian seabass reared on the non coated aqua-feeds in this experiment was very similar to the performance of Asian seabass reared on the same diets in the 2010 USB experiment (Booth et al., 2010a). Given that the Asian seabass used in each experiment were sourced from different farms and had different nutritional histories, the similarity in the results strengthens the conclusions drawn from our earlier study. The trends from both studies are clearly reflected in Figure 2 (note FCR is represented on the right y-axis).

We were not expecting the addition of feed attractants to improve feed intake in Asian seabass fed the fishmeal diet or the SBM38 diet *per se*, ostensibly because these diets were high in fishmeal and probably already quite palatable to the fish. We have observed a similar lack of response in mulloway *Argyrosomus japonicus* (Sciaenidae sp.) fed a commercial aquafeed top-coated with various proprietary feeding stimulants (Harris, 2006), presumably because the commercial aqua-feed was high in fishmeal and other ingredients that made it palatable. Similar conclusions were reached by Smith et al. (2005) with respect to the inclusion of feed attractants on commercial feeds for black tiger prawn

Penaeus monodon (Smith et al., 2005). These authors hypothesized that addition of attractants to diets already containing a threshold level of suitable feeding effectors would have little impact on feed intake or growth response. Nonetheless we hypothesized that the feed intake of the SPC60 and blended diet (SBM/SPC30) would be improved by the addition of at least one of the attractants. This was not the case. Glencross et al. (2010b) have hypothesized that diets for Asian seabass might require at least 15% fishmeal to ensure optimal feed intake when diets contain plant proteins such as lupin protein concentrate. In addition, earlier work by Singh et al. (2005) found that feed stimulation response in juvenile Asian seabass was higher when using 1% fishmeal (Bombay duck meal; *Harpodon nehereus*) as an attractant as opposed to using 1% proline, glycine or lysine in feeds containing 30% SBM. This group went on to investigate the stimulant effects of various fishmeal sources including Bombay duck (*Harpadon nehereus,* Harpadontidae), anchovy (*Coilia dussumieri,* Coilinae), lesser sardine (*Sardinella fimbriata*, Clupeidae), ribbon fish (*Trichiurus lepturus*, Trichiuridae) and shrimp head meal (*Penaeus monodon*, Penaeidae) on the feeding behaviour of fry and juvenile Asian seabass (Singh et al., 2006). The latter study confirmed that Bomaby duck meal was more efficacious in stimulating feeding response and feed intake in juvenile Asian seabass than the other fishmeal products when included at 10% of the diet.

A novel experiment comparing growth of Asian seabass fed live mosquito fish (*Gambusia holbrooki*, Girard), a 50:50 mixture of live mosquito fish and a commercial feed or the commercial feed alone found that Asian seabass grew significantly faster and had a lower FCR on the live fish treatment. Growth on the mixed ration was intermediate between the live fish and pellet only ration (Michael et al., 2010). The aforementioned studies suggest that fishmeal itself is probably a powerful feeding stimulant for Asian seabass. The work of Singh et al. (2005; 2006), also suggests that fishmeal type or source may also play and important role in the efficacy of response. Moreover, the stimulant qualities that make fishmeals unique to Asian seabass do not appear to have been replicated to the same extent in the quality or the quantities of Actipal HC1, Actipal ML8 or Artemia hydrolysates we used, respectively.

The use of proprietary feed attractants to promote feed intake has not always been successful (Smith et al., 2005). For example, a recent study with juvenile cobia *Rachycentron canadum* investigating the palatability of feeds containing soy protein concentrate and soy protein isolate (ADM Soycomil® and ADM Ardex®) found a proprietary feed attractant (Finnstim-S™; anhydrous betaine/beet protein hydrolysate, Danisco, Copenhagen, Denmark) was ineffective at increasing intake or performance when included at 1% of the diet (Trushenski et al., 2011). However, the results of this study may be confounded due to the fact that feed intake was restricted and the feeding trial was carried out in a semi-closed recirculation system which may have dispersed the feed attractant throughout the culture system. Our experimental approach of purging and flushing individual experiment tanks during and after feeding and incorporation of carbon filtration ensured that residual concentrations of feed attractants were reduced or eliminated.

Comparative weight gain and feed intake was also significantly better in European seabass fed a fishmeal control diet (38% fishmeal) containing no feed attractants compared to experimental diets containing 40% soy protein concentrate and 2.5% amino acid attractant mixture (Dias et al., 1997). However, incorporation of the attractant mixture significantly increased growth rate (i.e. thermal growth coefficient) of soy diets compared to growth on diets without it, but it did not significantly increase the feed intake of either diet. Similarly, others have trialed the efficacy of amino acid mixtures to increase intake of diets high in several plant proteins such as solvent extracted SBM, corn gluten meal and wheat middlings (Papatryphon & Soares Jr, 2001). These authors increased the dietary concentration of a single amino acid mixture from 1.7 to 6.3% of the diet and trialed these additions on a high fishmeal diet (39% fishmeal) and the aforementioned plant protein based diet. The results indicated that relative feed intake was significantly higher in the fishmeal diets compared to the plant protein diet and that addition of the attractant mixture significantly increased relative feed intake by about 0.5% body weight per day, however there was no benefit in adding more than 1.7% of the attractant mixture to the feeds (i.e. two-way ANOVA). It is clear from the aforementioned studies that selection of an appropriate attractant for a species and a particular dietary formulation is a complicated procedure. Nonetheless, the olfactory (smell) and gustatory (taste) characteristics of diets containing more than 40% fishmeal have had a far stronger influence on feed intake in this study than any of the feed attractants we tested.

Actipal HC1 is manufactured from tuna co-products and is reported to be rich in free amino acids and peptides with a molecular weight less than 500 Daltons. Phosphoric acid (E338), citric acid (E330), ethoxyquin (E324) and potassium sorbate (E202) are listed as ingredients. Actipal ML8 is manufactured from poultry raw material waste, contains similar amino acids and has approximately 62% of peptides weighing less than 500 Daltons The proprietary information supplied with the Artermia hydrolysate did not include any compositional data (see attached proprietary information at rear of this report). A comparison of the dry basis amino acid composition of the three hydrolysates is presented in Figure 3. Artemia (brine shrimp) are used extensively in the larviculture of fish species and for this reason hydrolysates of this crustacean might be expected to contain certain chemicals which may enhance feed intake. Crustacean based hydrolysates such as krill meal have proved efficacious in other studies with larval fish species (Kolkovski et al., 2000). The low lipid content of the Artemia hydrolysate used in the present study may indicate the hydrolysate was processed from adult specimens as the exoskeleton of adults forms a proportionally greater amount of the dry mater than in newly hatched nauplii (Webster & Lovell, 1991).

Non-volatile compounds with low molecular weights such as free L-amino acids, nucleotides and nucleosides, quaternary ammonium compounds and organic acids are thought to be particularly important in fish olfaction and gustation (Jobling et al., 2001). Furthermore, fish species have been crudely classified as having a “limited response range” while others have been classified as having a “wide response range” and these differences probably reflect differences in ecological niche (Lamb, 2001). Carnivorous species tend to be more sensitive to alkaline and neutral amino acids (e.g. glycine, proline, taurine, valine) while herbivorous types seem to respond better to acidic amino acids (e.g. aspartic and glutamic acids) (De la Higuera, 2001). Moreover, fish appear to respond better to certain mixtures of amino acids rather than single amino acids (Papatryphon & Soares Jr, 2000) and stimulants that possess the same mixture of compounds that they might encounter in their natural sources of prey. The scientific literature clearly indicates that feeding stimulants and mixtures are species-specific which may explain the lack of improvement in feed intake and growth of Asian seabass on the specific feeding stimulants evaluated in our study.

The negative factors affecting feed intake of diets containing SPC (SPC60 and SBM/SPC30) were not ameliorated by the use of the feed attractants we selected. This may be due to the concentration of the attractants or the method of incorporation onto pellets. For example, each of the proprietary attractants was added at the recommended level on an “as received basis”, however each of the attractants was high in moisture content which likely resulted in dry matter addition rates of approximately 1.17%, 0.67% and 0.32% for the Actipal HC1, Actipal ML8 and Artemia hydrolysate, respectively. These addition rates are quite dissimilar and result in varying levels of free amino acids, fatty acids and other peptides being top-coated onto the pellets. While liquid based hydrolysates are easily handled and sprayed onto the surface of pellets, the addition of a powdered product would be far easier. Other researchers have found that surface coating pellets with krill meal hydrolysates in both liquid and dry forms significantly improved feed intake in juvenile rainbow trout compared to fish fed the same diets but where the attractants were incorporated into the mash during the pelleting procedure (Oikawa & March, 1997). Further evaluation of the most suitable method of incorporating feeding stimulants into or onto feeds for Asian seabass may be warranted if an efficacious stimulant can be identified.

In conclusion, none of the feed attractants used in this experiment had a significant influence on relative feed intake or growth of Asian seabass. The overwhelming driver of feed intake was diet type and the performance results of this study reflect that of an earlier experiment using the exact same diets. The lack of efficacy in the tested hydrolysates may be related to the chemical composition of the individual hydrolysates, the inclusion rates we selected for this trial or possibly the method of incorporation. The lack of efficacy demonstrated in this study has also been noted in other feeding trials of this nature, albeit using different feeding stimulants. This indicates that selection of an appropriate feed attractant or stimulant for any particular fish species is a difficult task. Further research with Asian seabass in this field should include screening of additional proprietary feed stimulants and dose response studies aimed at determining suitable inclusion rates of efficacious feeding stimulants.

Table 1. Formulation and measured analysis of un-coated experimental feeds (dry matter basis).

	Diet code			
	D2 Fishmeal	D5 SBM 38	D8 SPC 60	D10 SBM/SPC
Ingredient (%)				
Fish oil	10.00	10.40	10.00	11.98
Fish meal	55.00	40.64	10.00	16.11
SBM[1]	0.00	38.00	0.00	30.00
SPC[2]	0.00	0.00	60.00	30.00
Wheat	14.70	10.00	12.92	10.00
Meat meal	10.00	0.00	0.00	0.00
Poultry meal	10.00	0.66	6.48	1.41
L-methionine	0.00	0.00	0.29	0.20
Vit/min premix	0.30	0.30	0.30	0.30
Proximate analysis (%)				
Ash	13.20	9.50	7.00	7.50
Nitrogen	8.64	8.28	9.00	8.21
Crude protein	54.00	51.75	56.25	51.31
Fat	16.70	15.20	10.90	14.50
NFE	16.10	23.55	25.85	26.69
Gross energy (MJ kg^{-1})	21.87	21.92	22.39	22.25
Amino acid (g kg^{-1})				
Alanine	32.34	27.83	26.25	24.94
Arginine	33.56	35.41	41.89	38.96
Aspartic acid	41.75	49.95	57.29	54.36
Cystine	4.38	5.33	6.80	5.90
Glutamic acid	68.63	81.03	98.20	91.57
Glycine	37.22	26.67	26.46	24.35
Histidine	15.36	15.58	14.57	14.18
isoLeucine	19.97	21.76	25.00	22.65
Leucine	37.08	37.71	43.3	38.68
Lysine	35.84	36.42	35.43	33.69
Methionine	10.28	9.27	10.39	8.94
Phenylalanine	20.12	22.32	26.91	24.34
Proline	25.89	23.64	28.49	25.96
Serine	19.82	22.08	26.43	24.57
Threonine	20.41	21.1	22.10	20.86
Tryptophan	5.75	6.18	6.50	6.19
Tyrosine	15.00	16.48	18.44	17.16
Valine	24.35	24.60	26.33	24.64
∑AAs (%)	46.77	48.34	54.08	50.19

1. SBM; solvent extracted soybean meal, Argentinean origin.
2. SPC; soy protein concentrate; Soycomil-K, Archer Daniels Midland (ADM)

Table 2. Measured proximate and amino composition of different hydrolysates (dry matter basis).

	Hydrolysate		
	Actipal HC1	Actipal ML8	Artemia
Total dry matter %	53.50	30.60	14.90
Ash %	21.50	14.40	31.10
Nitrogen %	9.38	4.82	3.11
Gross energy MJ Kg^{-1}	18.92	26.43	13.07
Total lipid (%)	15.29	52.29	2.46
Crude protein %	58.62	30.12	19.43
Amino acid (g kg^{-1})			
Alanine	30.37	14.31	11.20
Arginine	34.58	17.13	1.78
Aspartic acid	42.50	21.09	17.35
Cystine	5.31	3.50	1.33
Glutamic acid	61.77	33.59	19.91
Glycine	30.45	18.53	8.38
Histidine	14.08	6.31	3.39
isoLeucine	22.65	11.40	7.81
Leucine	37.57	20.03	10.99
Lysine	35.99	16.23	8.57
Methionine	13.41	6.42	2.89
Phenylalanine	20.49	10.99	4.89
Proline	23.45	12.78	8.70
Serine	23.31	11.20	7.00
Threonine	24.05	12.58	5.96
Tryptophan	3.26	1.59	0.61
Tyrosine	18.35	8.31	1.39
Valine	27.69	14.02	9.30

Table 3. Measured fatty acid composition of hydrolysates (dry matter basis).

	Hydrolysate		
	Actipal HC1	Actipal ML8	Artemia
Fatty acid (mg g^{-1} of dry sample)			
14	1.65	3.09	0.06
14:1n-5	-	0.86	-
15	0.65	0.54	0.02
16	19.69	99.25	0.97
16:1n-7	2.15	24.26	0.15
17	1.08	0.81	0.05
18	7.16	29.99	0.58
18:1n-9	9.67	196.72	1.31
18:1n-7	1.97	11.90	0.52
18:2n-6	0.95	49.79	0.61
18:3n-3	0.28	8.99	0.88
18:4n-3	0.44	0.30	0.11
20	0.36	0.72	-
20:1n-9	0.56	2.15	0.03
20:1n-7	0.06	-	-
20:2n-6	0.25	1.13	0.02
20:3n-6	-	-	-
20:4n-6	2.87	2.23	0.04
20:3n-3	0.09	-	0.03
20:4n-3	-	-	-
20:5n-3	5.36	0.41	0.14
22:1n-9	-	-	0.02
22:4n-6	0.25	0.35	-
22:5n-6	1.67	-	-
22:5n-3	1.24	0.52	-
22:6n-3	22.52	0.45	0.09
24:1n-9	0.53	-	-
% SFA	37.55	30.93	29.80
% MUFA	18.34	54.29	36.10
% n3 PUFA	36.74	2.46	22.14
% n6 PUFA	7.36	12.31	11.96

Note: "-" indicates <0.01mg g^{-1}

Table 4. Mean ± SD performance of Asian seabass fed test diets top-coated with 2% w/w hydrolysates for 6 weeks.

Hydrolysate	Diet type	Stock biomass (g/tank)	Harvest biomass (g/tank)	Biomass gain (g/tank)	Ind. stock weight (g/fish)	Ind. harvest weight (g/fish)	Ind. wt. gain (g/fish)	Feed* intake (g/tank)	AFCR *	Rel. feed intake *	Rel. wt gain
None	FM	1259.67	2832.67	1573.00	125.97	283.27	157.30	1413.75	0.90	12.74	14.16
	stdev	*16.04*	*289.98*	*276.57*	*1.60*	*29.00*	*27.66*	*195.78*	*0.04*	*1.23*	*1.90*
Artemia	FM	1277.00	2989.67	1712.67	127.70	298.97	171.27	1492.35	0.87	13.11	15.04
	stdev	*20.81*	*205.05*	*218.57*	*2.08*	*20.50*	*21.86*	*130.15*	*0.03*	*0.84*	*1.57*
Actipal ML8	FM	1274.00	2938.67	1664.67	127.40	293.87	166.47	1484.58	0.89	13.15	14.75
	stdev	*23.07*	*72.70*	*50.29*	*2.31*	*7.27*	*5.03*	*32.91*	*0.01*	*0.10*	*0.20*
Actipal HC1	FM	1266.33	2884.33	1618.00	126.63	288.43	161.80	1426.49	0.88	12.76	14.47
	stdev	*6.51*	*118.19*	*124.59*	*0.65*	*11.82*	*12.46*	*73.06*	*0.03*	*0.47*	*0.90*
None	SBM38	1257.00	2794.00	1537.00	125.70	279.40	153.70	1522.70	0.99	13.84	13.97
	stdev	*20.07*	*97.32*	*115.11*	*2.01*	*9.73*	*11.51*	*96.71*	*0.02*	*0.76*	*0.94*
Artemia	SBM38	1262.33	2821.00	1558.67	126.23	282.10	155.87	1493.92	0.96	13.50	14.09
	stdev	*7.02*	*52.85*	*46.69*	*0.70*	*5.28*	*4.67*	*25.94*	*0.01*	*0.12*	*0.29*
Actipal ML8	SBM38	1259.33	2733.00	1473.67	125.93	273.30	147.37	1412.49	0.96	12.94	13.50
	stdev	*22.50*	*115.66*	*117.60*	*2.25*	*11.57*	*11.76*	*79.50*	*0.03*	*0.51*	*0.88*
Actipal HC1	SBM38	1273.00	2813.67	1540.67	127.30	281.37	154.07	1491.61	0.97	13.45	13.89
	stdev	*20.81*	*98.44*	*119.23*	*2.08*	*9.84*	*11.92*	*65.75*	*0.04*	*0.49*	*0.97*
None	SPC60	1277.67	2353.67	1076.00	127.77	235.37	107.60	1223.66	1.14	11.83	10.40
	stdev	*14.29*	*119.82*	*114.01*	*1.43*	*11.98*	*11.40*	*63.64*	*0.06*	*0.35*	*0.87*
Artemia	SPC60	1264.33	2336.00	1071.67	126.43	233.60	107.17	1193.65	1.11	11.63	10.44
	stdev	*5.51*	*61.25*	*65.90*	*0.55*	*6.12*	*6.59*	*52.91*	*0.03*	*0.42*	*0.55*
Actipal ML8	SPC60	1259.33	2285.67	1026.33	125.93	228.57	102.63	1209.11	1.18	11.91	10.09
	stdev	*17.79*	*103.20*	*85.62*	*1.78*	*10.32*	*8.56*	*1.61*	*0.10*	*0.29*	*0.61*
Actipal HC1	SPC60	1279.00	2325.00	1046.00	127.90	232.50	104.60	1232.02	1.18	11.96	10.16
	stdev	*15.72*	*96.06*	*95.91*	*1.57*	*9.61*	*9.59*	*79.46*	*0.08*	*0.60*	*0.77*
None	SBM/SPC30	1269.67	2416.33	1146.67	126.97	241.63	114.67	1285.86	1.12	12.32	10.99
	stdev	*17.10*	*153.64*	*143.18*	*1.71*	*15.36*	*14.32*	*141.58*	*0.03*	*1.03*	*1.05*
Artemia	SBM/SPC30	1254.67	2297.33	1042.67	125.47	229.73	104.27	1200.29	1.15	11.81	10.26
	stdev	*12.66*	*21.36*	*31.21*	*1.27*	*2.14*	*3.12*	*8.09*	*0.04*	*0.11*	*0.31*
Actipal ML8	SBM/SPC30	1274.33	2431.67	1157.33	127.43	243.17	115.73	1298.63	1.12	12.40	11.05
	stdev	*41.55*	*118.45*	*79.22*	*4.15*	*11.84*	*7.92*	*88.44*	*0.03*	*0.45*	*0.43*
Actipal HC1	SBM/SPC30	1249.67	2388.00	1138.33	124.97	238.80	113.83	1273.72	1.12	12.36	11.05
	stdev	*35.50*	*26.63*	*34.67*	*3.55*	*2.66*	*3.47*	*25.05*	*0.02*	*0.26*	*0.43*

* Rel. weight gain and feed intake presented as g $kgBW^{-0.8}day^{-1}$. All feed intake and feed conversion data calculated on a dry matter basis.

Table 5. Results of two-way *ANOVA* on selected performance criteria.

Criterion	Hydrolysate (A)	Diet type (B)	A x B	Residual
Relative feed intake				
Mean square	0.0563	6.1691	0.2590	0.3494
F ratio	0.16	17.66	0.74	
P-value	0.9217	<0.0001	0.6686	
Relative weight gain				
Mean square	0.0307	55.5935	0.3787	0.8133
F ratio F	0.04	68.35	0.47	
P-value	0.9900	<0.0001	0.8867	
Log (AFRC)				
Mean square	0.0006	0.1892	0.0013	0.0016
F ratio	0.38	117.82	0.83	
P-value	0.7665	<0.0001	0.5964	
Ind. weight gain				
Mean square	5.8403	10217.5	81.4342	155.387
F ratio	0.04	65.75	0.52	
P-value	0.9901	<0.0001	0.8461	
Ind. feed intake				
Mean square	5.7369	2135.11	58.6747	77.5065
F ratio	0.07	27.55	0.76	
P-value	0.9735	<0.0001	0.6554	

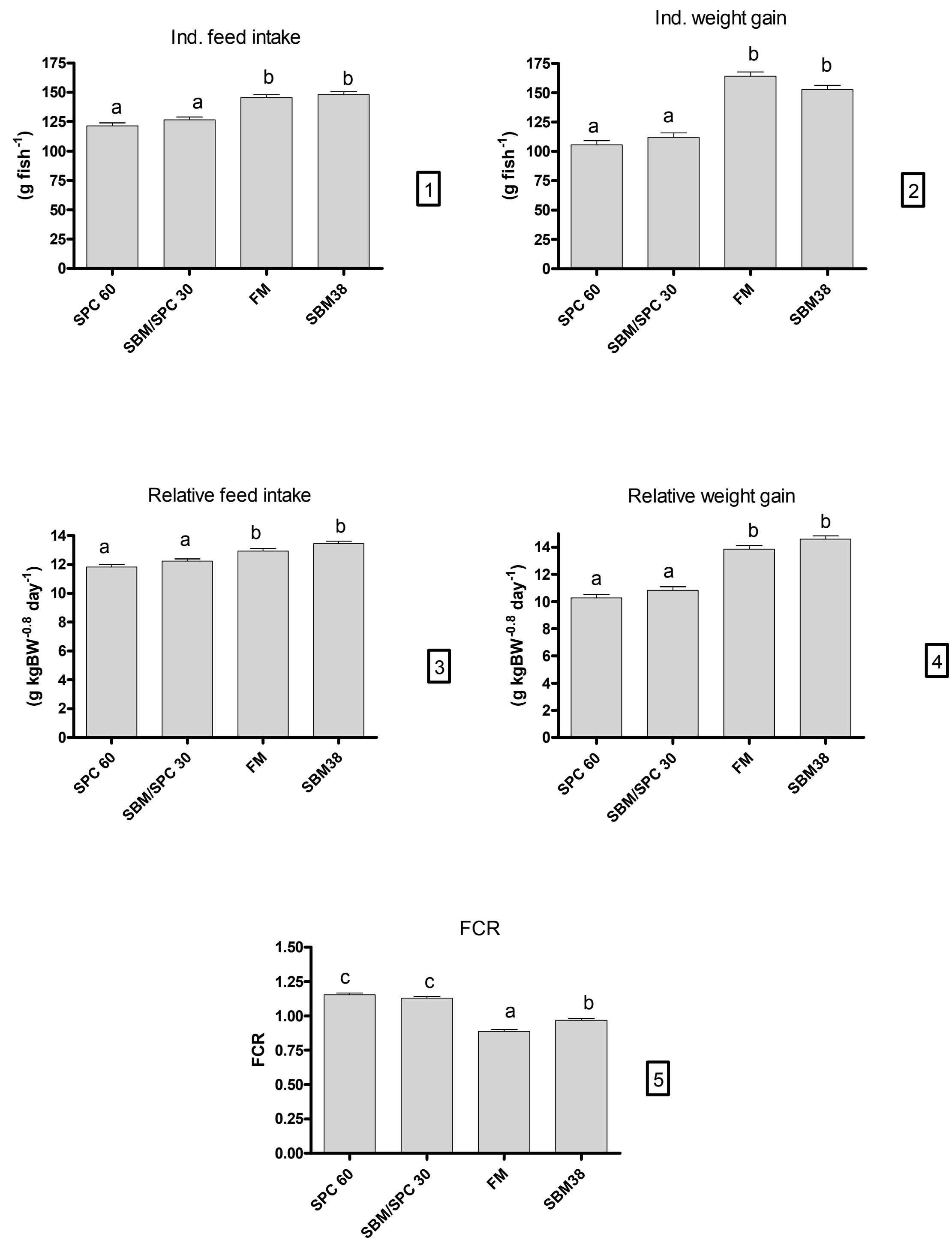


Figure 1. Performance characteristics of Asian seabass fed different attractants.

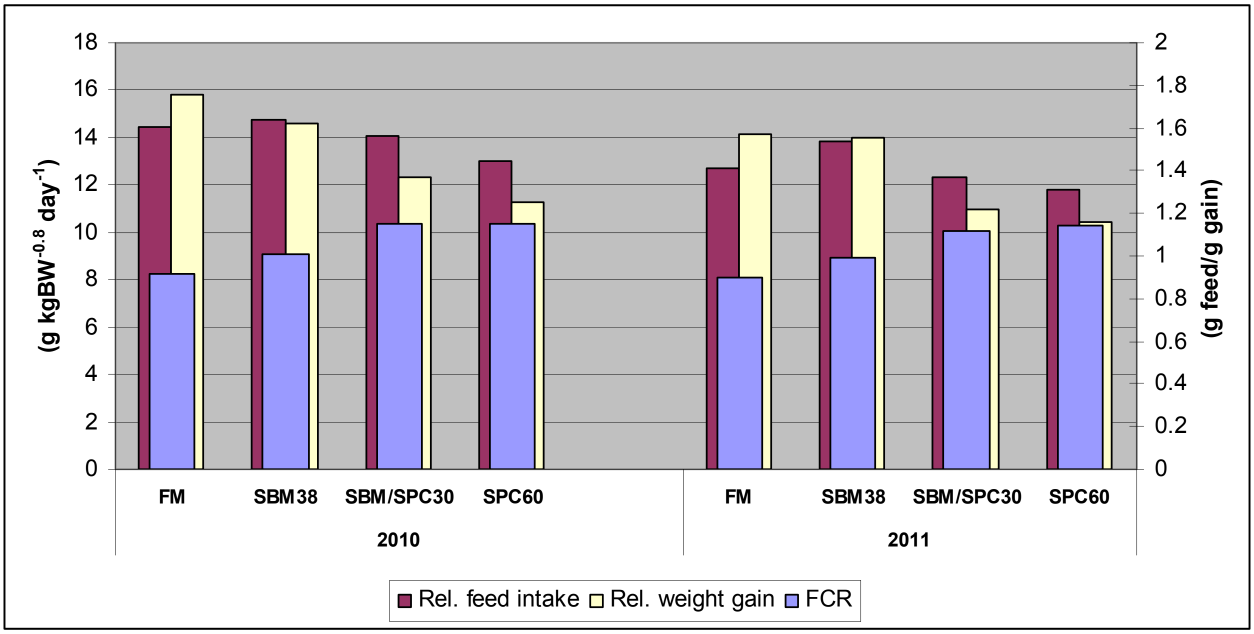


Figure 2. Comparison of performance characteristics of Asian seabass fed the same test feeds in 2010 and 2011. Test feeds were not coated with attractants.

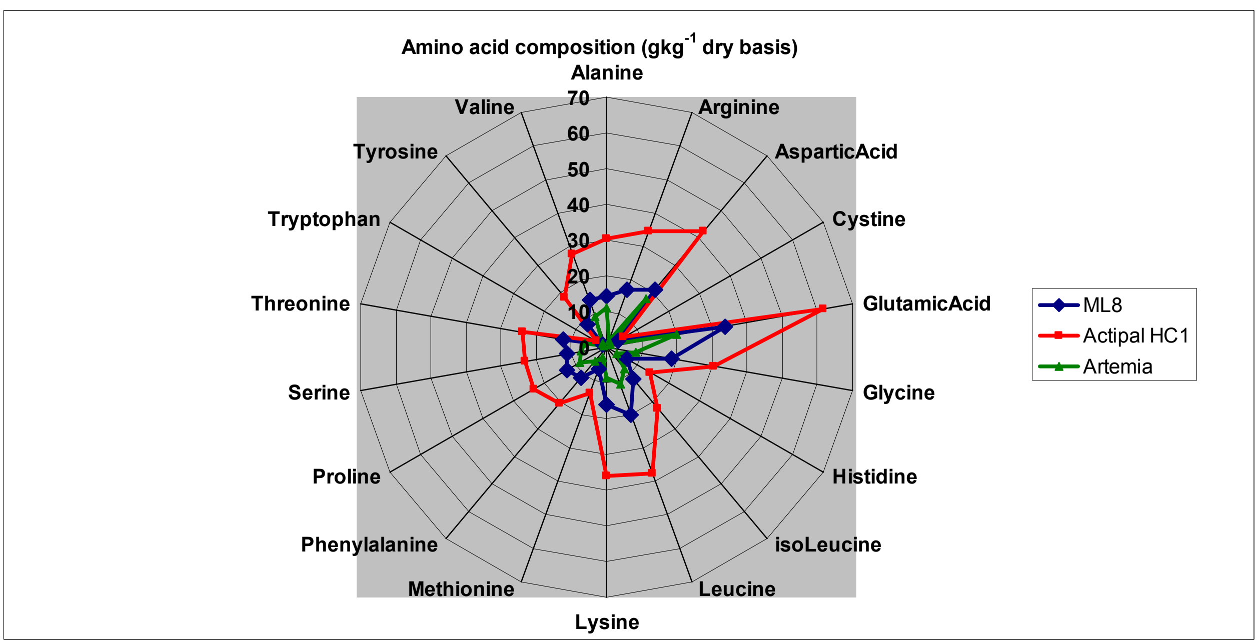


Figure 3. Dry basis (g kg^{-1}) amino acid profiles of different feed attractants.

Determination of the apparent digestibility of key feed ingredients from ASA-IM identified target markets in SEA such as Thailand, Philippines, Indonesia, Vietnam or Malaysia.

Introduction

South East Asia (SEA) is leading the aquaculture production of Asian seabass *Lates calcarifer,* producing approximately 40,000 metric tonnes in 2009 (FAO, 2011). Production of Asian seabass is mostly done in marine environments and farmers are steadily moving towards use of dry commercial aquafeeds rather than using farm based feeds based on industrial fish. Development of commercial aquafeeds for this species in SEA is being assisted by the American Soybean Association International Marketing (ASA-IM) group and research conducted by the United Soybean Board (USB). These groups, assisted by NSW DPI have identified that development of aquafeeds for Asian seabass in SEA is being constrained by a lack of information on feed ingredients, particularly basic information on the nutrient and energy digestibility of so called "backbone ingredients"; the most commonly available ingredient stocks. Other issues facing feed formulators in SEA include the inability to access high quality ingredients, particularly fishmeal, which is often only available in fair average quality (FAQ) or lower specifications. These constraints can only be overcome by determining the digestibility of basic feed ingredients and understanding how these ingredients interact and impact on feed formulation and pellet quality. This information will also assist feed formulators in rapidly reducing or eliminating fishmeal as a basic ingredient in diets for Asian seabass and assist in increasing the level of alternative proteins such as SBM and SPC.

In previous research for the United Soybean Board (USB), NSW DPI successfully determined the proximate and amino acid digestibility coefficients of fishmeal and two levels each of solvent extracted soybean meal (SBM) and soy protein concentrate (SPC) by Asian seabass (Booth & Allan, 2010). These coefficients were combined with reliable literature values on the apparent digestibility of other feed ingredients to construct an ingredient data base which could be used to formulate aqua-feeds for this species on a digestible nutrient basis. Although basic apparent digestibility coefficients for several feed ingredients were available, the vast majority of literature studies had only determined coefficients for protein and energy and data on the apparent digestibility of amino acids was missing. Data on amino acid digestibility is critical in correctly formulating feeds for fish and crustaceans containing SBM and SPC as soybeans are deficient in the sulphur amino acids. In addition, formulating to amino acid digestibility becomes increasingly important as formulators strive to reduce fishmeal content in aquafeeds by using other complimentary protein and energy sources, especially as these sources often have their own inherent amino acid deficiencies. Formulating feeds for Asian seabass on a digestible amino acid and digestible energy basis should result in feeds which meet but do not exceed the digestible protein and energy requirements of this species. Furthermore, knowledge of the amino acid digestibility of individual ingredients will also give feed formulators far greater flexibility and confidence in manipulating feed recipes and reducing levels of fishmeal in diets for this species.

The aim of this study is to determine the proximate and amino acid digestibility of ingredients typical of those used in SEA feed mills. Ingredients selected for this trial include two sources of fishmeal (low and high protein specification), poultry meal, blood meal, corn gluten meal, raw wheat and pre-gelatinized wheat starch. Data from this experiment will be combined with earlier research on the digestibility of SBM and SPC to update and refine the USB feed ingredient formulation data base for Asian seabass.

Methods

Overview of digestibility experiment

Several major protein and energy sources were selected for evaluation based on information provided by ASA-IM Manager in South East Asia (Lukas Manomaitis) and to compliment the data base of digestibility coefficients for Asian seabass presented in earlier work (Booth & Allan, 2010). As quarantine restrictions precluded NSW DPI importing these ingredients into Australia, similar ingredients were sourced from Ridley Aquafeed Pty Ltd (Narangba, QLD), a collaborator on the current USB investigation. The selected ingredients and their nutrient compositions are detailed in Table 6.

The apparently digestibility of test ingredients was determined using indirect techniques that rely on feeding diets which contain a mixture of a reference diet (69.3%), the test ingredient (30%) and chromium oxide as inert marker (0.7%). Test diets were fed to

Asian seabass for 7-8 days before faecal material was collected from the distal intestine using manual stripping techniques. Ingredients, diets and faecal material were chemically analysed to determine nutrient and energy content as well as concentration of chromium. These values were subsequently used to calculate diet and ingredient digestibility coefficients for each ingredient.

Ingredients and diet preparation

A commercial Asian seabass (barramundi) feed was used as the reference diet in the digestibility experiment (Ridley AquaFeed Pty Ltd; Marine Float; stated min. crude protein 50%, min. crude fat 14% and max. crude fibre 2.5%). This feed was from the same physical batch of feed used in the previous trial with Asian seabass and was used to provide continuity between the present and previous digestibility experiment. The measured nutrient, amino acid and energy content of the reference diet is presented in Table 6. Prior to inclusion in test feeds the reference diet and all ingredients were ground in a hammer mill fitted with a 1.6 mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia). The reference diet, test ingredients and marker were then combined on a dry matter basis and thoroughly mixed (Hobart Mixer; Troy Pty Ltd, Ohio, USA) before the addition of distilled water. Each mash was then formed into pellets using a meat grinder fitted with an 6mm diameter die plate (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were dried for 5 to 6 h (≈ 35º C) in a simple convection drier until moisture content was < 100 g kg^{-1} diet. Following preparation, all diets were labelled and stored frozen at < -15ºC until required. The ingredient and measured nutrient composition of the test diets is presented in Table 7.

Fish stocks & handling protocols

Three hundred size graded Asian seabass were collected from Tailor Made Fish Farm (Marsh Road, Bobs Farm, NSW Australia) and transported to Port Stephens Fisheries Institute (PSFI) in 2 x 600L rectangular fish transporters containing freshwater. During the short journey the transport tanks were supplied with medical grade oxygen (BOC Australia) via regulated flow meters (Ezi-Flow; Comweld Medical Cigweld Pty Ltd Preston, Victoria, Australia) feeding submerged ceramic diffusers. This ensured the dissolved oxygen concentration in either tank did not fall below 6 mg L^{-1}. On arrival at

PSFI, fish were lightly anaesthetised (10 ppm Aquis-S®) in transport tanks, weighed in groups and systematically stocked into experiment tanks until each tank contained 12 fish (mean±sd body weight = 297±12 g). During the first few two days after stocking the fish were fed once daily on a sinking commercial Asian seabass feed provided by Ridley Aqua-Feeds Pty. Ltd (Narangba, QLD, Australia).

Laboratory facility

The digestibility experiment was performed in a laboratory that housed 48 x 200L white polyethylene aquaria supplied by two interlinked recirculating bio-filtration units of 1,700 litre capacity. Water flow to each experimental tank was controlled by small PVC taps that provided a weak centripetal current within each tank that assisted the removal of waste through a central, vertical PVC standpipe (32 mm diameter) fixed approximately 10 mm from the base. Water temperature was controlled in each of the bio-filtration units using a reverse cycle refrigeration unit to allow precise temperature control. Each unit was enriched with medical grade oxygen which was injected directly into each of the main supply manifolds. Each of the 48 experimental aquaria contained a central air-stone to provide additional aeration. Black plastic was wrapped around each of the 48 aquaria and a black plastic lid, which covered half the tank opening was fitted to minimise external disturbance of fish. Black ‘bird-mesh’ was installed over each experiment tank to prevent fish escaping. Although the laboratory contained 48 polyethylene tanks, replicate groups of fish were only stocked into alternate tanks (24 tanks). This was done to provide a spare, well oxygenated tank in which to recover anaesthetised fish post-stripping.

Faecal collection procedure

Dietary treatment was randomly assigned to n=3 tanks and fish were switched directly to test feeds on the third day after stocking. All fish were acclimated to their respective test diets for 8 days prior to faecal stripping. During this time they were hand fed to apparent satiation at 0800h and 1430h. After the acclimation period, fish were hand fed to apparent satiation at 0630h and 1500h. Fish were fed early to allow approximately 8 h before faecal collection was attempted. Feed was also offered to fish after the stripping event to ensure continuity of feeding.

Faecal collection was undertaken in stages, with 50% of tanks stripped on Mondays and the remaining 50% stripped on Tuesdays. This procedure was repeated on Thursdays and Fridays. In this way fish could be stripped twice weekly and rested for approximately 72 h between stripping events. Faecal stripping was continued until sufficient sample was collected to conduct the range of chemical analyses required (e.g. >6 g of dry matter).

Faecal material was collected at approximately 1430 h by manually stripping individual fish after they had been anaesthetised within their respective experiment tanks (25-30 ppm Aqui-S®). Faecal matter was expressed from the distal portion of the digestive tract by applying firm pressure to the abdominal region; running the thumb and forefinger from the pelvic fin region to the vent. Prior to faecal collection the same technique was used to expel urinary products and the area around the vent was wiped clean with a damp cloth. Faecal matter was expressed into 70 mL plastic sample jars held by and assistant and immediately transferred to a freezer. After stripping fish were then placed in the adjacent experiment tank to recover in well oxygenated water. Daily faecal collections from individual tanks were pooled and kept frozen (< -15ºC). At the conclusion of the experiment all faecal samples were oven dried for 24 h (80˚C) before samples were finely ground in a mortar and pestle and re-dried (as described). Samples were divided for dispatch to different service providers (see chemical analysis).

The digestibility experiment was run for 57 days (17th August - 13th October 2011). At the end of the experiment all fish were anaesthetised (10 ppm Aqui-S), weighed and returned to dedicated holding tanks at PSFI. No fish died during the experiment.

Water quality

Water quality was monitored on a daily basis during the experiment using a Horiba water quality analyser (Model U10). Mean±SD for water temperature, dissolved oxygen, salinity and pH were 26.98±1.10˚C, 7.18±0.85 mg L^{-1}, 31.73±2.22 g L^{-1} and 7.8±0.15 units, respectively.

Chemical analyses

Proximate analysis of ingredients, diets and faecal material was done by the NSW Department of Primary Industries Diagnostic & Analytical Services, Wagga Wagga, NSW, Australia while analysis of chromium was performed by Ecoteam Environmental Services (University of Sunshine Coast, Qld). These analyses were conducted according to specific laboratory methodologies or AOAC methods (AOAC, 2005). The crude protein content of ingredient, diet or faecal samples was determined by multiplying the measured nitrogen content of each sample by 6.25. Crude fat was determined by ether extraction. Quantitative amino acid analysis was done after samples underwent 24 h liquid hydrolysis in 6M HCl at 110°C (note: cysteine and tryptophan are not analysed by this method). After hydrolysis all amino acids were analysed using the Waters AccQTag Ultra chemistry method. As asparagine is hydrolysed to aspartic acid and glutamine to glutamic acid, the amount of these acids is the sum of those respective components. Samples were analysed in duplicate and results are expressed as the average. Amino acid analysis was done by the Australian Proteome Analysis Facility Ltd, Macquarie University, Sydney, NSW, Australia.

Calculation of digestibility coefficients

Apparent digestibility coefficients (ADC) of specific nutrients, amino acids and gross energy for each of the reference and test diets were calculated on a dry matter basis. Prior to calculation of ADC's the analysed nutrient or energy content of test and reference diets was cross checked by deriving the same values from the analysed nutrient or energy contents of individual ingredients as well as ingredient x reference diet combinations. This was done to ensure that spurious ADC's were not determined (Bureau & Hua, 2006). The ADC's for diets were calculated according to equation 1:

$$ADC\ (\%) = 100 \times [1 - (F/D \times DCr/FCr)], \qquad \text{Eq. 1}$$

where F = % nutrient or gross energy in faeces; D = % nutrient or gross energy in diet; DCr = % chromic oxide in diet; FCr = % chromic oxide in faeces (Cho et al., 1982).

ADCs for ingredients were calculated according to equation 2:

ADCING (%) = [(NutrTD*ADTD) – (PRD*NutrRD*ADRD)] / [(PING*NutrING)] Eq. 2

where ADCING = apparent digestibility of nutrient or gross energy in the test ingredient; NutrTD = the nutrient or gross energy concentration in test diet; ADTD = the apparent digestibility of the nutrient or gross energy in the test diet; PRD = proportional amount of reference diet; NutrRD = the nutrient or gross energy concentration in the reference diet; ADRD is the apparent digestibility of nutrient or gross energy in the reference diet; PING = proportional amount of test ingredient; NutrING is the nutrient or gross energy concentration in the test ingredient (Sugiura, Dong, Rathbone & Hardy, 1998).

Statistical analyses

The weight gain of Asian seabass fed different diets was compared with one-way ANOVA after setting alpha = 0.05. Cochran's test was applied to ensure variances were homogenous and the Tukey-Kramer multiple comparison procedure was used to separate treatment means at the 95% confidence interval. Statistical analyses were performed using Statgraphics Plus Version 4 software (Manugistics Inc. 1998).

Results / Discussion

No fish died during the experiment and fish gained between 1.7 to 3.4 g day^{-1}. These gains are slightly lower than the weight gain predicted for Asian seabass of this size (Glencross, 2008), however, it should be noted that this experiment was not designed as a growth study. The fact that all fish recorded positive growth indicates nutrient and energy intake exceeded maintenance requirements and that the impact of stripping procedures on the fish was minimal. Figure 4 summarises the average individual weight gain of each treatment (n=3). An exploratory one-way ANOVA revealed significant differences between the weight gain of Asian seabass fed different diets, with greatest gains recorded in fish fed the reference diet substituted with either of the fishmeal products or the reference diet substituted with poultry meal.

Collection of faecal material from fish proceeded efficiently, however the daily amount of faecal material varied from treatment to treatment and from tank to tank. Material was usually obtained from all fish within a tank, however on some occasions individual fish produced no faecal material. During the collection of faecal material we noticed a distinct variation between the faecal consistencies of fish fed the wheat and pregelatinized starch diets compared to other treatments. Faecal material from the wheat diet and from those fed the starch diet was extremely aqueous while that from the other treatments was more viscous. The observations we made with respect to the faecal consistency of fish fed wheat and pregelatinized starch was in agreement with observations reported on Asian seabass fed diets containing 30% barley, wheat or tapioca (Glencross et al., 2011a). In contrast, the faecal consistency of Nile tilapia fed diets high in gelatinized starch improved (Amirkolaie et al., 2006).

The apparent digestibility coefficients of the reference and experimental diets are presented in Table 8. The apparent digestibility coefficients of the reference diet from this experiment were very similar to the coefficients presented for the same reference diet examined in 2009 (Booth & Allan, 2010) and confirms that differences between the two digestibility experiments were relatively minor. Accordingly, this diet served as an inter-experimental control. Data on the digestibility of the reference diet used in 2009 has been included in Table 8 for comparison.

The apparent digestibility coefficients of test diets containing poultry meal or either of the diets containing fishmeal tended to be higher than the same coefficients for the reference diet, indicating these ingredients had a positive influence on digestibility. This was reflected in the high dry matter, energy and protein digestibility coefficients of these ingredients. In contrast, the addition of blood meal, corn gluten, wheat or pregelatinized wheat starch tended to lower the digestibility of the reference diet, indicating these ingredients had a somewhat negative impact on digestibility of the experimental diets Table 8).

Almost all proximate digestibility coefficients calculated for fishmeal FM1 (prime quality) were greater than 100%. Availability of most of the amino acids for this ingredient were also greater than 100% indicating this source of fishmeal was either completely digested or that minor errors in the chemical analysis of samples have been compounded by the sensitive nature of the equation used to calculate the digestibility coefficients of ingredients. This phenomenon is common in the literature and proximate coefficients greater than 100% have been presented for Asian seabass fed various ingredients (McMeniman, 2003; Booth & Allan, 2010; Glencross, 2011; Glencross et al., 2011a), especially for fat (Williams, 1998b). Compared to the prime fishmeal source the tuna meal (FM3) was not as well digested, especially with regard to dry matter digestibility. This result is similar to comparisons made between the digestibility of Danish fishmeal and tuna meal by Asian seabass in preliminary work on this species (Williams, 1998a). This may be indicative of the slightly higher ash and lower protein composition of the tuna meal. The prime fishmeal source we have examined in the present study is likely similar to the high quality steam dried South American fishmeal source (i.e. Empresa Pesquera Polar, Ecuador) examined in 2009 (included in table 8 for reference) (Booth & Allan, 2010). However, the range in coefficients for each of the fishmeal products we have examined clearly demonstrates there is considerable variation in the nutritional quality of fishmeal (Allan et al., 2000). This variation in nutritional quality can exist even when the proximate composition of the various fishmeals is similar and obviously has major implications for the accurate formulation of aquafeeds, especially on a digestible nutrient and energy basis.

Poultry meal was well digested and in-line with coefficients presented for Asian seabass by Boonyaratpalin & Williams (2002) and significantly higher than the digestibility of poultry offal meal examined by Glencross (2011), who presented coefficients for dry matter, protein and energy of 10, 40 and 53%, respectively. Blood meal was poorly digested by Asian seabass in this study as indicated by the low proximate digestibility and amino acid availability (Table 9). This could mean that the batch of blood meal tested in this trial was overheated during the rendering and drying process (Booth et al., 2005). Reported digestibility coefficients for blood meal vary considerably. For example, protein and energy digestibility of a steam-dried blood meal (ruminant origin) was as low as 46 and 58%, respectively, (Nengas et al., 1995), but as high as 90 and 83% for a spray-dried meal (Lupatsch et al., 1997) fed to gilthead sea bream. Other processes appear to affect the protein digestibility of blood meal including the application of organic acid preservatives (Laining et al., 2003). It is possible that the 30% substitution of the reference diet with blood meal may have impacted the digestibility of this ingredient. For example, an experiment evaluating blood meal inclusion content on the digestibility and performance of Murray cod *Maccullochella peelii peelii*, a carnivorous freshwater native fish found that both digestibility and performance decreased as the dietary levels of blood meal were increased from 8% to 32% of the diet (Abery et al., 2002). Semi-commercial diets for Asian seabass based on meat meal but also containing between 7-9% ring dried blood meal have been trialled with some success (Williams et al., 2003). However the effect of blood meal inclusion on digestibility in Asian seabass remains to be examined.

Asian seabass digested corn gluten meal better than blood meal in the present trial. However they appear to digest wheat gluten far more efficiently than corn gluten according to coefficients cited in other reports (Boonyaratpalin & Williams, 2002). Booth et al. (Booth et al., 2010b) also noted that yellowtail kingfish *Seriola lalandi*, a pelagic marine carnivore appear to digest wheat gluten more efficiently than maize gluten. The apparent availability of nearly all amino acids from corn gluten was lower than 51% for yellowtail *S. quinqueradiata*. The authors of that study hypothesized that the low pH of the material they tested (pH ≈ 3.2) may have affected digestibility (Masumoto et al., 1996).

The protein digestibility of wheat was high (90%), however the concomitant dry matter, organic matter and gross energy digestibility of wheat was low (all close to 30%) indicating wheat will not serve as a suitable source of dietary energy for Asian seabass. The calculated coefficients for dry matter, organic matter and energy for the pregelatinized wheat starch were close to zero or negative indicating that this starch product was indigestible to Asian seabass. A recent publication on the digestibility of cereal grains by Asian seabass has provided new comparative data by which to assess the digestibility coefficients of wheat and wheat starch from the current experiment (Glencross et al., 2011a). The latter study employed similar ingredient inclusion levels and also used stripping techniques to collect faecal material. Apparent digestibility coefficients cited by Glencross et al. (2011) for dry matter, protein and energy of wheat were 65.5, 100.2 and 65.2%, respectively compared to 30.2, 90.7 and 31.1%, for the same coefficients in our trial. There was some agreement in the digestibility of starch from wheat with a value of 29.7% cited by Glencross et al. (2011) and a value of 31.1% found in the present experiment. It is obvious the major discrepancy between trials is in the dry matter and energy digestibility. This may be related to the reference diet used in each study. In our case we used a commercial barramundi feed as a reference diet while Glencross et al. (2011) used a reference diet made of 64% fishmeal, 10% fish oil, 12.4%

cellulose and 13% wheat gluten. One premise behind the calculation of apparent digestibility coefficients using the methods described in our study is that there are no interactions or associative effects between the reference diet and the test ingredient/s. That is, apparent digestibility coefficients are assumed to be additive. This is not always the case, especially for carbohydrate sources (Booth et al., 2006; Glencross et al., 2007). It is known that the commercial "reference diet" contained approximately 20% wheat and 5% SBM (Booth et al., 2010a). This meant that in total, the wheat and pregel-starch test feeds would have contained approximately 44% wheat (i.e. 33% starch) or 41% starch, respectively which could have indirectly affected the apparent dry matter and energy digestibility of the wheat or pregelled starch ingredients by overwhelming the digestive capacity of the fish.

Other workers, using similar methods to those mentioned above have also investigated the effect of starch inclusion on digestibility of starch by Asian seabass (McMeniman, 2003). However, this author used a reference diet based on 80% fishmeal, 11% corn gluten and 7% fish oil. McMeniman (2003) found that increasing the level of wheat starch substitution of the reference diet from 15 to 30% resulted in clear reductions in both dry matter and energy digestibility of the test diets and the ingredient itself (see inserted tables below). The digestibility of other starch sources, including pregelatinized wheat starch was also determined by McMeniman (2003) and confirm that wheat starch and pregelatinized wheat starch are poorly or even negatively digested by Asian seabass and tend to act as dietary diluents rather than as a source of energy in feeds (McMeniman, 2003).

One of the reasons for including the raw wheat and pregelatinized wheat starch ingredients in our trial was to simulate the cooking-extrusion process on the inevitable gelatinization of wheat starch in finished feeds. It would appear from our results that gelatinization of wheat (starch) through extrusion-cooking will not enhance the digestibility of finished aquafeeds for Asian seabass. In addition, there were clear impacts of wheat and wheat starch on the physical quality of the faecal material produced when test feeds contained 30% of either ingredient. This may indicate that these levels of wheat or starch were affecting the passage rate of digesta and by inference the digestibility of diets and ingredients. It appears therefore that the inclusion of wheat and wheat starch products in aquafeeds for Asian seabass will need to be limited to levels that allow the satisfactory production of finished pellets and not for nutritional reasons *per se*.

Metabolic studies with Asian seabass have indicated they suffer prolonged hyperglycaemia after feeding or injection with glucose and have only limited ability to metabolise galactose or xylose, the normal end products of non-starch polysaccharide digestion (Anderson, 2003). In addition, the post-prandial blood glucose levels of Asian seabass fed diets containing glucose, dextrin, raw wheat starch and pregelatinized wheat starch indicated that pregelatinized wheat starch was more quickly digested and absorbed than raw starch and that digestion and absorption of dextrins and maltose did not proceed any faster than the absorption of raw starch. For this reason attempts to improve dry matter and energy digestion of raw starches or NSP's by pre-digestion, gelatinization or dextrinisation may not be particularly successful in Asian seabass (Anderson, 2003).

Despite poor dry matter and energy digestibility, wheat protein was well digested by Asian seabass (Table 8) and is reflected in the almost 100% apparent digestibility of dry matter, protein and energy from wheat gluten by this species (McMeniman, 1998; Boonyaratpalin & Williams, 2002). Further research on the apparent availability of amino acids from wheat gluten by Asian seabass will be beneficial, however in the absence of this data a conservative estimate for amino acid coefficients of approximately 90% should be used. Confidence in this approach is gained from the high correlation between the average amino acid availability of diets or ingredients and their respective protein ADCs derived from the present experiment (Figure 5). However, formulation of aquafeeds using individual amino acids will be more accurate.

This experiment has determined the proximate digestibility of poultry meal, blood meal, corn gluten, two sources of fishmeal, raw wheat and pregelatinized wheat starch. Apart from previous research for the USB, to our knowledge this is the first time data on the availability of amino acids for these ingredients has been determined. This data will be used to expand the existing formulation data base of feed ingredients for Asian seabass which already includes similar information on the digestibility of soybean meal, soy protein concentrate and South American fishmeal.

Table 6. Measured composition of reference diet and feed ingredients used in digestibility experiment (dry matter basis).

	Reference	Poultry meal	Blood meal	Corn gluten	Fishmeal (Std) FM1	Tuna meal FM3	Raw wheat	Pregel wheat starch
Nutrient (%)								
Ash	8.2	15.5	2.2	3.1	17.0	21.6	1.7	0.4
Organic matter	91.8	84.5	97.8	96.9	83.0	78.4	98.3	99.7
Fat	12.9	15.0	0.5	3.0	8.2	7.9	1.1	0.5
Energy (MJ kg^{-1})	22.3	21.8	21.1	23.0	20.6	19.1	17.0	12.9
Nitrogen	8.8	10.1	15.8	10.8	11.9	10.8	2.1	0.2
Crude protein	54.8	63.3	98.9	67.5	74.1	67.4	13.3	1.4
TNSC	19.0	3.0	1.0	16.0	5.0	4.0	76.0	100.0
NFE	24.2	6.3	-1.6	26.4	0.7	3.1	84.0	97.7
Amino acid (%)								
Hydroxyproline	0.40	2.12	0.00	0.08	0.56	0.91	0.00	0.00
Histidine	1.93	1.34	5.23	1.44	1.99	2.15	0.27	0.01
Taurine	0.21	0.34	0.05	0.03	0.55	0.28	0.00	0.00
Serine	2.53	2.64	4.27	3.58	2.73	2.88	0.56	0.01
Arginine	2.91	4.25	5.31	2.56	4.07	4.15	0.46	0.02
Glycine	2.96	5.96	3.84	2.22	4.10	4.63	0.48	0.01
Aspartic acid	4.58	4.55	7.95	3.91	5.80	5.70	0.57	0.02
Glutamic acid	6.66	7.58	9.03	12.67	8.35	7.77	3.58	0.04
Threonine	2.21	2.42	4.82	2.38	2.98	3.03	0.33	0.01
Alanine	3.25	3.79	6.70	5.17	4.03	4.08	0.40	0.01
Proline	2.55	3.93	3.78	5.82	2.76	3.21	1.18	0.01
Lysine	3.80	3.77	8.01	1.59	5.19	4.88	0.32	0.02
Tyrosine	1.18	1.67	3.11	2.70	2.17	2.13	0.18	0.01
Methionine	0.94	1.25	1.37	1.37	2.02	1.83	0.13	0.00
Valine	3.28	2.93	6.60	3.22	3.62	3.63	0.51	0.01
Isoleucine	1.64	2.34	3.21	2.69	2.98	2.98	0.40	0.01
Leucine	4.67	4.20	10.33	10.08	5.08	5.08	0.77	0.02
Phenylalanine	2.64	2.31	5.98	4.02	2.79	2.78	0.53	0.01
Sum AA's	*48.36*	*57.39*	*89.59*	*65.55*	*61.77*	*62.10*	*10.67*	*0.22*

Crude protein = nitrogen x 6.25; TNSC = total non-structural carbohydrate; NFE by difference = 100 – (ash + fat + crude protein); Note: With the exception of pregelatinized wheat starch, all ingredients and diets were provided by Ridley Aquafeed Pty Ltd, Narangba, QLD, Australia. Commercial barramundi diet; Ridley Aquafeed Pty Ltd product code 875630625, run 90332. Pregel starch = 100% pregelatinized wheat starch; Penfords Pty Ltd, Australia.

Table 7. Formulation and measured composition of experimental diets used in digestibility experiment (dry matter basis).

	Reference	Poultry meal	Blood meal	Corn gluten	Fishmeal (Std) FM1	Tuna meal FM3	Raw wheat	Pregel wheat starch
Ingredient (g kg^{-1})								
Commercial diet	990	693	693	693	693	693	693	693
Poultry meal	0	300	0	0	0	0	0	0
Blood meal	0	0	300	0	0	0	0	0
Corn gluten	0	0	0	300	0	0	0	0
Fishmeal FM1	0	0	0	0	300	0	0	0
Fishmeal FM3	0	0	0	0	0	300	0	0
Wheat	0	0	0	0	0	0	300	0
Pregel starch	0	0	0	0	0	0	0	300
Chromium oxide	10	7	7	7	7	7	7	7
Nutrient (%)								
Ash	8.6	11.1	6.6	6.8	10.8	12.6	6.5	6.2
Organic matter	91.4	88.9	93.4	93.2	89.2	87.4	93.5	93.8
Fat	12.8	12.9	8.7	10.3	11.3	11.5	9.2	7.4
Energy (MJkg^{-1})	22.0	22.1	22.8	22.6	21.9	21.4	20.9	20.8
Nitrogen	8.7	9.1	10.7	9.2	9.6	9.2	6.7	6.1
Crude protein	54.1	57.0	66.8	57.7	60.2	57.2	42.1	38.2
TNSC	19.0	15.0	12.0	18.0	13.0	14.0	33.0	42.0
NFE	24.5	19.0	18.0	25.2	17.7	18.7	42.2	48.2
Amino acid (%)								
Hydroxyproline	0.37	0.83	0.26	0.29	0.45	0.54	0.26	0.27
Histidine	1.93	1.65	2.81	1.81	2.03	2.00	1.42	1.37
Taurinea	0.22	0.24	0.17	0.17	0.34	0.23	0.15	0.16
Serine	2.51	2.40	3.03	2.90	2.72	2.68	1.94	1.81
Arginine	2.91	3.08	3.54	2.87	3.41	3.29	2.11	2.00
Glycine	2.92	3.59	3.16	2.83	3.43	3.49	2.19	2.09
Aspartic acid	4.58	4.22	5.59	4.51	4.80	4.97	3.26	3.25
Glutamic acid	6.66	6.44	7.35	8.58	7.16	7.04	5.61	4.72
Threonine	2.20	2.12	2.93	2.30	2.55	2.47	1.63	1.58
Alanine	3.22	3.17	4.26	3.83	3.52	3.53	2.36	2.31
Proline	2.53	2.77	2.89	3.62	2.72	2.79	2.13	1.81
Lysine	3.77	3.43	4.91	3.13	4.13	4.06	2.56	2.64
Tyrosine	1.20	1.27	1.66	1.58	1.58	1.49	0.77	0.76
Methionine	0.95	0.95	1.05	0.97	1.32	1.20	0.64	0.65
Valine	3.28	2.98	4.21	3.32	3.50	3.41	2.42	2.34
Isoleucine	1.63	1.71	2.07	1.95	2.11	2.01	1.22	1.14
Leucine	4.66	4.22	6.24	6.26	4.96	4.82	3.44	3.32
Phenylalanine	2.64	2.39	3.57	3.06	2.82	2.72	1.98	1.89
Sum AA's	*48.16*	*47.45*	*59.70*	*54.01*	*53.54*	*52.74*	*36.10*	*34.09*

Crude protein = nitrogen x 6.25.; TNSC = total non-structural carbohydrate; NFE by difference = 100 – (ash + fat + crude protein). Chromium oxide = Cr_2O_3; Technipur, Merck, Damstadt, Germany.

Table 8. Apparent digestibility coefficients (ADC) of diets and ingredients fed to Asian seabass. Data are mean ± SD.

	Dry matter	Organic matter	Fat	Gross energy	Crude protein	TNSC
Test diet ADCs (%)						
Reference	61.0	67.0	87.0	75.8	74.2	73.3
	3.2	*2.7*	*0.9*	*2.2*	*2.2*	*2.8*
Poultry	65.7	72.6	92.6	80.2	76.1	77.5
	0.0	*0.4*	*2.6*	*0.7*	*0.6*	*1.4*
Blood meal	60.8	64.8	90.3	70.1	65.1	81.7
	2.4	*2.2*	*0.9*	*2.1*	*1.9*	*2.5*
Corn gluten	59.8	65.9	92.7	73.2	75.8	75.6
	2.2	*1.8*	*1.6*	*2.6*	*1.4*	*3.2*
Fishmeal FM1	70.9	78.1	96.8	83.8	82.5	89.5
	0.1	*0.3*	*0.1*	*1.3*	*0.6*	*2.3*
Fishmeal FM3	63.2	71.9	94.9	79.3	77.8	83.1
	0.9	*1.0*	*0.5*	*0.6*	*1.3*	*1.8*
Raw wheat	51.7	55.0	94.1	63.6	74.1	46.6
	1.6	*1.6*	*1.4*	*2.3*	*1.5*	*1.2*
Pregel starch	44.0	48.2	89.9	55.5	71.5	41.5
	1.4	*1.2*	*2.0*	*1.3*	*1.6*	*1.2*
Reference (2009)	65.9	-	89.4	75.2	77.0	-
	4.5	-	*2.0*	*2.7*	*3.1*	-
Ingredient ADCs (%)						
Poultry	76.7	88.1	89.7	95.1	83.8	-
	0.0	*1.3*	*7.5*	*2.5*	*1.9*	-
Blood meal	60.3	61.1	-	60.2	55.6	-
	8.1	*6.9*	-	*7.4*	*4.3*	-
Corn gluten	57.2	64.9	-	71.2	82.3	81.7
	7.4	*5.8*	-	*8.5*	*4.0*	*12.1*
Fishmeal FM1	94.0	108.2	133.1	108.5	100.3	-
	0.3	*1.2*	*0.6*	*4.4*	*1.7*	-
Fishmeal FM3	68.3	86.9	125.1	93.5	88.3	-
	3.2	*3.8*	*2.2*	*2.4*	*3.8*	-
Raw wheat	30.2	30.0	-	31.1	90.7	31.0
	5.3	*5.1*	-	*9.4*	*16.3*	*1.9*
Pregel starch	4.4	9.0	-	-20.2	-	26.6
	4.7	*3.9*	-	*6.4*	-	*1.7*
Fishmeal (2009)	98.2	-	98.9	105.0	95.6	-
	5.9	-	*2.4*	*4.7*	*3.7*	-

TNSC = total non-structural carbohydrates. Missing fat and TNSC values for some ingredients is due to the low concentration of these nutrients in the test ingredients and the effect this has on the calculation of digestibility coefficients.

Table 9. Availability of amino acids from experimental diets and test ingredients. Data are average of n=3 replicate samples.

	Reference	Poultry meal	Blood meal	Corn gluten	Fishmeal (Std) FM1	Tuna meal FM3	Raw wheat	Pregel wheat starch
Diet ADC (%)								
Hydroxyproline	75.78	78.46	83.27	75.06	80.77	71.75	78.46	81.37
Histidine	75.53	77.81	63.72	78.81	84.76	81.08	76.28	77.17
Taurine	25.84	44.94	11.13	2.65	60.44	44.78	13.82	10.59
Serine	73.98	74.16	66.68	78.14	82.67	78.55	75.09	73.64
Arginine	84.09	84.97	71.79	85.55	89.71	86.03	84.40	85.23
Glycine	74.42	76.39	67.47	75.49	82.00	77.01	75.75	75.71
Aspartic acid	69.32	68.93	58.20	71.90	78.17	74.63	71.04	69.48
Glutamic acid	82.38	82.52	69.27	83.72	88.35	84.78	84.78	81.78
Threonine	75.51	77.08	65.06	76.95	84.71	80.39	75.84	74.87
Alanine	77.26	79.51	63.12	81.03	86.18	81.34	77.04	76.93
Proline	77.09	78.17	67.35	80.87	84.66	79.75	79.69	75.36
Lysine	80.43	81.76	66.78	80.61	87.67	84.10	80.04	84.07
Tyrosine	77.67	79.51	67.39	81.76	86.44	83.30	79.40	77.99
Methionine	82.00	83.87	75.39	83.61	91.75	89.08	84.81	84.63
Valine	74.39	76.03	61.45	76.48	84.34	80.21	74.27	73.57
Isoleucine	83.97	82.83	66.52	82.41	88.71	85.32	83.70	81.52
Leucine	77.56	79.59	63.88	82.61	86.71	82.74	77.59	77.06
Phenylalanine	77.89	79.91	65.67	82.03	86.66	82.56	78.73	78.11
Average of ADC's	*74.73*	*77.03*	*64.12*	*75.54*	*84.15*	*79.30*	*75.04*	*74.39*
Ingredient ADC (%)								
Hydroxyproline	-	79.63	0.00	66.20	88.97	67.65	0.00	-
Histidine	-	85.48	53.55	89.05	105.70	92.73	88.99	-
Taurine	-	73.39	-131.76	-356.49	91.80	79.25	0.00	-
Serine	-	74.57	56.58	85.02	101.47	87.95	86.82	-
Arginine	-	86.37	56.06	89.39	99.08	89.21	88.79	-
Glycine	-	78.67	54.94	78.82	94.75	80.86	95.09	-
Aspartic acid	-	68.02	43.25	78.96	94.48	84.58	103.14	-
Glutamic acid	-	82.81	46.70	85.38	99.48	89.60	95.22	-
Threonine	-	80.43	53.89	80.06	100.66	88.71	80.90	-
Alanine	-	84.02	47.12	86.57	102.99	88.93	72.89	-
Proline	-	79.80	52.00	84.73	100.97	84.68	92.76	-
Lysine	-	84.89	51.67	81.59	100.05	90.77	69.25	-
Tyrosine	-	82.56	58.30	85.93	97.59	90.60	106.40	-
Methionine	-	87.17	64.77	86.20	102.39	97.60	131.36	-
Valine	-	80.32	46.43	81.45	105.36	92.48	72.33	-
Isoleucine	-	80.96	45.66	80.18	94.81	87.06	81.02	-
Leucine	-	84.86	49.45	88.07	106.34	93.84	78.01	-
Phenylalanine	-	85.31	53.09	88.39	105.99	92.90	88.56	-
Average of ADC's	*-*	*81.52*	*49.03*	*83.29*	*100.06*	*88.24*	*84.21*	*-*

Average of ADC's for ingredients excludes data for taurine.

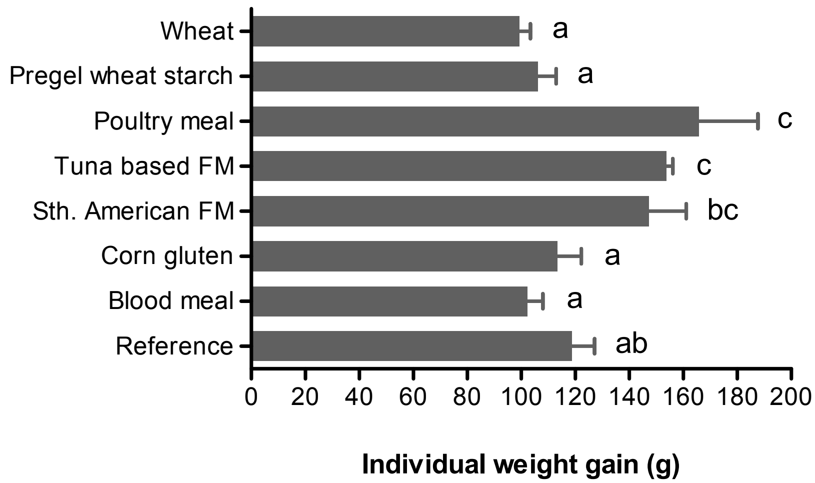
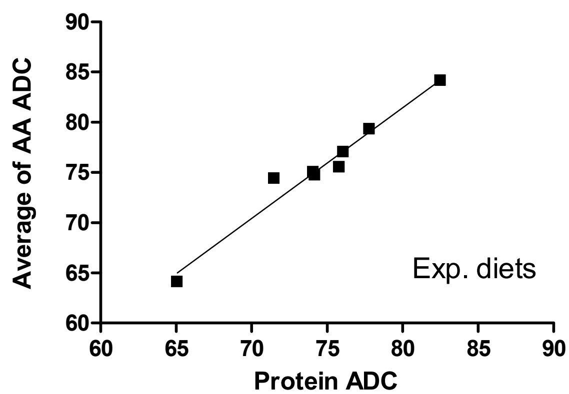


Figure 4. Weight gain of Asian seabass at the end of the digestibility experiment.



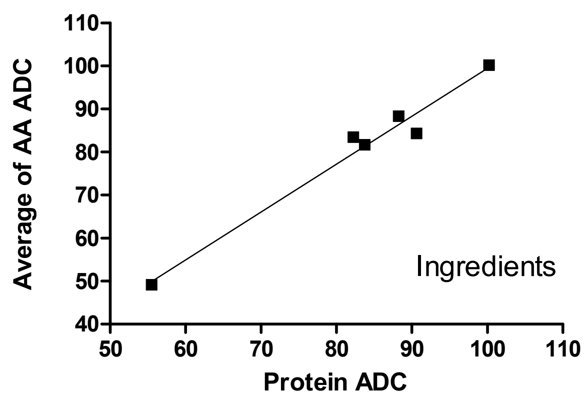


Figure 5. Relationship between protein digestibility and average amino acid availability in experimental diets (upper figure) and ingredients (lower figure).

Update the feed ingredient formulation data base for Asian seabass

Introduction

Previous investigation of the literature on the digestibility of feed ingredients by Asian seabass found that while there was some information on the digestibility of protein and energy from a range of ingredients there was little if any data on the availability of amino acids or the digestibility of carbohydrates (Booth & Allan, 2010). Amino acid availability data is critical to correctly formulating feeds for Asian seabass that contain high levels of soybean meal (SBM) and soy protein concentrate (SPC) as these ingredients are low in the sulphur amino acids. In addition many other alternative feed ingredients are themselves deficient in particular amino acids so the complimentary blending of feed ingredients to supply the correct concentration of amino acids at a dietary level, especially the limiting amino acids, is critical. Many published studies investigating ingredient replacement for Asian seabass have determined the digestibility of their experimental feeds at the end of growth assays but have failed to determine and present data on individual dietary ingredients (Eusebio & Coloso, 2000). To maximise use of soybean products, knowledge of digestibility and utilisation of other components in the diet must also be known so formulations can match digestible nutrient and energy requirements. Expansion of the current Asian seabass feed ingredient data base to include data on digestible amino acids will greatly improve the probability of formulation success.

The objective of this activity was to undertake a brief literature survey to assess recent publications on the digestibility of feed ingredients by Asian seabass and integrate new data into the current USB feed formulation data base for this species.

Methods

An investigation of peer reviewed literature on the digestibility of SBM, SPC and other feed ingredients by Asian seabass was undertaken using NSW DPI Library access to the ISI Web of Knowledge (Thomson Reuters) search engine. A preliminary “title” search for the terms *Lates calcarifer,* Asian seabass or barramundi was undertaken by searching documents published since 2000. For each of the scientific and common names additional keywords such as soybean, soybean meal, soy protein concentrate, ingredient, digestibility, diet and availability were included to refine the searches. Individual title searches for *Lates calcarifer*, Barramundi and Asian seabass produced about 205, 122 and 46 hits respectively. As a consequence of the limited number of results for each "title" search, all hits were examined for relevant nutritional data. Secondary searches were also made in peer reviewed journals not normally covered by the ISI Web of Knowledge to ensure all relevant data was captured.

Results / Discussion

Little new information on the digestibility of feed ingredients for Asian seabass was found in the peer reviewed literature. References, including several important nutrition publications predating the year 2000 search criteria are listed at the rear of this report. Of the manuscripts listed, only two recent papers were dedicated to the determination of apparent digestibility coefficients for Asian seabass. One paper investigated the digestibility of different cereal grains and the other investigated the digestibility of various ingredients such as lupin meals and lupin concentrates, soybean meal, canola meal, poultry meal and feather meal (Glencross, 2011; Glencross et al., 2011a). The latter paper also investigated using rainbow trout *Oncorhynchus mykiss* as a surrogate for determining digestibility coefficients for Asian seabass due to the difficulty in collecting faecal material from the latter species (Glencross, 2011). These difficulties include the lack of a bound faecal pellet and the often diffuse and aqueous nature of the faecal matter. While the new data from Glencross and colleagues is welcome, unfortunately neither of their studies determined the availability of amino acids from any of the ingredients. The relevant data on Asian seabass from each of their publications is presented below after back calculations to convert dry matter values to "as received" values (Table 10). Inspection of their results indicates that protein is well digested from most of the ingredients they evaluated, however protein digestibility was low in one cultivar of Barley (Barley 871), expeller canola meal, poultry offal meal and hydrolysed feather meal. Not surprisingly, the energy digestibility from most of the cereals, legumes and oilseeds was low, but protein concentration of two lupin varieties significantly elevated energy and dry matter digestibility (Table 10). Lower digestibility of the cereal grains is likely related to their high starch content (see previous section) while that of the legume meals such as lupins is likely related to their naturally high non-starch polysaccharide content, which forms the major part of the carbohydrate in this grain

(Glencross, 2001). Asian seabass are known to be inefficient at digesting starch (Williams & Barlow, 1998), whether it is gelatinized or not and inefficient at utilising the breakdown products of starch (i.e. glucose) once it enters the bloodstream (Allan et al., 2003). Glencross et al. (2011a) have indicated that the digestibility of cereal starches by Asian seabass may be related to the amylopectin / amylase ratio of the grain, decreasing as the amylopectin content of the grain increases. This hypothesis requires further examination.

Updated digestible nutrient values for the proximate and amino acid availability of fishmeal, soybean meal, soy protein concentrate and other ingredients were complied in two forms; a basic formulation data base which included the proximate values and digestible nutrients of feed ingredients examined in trials for the USB as well as from the literature (Table 11) and a new formulation data base which included the content and availability of amino acids from ingredients investigated in research conducted exclusively for the USB (Table 12). Together these two tables provide the most recent and up to date information on the digestibility of common feed ingredients for Asian seabass. Included with this information is a recent comparison of the nutrient and energy composition of different sources of soybean meal and soy protein concentrates being used by other USB researchers in the USA and the Philippines (Table 13; data kindly provided by Dr Rick Barrows). Inspection of this table indicates that the nutrient composition of the various soybean meals and soy concentrates are remarkably similar on a dry matter basis.

At the planning stages of the FY2011 research on Asian seabass the ASA-IM Manager in South East Asia (Lukas Manomaitis) and other researchers linked to this project (Mark Newman) were consulted regarding their opinions on which feed ingredients from SEA should be targeted for determination of digestibility. A short list of “backbone” ingredients was compiled which included a good quality anchovy or sardine meal, tuna meal, poultry by-product meal, blood meal and corn gluten meal. Digestibility data for these types of ingredients (Australian analogues) was presented in an earlier section of this report. Other ingredients typical of those used in an aquafeed mill in SEA were also prioritised in order of their perceived importance to the formulation of aquafeeds for Asian seabass (see Table 14). Review of this table indicates that the determination of proximate and amino acid availability for many ingredients remains to be examined including those for squid liver powder or meal, broken rice, cassava meal, pork meat meal, shrimp head meal and wheat gluten. The priority table also list several energy sources such as tapioca and corn starch. Based on the digestibility of wheat and pregelatinized wheat starch presented in previous sections of this report the cost-benefit ratio of exploring the digestibility of additional starch sources for use in Asian seabass feeds is probably quite low. The expense of evaluating the digestibility of additives such as anti-oxidants, premixes and synthetic amino acids is unwarranted and best guess values for the digestibility and availability of these minor feed constituents should suffice.

The updated formulation data bases, especially the data base on the availability of amino acids will be useful to manufacturers of aquafeeds for Asian seabass in South East Asia and Australia. It will also be vital for formulating and validating use of new commercial aquafeeds for this species in laboratory and field based trials in FY2012. The updated information should allow the manufacture of extruded aquafeeds that contain little if any fishmeal and optimal inclusion levels of SBM and SPC.

Table 10. Composition (as is basis) and apparent digesibility of feed ingredients by Asian seabass. Tabulated values based on data presented by (Glencross, 2011; Glencross et al., 2011a).

Ingredient	Composition									
	Dry Matter %	Moisture %	Ash %	Crude protein %	Fat %	NFE %	Gross energy MJ kg^{-1}	ADC CP %	ADC GE %	ADC DM %
Barley, 871[1]	91.4	8.6	2.6	24.6	7.4	56.9	18.4	62.6	56.5	49.9
Barley, Waxiro[1]	89.5	10.5	1.7	16.5	3.7	67.7	16.6	94.0	63.5	59.5
Barley, Torrens[1]	88.9	11.1	2.1	22.4	3.2	61.1	16.6	79.1	77.4	84.6
Barley[2]	89.7	10.3	1.9	13.5	3.9	70.3	16.5	100.0	54.7	46.9
Wheat[2]	89.8	10.2	1.3	17.6	2.8	68.1	16.4	100.0	65.2	65.5
Oats[2]	90.6	9.4	2.3	12.2	8.2	67.9	17.5	98.0	52.4	57.6
Sorghum[2]	90.9	9.1	1.4	12.5	3.5	73.4	16.8	100.0	53.7	55.5
Tapioca[2]	87.1	12.9	0.3	0.6	0.3	85.9	14.8	100.0	58.0	73.7
Triticale[2]	89.5	10.5	1.8	18.3	2.3	67.0	16.5	100.0	57.3	63.5
Corn[2]	88.3	11.7	1.2	4.2	2.3	80.6	16.4	100.0	43.2	81.2
Faba beans[2]	89.8	10.2	3.2	34.1	5.7	46.8	16.7	100.0	61.6	65.2
Lupin, luteus kernal meal[3]	91.3	8.7	3.6	51.8	6.1	29.9	19.6	81.2	82.7	64.7
Lupin, luteus protein conc.[4]	93.7	6.3	2.2	70.6	14.3	6.6	24.5	98.6	100.0	92.3
Lupin, angustifolius kernal meal[3]	91.6	8.4	3.2	37.7	5.9	44.8	18.7	96.1	73.4	36.8
Lupin, angustifolius protein conc.[4]	93.1	6.9	2.7	76.2	10.4	3.7	22.3	86.0	100.0	89.0
Soybean meal, sol. extracted[5]	89.6	10.4	7.7	44.8	1.5	35.6	17.3	100.0	65.5	56.7
Canola, expeller extracted[6]	91.6	8.4	4.9	35.5	12.2	51.2	21.5	63.3	59.7	20.9
Poultry offal meal[7]	91.9	8.1	14.7	55.9	10.9	10.4	21.3	39.7	52.5	10.2
Hydrolysed feather meal[7]	91.6	8.4	1.6	73.5	13.2	3.4	22.3	74.8	67.9	36.9

NFE = nitrogen free extract; ADC = apparent digestibility coefficients determined by indirect methods and stripping procedures.
[1] CSIRO Plant Industries, Black Mountain, ACT, Australia; [2] Ridley Aquafeed, Narangba, QLD, Australia; [3] Coorow Seed Cleaners, Coorow, WA, Australia; [4] Department of Agriculture, South Perth, WA, Australia; [5] Wesfeeds, Bentley WA, Australia; [6] Riverland Oilseeds, Pinjarra, WA, Australia; [7] Skretting Australia, Cambridge, TAS, Australia.

Table 11. Basic ingredient formulation data base for Asian seabass (as is basis).

	Dry matter %	Ash %	Nitrogen %	Crude protein %	Fat %	NFE %	Gross energy MJ kg^{-1}	DP[1] %	DE[2] MJ kg^{-1}	DDM[3] %	Reference
Ingredient											
Fishmeal Ecuador	94.50	13.23	11.60	72.52	10.11	0.00	20.20	69.33	20.20	92.80	USB 2009
Soybean meal 30, Argentine	87.90	5.98	7.43	46.47	2.11	33.34	17.40	41.31	12.48	52.83	USB 2009
Soybean meal 50, Argentine	87.90	5.98	7.43	46.47	2.11	33.34	17.40	38.66	10.93	39.56	USB 2009
ADM Soy PC 30	91.40	5.67	10.57	66.03	0.46	19.24	18.59	63.32	15.11	68.00	USB 2009
ADM Soy PC 50	91.40	5.67	10.57	66.03	0.46	19.24	18.59	63.12	15.50	68.46	USB 2009
Fishmeal	91.90	15.35	10.76	67.26	8.09	1.21	19.11	64.30	19.11	90.25	USB 2010
Poultry meal	97.20	13.22	10.52	65.73	12.73	5.52	21.55	51.80	16.53	47.63	USB 2010
Meat meal	96.20	33.67	8.23	51.41	9.14	1.98	15.71	32.64	10.45	40.98	USB 2010
Wheat	89.20	1.34	2.24	13.99	1.25	72.62	16.45	9.47	6.36	38.62	USB 2010
Soybean meal 30, Argentine	88.10	6.17	7.39	46.20	1.76	33.97	17.50	41.07	12.55	52.95	USB 2010
Soybean meal 50, Argentine	88.10	6.17	7.39	46.20	1.76	33.97	17.50	38.44	10.99	39.65	USB 2010
ADM Soy PC 30	87.90	5.54	10.13	63.34	0.08	18.94	18.08	60.75	14.70	65.40	USB 2010
ADM Soy PC 50	87.90	5.54	10.13	63.34	0.08	18.94	18.08	60.56	15.08	65.84	USB 2010
Fish oil	99.00	1.00	0.01	0.01	97.50	0.49	38.00	0.01	36.40	98.01	USB 2010
Methionine	95.00	2.00	9.30	58.00	0.01	1.00	13.00	56.84	12.74	93.10	USB 2010
DSM Premix vit/min	97.80	52.71	0.85	5.32	6.85	32.92	8.14	4.25	6.51	48.90	USB 2010
Poultry meal	95.20	14.76	9.63	60.21	14.28	5.95	20.75	50.46	19.74	73.02	USB 2011
Blood meal	96.97	2.13	15.35	95.94	0.48	0.50	20.46	53.34	12.32	58.47	USB 2011
Corn Gluten	92.78	2.88	10.02	62.62	2.78	24.49	21.34	51.54	15.19	53.07	USB 2011
Fishmeal FM1Prime	94.15	16.00	11.16	69.73	7.72	0.69	19.39	69.73	19.39	88.50	USB 2011
Fishmeal FM3 Tuna	94.47	20.41	10.19	63.71	7.46	2.89	18.04	56.25	16.87	64.52	USB 2011
Raw wheat	90.53	1.54	1.92	12.00	1.00	76.00	15.39	10.88	4.79	27.34	USB 2011
Pregel-starch	95.06	0.38	0.21	1.31	0.48	92.90	12.26	1.24	<0.05	4.18	USB 2011
Extruded wheat***	87.90	2.60	2.30	14.30	4.40	66.60	17.10	9.68	6.61	38.06	Williams & Barlow 1998
Wheat flour (gelled, 12% CP)	88.69	0.46	2.06	12.86	1.40	73.97	10.00	8.36	5.00	48.78	Williams & Barlow 1998
Starch (gelled <30% diet)	91.00	3.64	0.78	4.87	1.27	81.22	15.28	3.80	5.50	36.40	Williams & Barlow 1998
Mill run	89.67	3.88	3.20	20.00	4.04	61.75	17.61	12.00	5.28	31.38	Williams & Barlow 1998
Meat meal	95.50	22.92	8.71	54.44	13.85	0.00	14.33	34.57	9.53	40.68	McMeniman 1998
Poultry offal meal	94.70	11.08	10.00	62.50	16.10	0.00	22.16	49.25	17.00	46.40	McMeniman 1998
Full fat soybean meal	91.00	4.82	6.52	40.78	16.65	20.56	19.75	34.58	14.99	62.79	McMeniman 1998
Canola meal	95.10	6.47	6.22	38.87	2.95	42.16	18.73	31.48	10.51	46.50	McMeniman 1998
Dehulled lupin	90.20	2.35	6.18	38.61	6.58	33.82	18.76	37.88	11.54	54.66	McMeniman 1998
Wheat gluten meal	93.10	0.84	12.23	76.44	1.02	8.38	21.41	76.44	21.16	93.10	McMeniman 1998

Lupin luteus kernal meal	91.30	3.56	8.28	51.77	6.12	29.86	19.63	42.03	16.23	59.07	Glencross 2011
Lupin luteus Prot. Conc.	93.70	2.16	11.30	70.65	14.34	6.56	24.55	69.66	24.55	86.49	Glencross 2011
Lupin angustifolius kernal meal	91.60	3.21	6.04	37.74	5.86	44.79	18.69	36.27	13.72	33.71	Glencross 2011
Lupin angustifolius Prot. Conc.	93.10	2.70	12.20	76.25	10.43	3.72	22.34	65.57	22.34	82.86	Glencross 2011
Soybean meal – Sol. ext.	89.60	7.71	7.17	44.80	1.52	35.57	17.29	44.80	11.33	50.80	Glencross 2011
Canola meal – expeller ext.	91.60	4.85	5.69	35.54	12.18	51.20	21.53	22.50	12.85	19.14	Glencross 2011
Poultry offal meal	91.90	14.70	8.94	55.88	10.94	10.38	21.32	22.18	11.19	9.37	Glencross 2011
Feather meal - hydrolysed	91.60	1.56	11.75	73.46	13.19	3.39	22.26	54.95	15.11	33.80	Glencross 2011
Barley 871	91.40	2.56	3.93	24.59	7.40	56.85	18.40	15.39	10.40	45.61	Glencross et al. 2011
Barley Waxiro	89.50	1.70	2.63	16.47	3.67	67.66	16.62	15.48	10.55	53.25	Glencross et al. 2011
Barley Torrens	88.90	2.13	3.58	22.40	3.20	61.07	16.63	17.72	12.87	75.21	Glencross at al. 2011
Barley - Ridley	89.70	1.88	2.17	13.54	3.95	70.32	16.45	13.54	9.00	42.07	Glencross et al. 2011
Wheat - Ridley	89.80	1.35	2.82	17.60	2.78	68.07	16.39	17.60	10.69	58.82	Glencross et al. 2011
Oats - Ridley	90.60	2.27	1.96	12.23	8.24	67.86	17.55	11.99	9.20	52.19	Glencross et al. 2011
Sorghum - Ridley	90.90	1.36	2.01	12.54	3.55	73.45	16.80	12.54	9.02	50.45	Glencross et al. 2011
Tapioca - Rildey	87.10	0.35	0.10	0.61	0.26	85.88	14.77	0.61	8.57	64.19	Glencross et al. 2011
Triticale - Ridley	89.50	1.79	2.94	18.35	2.33	67.04	16.52	18.35	9.47	56.83	Glencross et al. 2011
Corn - Ridley	88.30	1.24	0.66	4.15	2.30	80.62	16.44	4.15	7.10	71.70	Glencross et al. 2011
Faba bean - Ridley	89.80	3.23	5.46	34.12	5.66	46.79	16.68	34.12	10.27	58.55	Glencross et al. 2011

[1] digestible protein; [2] digestible energy, [3] digestible dry matter; [***] based on the average ADCs of dry matter, protein and energy, respectively for flour, starch and mill run.

Table 12. Ingredient formulation data base for Asian seabass based on availability of amino acids (as is basis).

	Fishmeal 2009	SBM 30 2009	SBM 50 2009	SPC 30 2009	SPC 50 2009	Poultry meal 2011	Blood meal 2011	Corn gluten 2011	FM1 prime 2011	FM3 tuna 2011	Whole wheat 2011	PGN 2011
As rec'd composition (%)												
Dry matter	94.50	87.90	87.90	91.40	91.40	95.20	96.97	92.78	94.15	94.47	90.53	95.06
Moisture	5.50	12.10	12.10	8.60	8.60	4.80	3.03	7.22	5.85	5.53	9.47	4.94
Ash	13.23	5.98	5.98	5.67	5.67	14.76	2.13	2.88	16.00	20.41	1.54	0.38
Nitrogen	11.60	7.43	7.43	10.57	10.57	9.63	15.35	10.02	11.16	10.19	1.92	0.21
Crude protein	72.52	46.47	46.47	66.03	66.03	60.21	95.94	62.62	69.73	63.71	12.00	1.31
Fat	10.11	2.11	2.11	0.46	0.46	14.28	0.48	2.78	7.72	7.46	1.00	0.48
NFE	0.00	33.34	33.34	19.24	19.24	5.95	0.50	24.49	0.69	2.89	76.00	92.90
Energy ($MJkg^{-1}$)	20.20	17.40	17.40	18.59	18.59	20.75	20.46	21.34	19.39	18.04	15.39	12.26
Alanine	3.86	1.97	1.97	2.78	2.78	3.61	6.50	4.80	3.79	3.86	0.36	0.01
Arginine	4.48	3.59	3.59	5.24	5.24	4.05	5.15	2.38	3.83	3.92	0.42	0.02
Aspartic acid	5.28	5.48	5.48	7.26	7.26	4.33	7.71	3.63	5.46	5.39	0.52	0.02
Cystine	0.74	0.68	0.68	0.86	0.86	0.84	1.24	1.20	0.59	0.47	0.27	0.00
Glutamic acid	8.11	8.72	8.72	11.90	11.90	7.22	8.76	11.76	7.86	7.34	3.24	0.04
Glycine	4.26	1.96	1.96	2.78	2.78	5.67	3.72	2.06	3.86	4.38	0.43	0.01
Histidine	3.09	1.18	1.18	1.69	1.69	1.28	5.07	1.34	1.87	2.03	0.24	0.01
isoLeucine	2.91	2.04	2.04	2.99	2.99	2.23	3.11	2.50	2.80	2.82	0.36	0.01
Leucine	5.09	3.46	3.46	5.06	5.06	4.00	10.02	9.35	4.78	4.80	0.70	0.02
Lysine	4.92	2.78	2.78	3.99	3.99	3.59	7.77	1.48	4.88	4.61	0.29	0.02
Methionine	1.99	0.60	0.60	0.86	0.86	1.19	1.33	1.27	1.90	1.73	0.12	0.00
Phenylalanine	2.73	2.31	2.31	3.32	3.32	2.20	5.80	3.73	2.63	2.63	0.48	0.01
Proline	2.80	2.42	2.42	3.42	3.42	3.74	3.67	5.40	2.60	3.03	1.07	0.01
Serine	2.73	2.38	2.38	3.44	3.44	2.51	4.14	3.32	2.57	2.72	0.51	0.01
Threonine	3.02	1.85	1.85	2.72	2.72	2.30	4.68	2.21	2.80	2.86	0.30	0.01
Tryptophan	0.83	0.60	0.60	0.82	0.82	0.62	1.20	0.50	0.83	0.65	0.17	0.01
Tyrosine	2.31	1.56	1.56	2.26	2.26	1.59	3.02	2.51	2.04	2.01	0.16	0.01
Valine	3.34	2.16	2.16	3.14	3.14	2.79	6.40	2.99	3.41	3.43	0.46	0.01
Hydroxyproline	0.53	0.00	0.00	0.00	0.00	2.02	0.00	0.07	0.53	0.86	0.00	0.00
Taurine	0.52	0.00	0.00	0.00	0.00	0.32	0.05	0.03	0.52	0.26	0.00	0.00
Digestible nutrients and amino acids (%)[1]												
DDM	92.80	52.83	39.56	68.00	68.46	73.02	58.47	53.07	88.50	64.52	27.34	4.18
DP	69.33	41.31	38.66	63.32	63.12	50.46	53.34	51.54	69.73	56.25	10.88	1.24
DE	20.20	12.48	10.93	15.11	15.50	19.74	12.32	15.19	19.39	16.87	4.79	0.78
Alanine	3.86	1.83	1.64	2.78	2.71	3.03	3.06	4.16	3.79	3.43	0.26	0.01
Arginine	4.47	3.48	3.46	5.24	5.14	3.50	2.89	2.13	3.79	3.50	0.37	0.02
Aspartic acid	5.28	5.09	4.66	6.83	6.54	2.95	3.33	2.87	5.16	4.56	0.52	0.02
Cystine	0.72	0.62	0.53	0.86	0.85	0.80	1.18	1.14	0.56	0.45	0.26	0.00
Glutamic acid	8.11	8.38	7.63	11.49	11.45	5.98	4.09	10.04	7.82	6.58	3.09	0.04
Glycine	4.26	1.74	1.60	2.73	2.65	4.46	2.04	1.62	3.66	3.54	0.41	0.01
Histidine	3.09	1.03	1.00	1.69	1.65	1.09	2.71	1.19	1.87	1.88	0.21	0.01
isoLeucine	2.87	1.85	1.84	2.83	2.87	1.81	1.42	2.00	2.65	2.46	0.29	0.01
Leucine	5.09	3.23	2.92	4.89	5.06	3.39	4.96	8.23	4.78	4.50	0.55	0.02
Lysine	4.92	2.57	2.47	3.82	3.87	3.05	4.01	1.21	4.88	4.18	0.20	0.02
Methionine	1.97	0.60	0.60	0.83	0.84	1.04	0.86	1.09	1.90	1.69	0.12	0.00
Phenylalanine	2.73	2.15	2.03	3.29	3.32	1.88	3.08	3.30	2.63	2.44	0.43	0.01
Proline	2.80	2.33	2.14	3.34	3.42	2.98	1.91	4.58	2.60	2.57	0.99	0.01
Serine	2.73	2.09	2.02	3.27	3.31	1.87	2.34	2.82	2.57	2.39	0.44	0.01
Threonine	3.02	1.65	1.56	2.63	2.64	1.85	2.52	1.77	2.80	2.54	0.24	0.01
Tryptophan	0.78	0.50	0.49	0.64	0.78	0.59	1.14	0.48	0.78	0.62	0.16	0.00
Tyrosine	2.29	1.54	1.36	2.10	2.26	1.31	1.76	2.16	1.99	1.82	0.16	0.01
Valine	3.34	1.90	1.77	3.07	3.14	2.24	2.97	2.44	3.41	3.17	0.33	0.01
Hydroxyproline	0.50	0.00	0.00	0.00	0.00	1.61	0.00	0.05	0.47	0.58	0.00	0.00
Taurine	0.49	0.00	0.00	0.00	0.00	0.23	0.05	0.03	0.48	0.21	0.00	0.00

[1] Digestible proximate and amino acid values calculated by multipling ingredient amino acid content x apparent digestibility coefficient. Note; ADC's greater than 100% have been limited to a maximum value of 100.

Table 13. United Soybean Board - Characterization of soy products from aquafeeds research; Rick Barrows

Investigator	Davis	Chong Lee	Booth	Coloso	Coloso	Booth	Clemente	Chong Lee
Country	USA	USA	Australia	Philippines	Philippines	Australia	USA	USA
Source	Faithway Feeds	HP12230	Argentine PTO			ADM Soycomil-k	Solae ProCon 2000	Solae Profine VF
Ingredient	SBM	SBM	SBM	SBM	Soy PC	Soy PC	Soy PC	Soy PC
Dry matter basis (%)								
Moisture	11.0	14.0	13.5	11.5	11.2	13.6	4.9	10.7
Dry matter	89.0	86.0	86.5	88.5	88.8	86.4	95.1	89.3
Crude Protein	47.7	42.7	46.3	46.8	64.3	63.3	67.0	65.3
Crude fat	1.2	1.8	2.4	1.3	0.3	0.7	1.0	0.9
Ash	6.2	6.5	6.5	7.2	5.9	5.2	6.6	5.9
Crude fibre	3.9	4.6	3.3	3.8	3.7	3.3	5.4	3.3
NFE	41.0	44.4	41.5	40.9	25.8	27.5	20.0	24.6
Starch	2.4	1.7	1.7	2.1	1.0	0.7	1.0	1.0
Calcium	0.26	0.64	0.32	0.77	0.37	0.31	0.35	0.38
Phosphorus	0.69	0.69	0.69	0.73	0.78	0.71	0.77	0.80

Table 14. List and priority of investigation of feed ingredients in South East Asian region provided by Lukas Manomaitis

	Priority				
	High		Medium		Low
Ingredient					
Antioxidant	1	Calcium Phosphate, monobasic	2	Banana Flour	3
Antioxidant	1	Choline Chloride 50% (powder)	2	Blood Meal (ring dried)	3*¥
Blood Meal (spray dried)	1*¥	Choline Chloride 75%	2	Coconut oil	3
Cassava Meal	1	DCP/MDCP 18% Phosphorous	2	Copra Meal	3
Choline Chloride 60%	1	FM Local (type?)	2*	Corn, whole, yellow	3
Corn Gluten Meal (CGM)	1*¥	Fish Oil Local (type?)	2	Corn Starch	3
DL-Methionine (methionine - 98.5%)	1	L-Lysine (lysine - 74%)	2	Crude Palm Oil	3
Fish Meal (FM) (Chilean/Peruvian)	1*¥	Meat & Bone Meal Argentina	2*	Limestone	3
Fish Oil Imported (type?)	1	Meat & Bone Meal USA	2*	NSP Enzyme	3
Mold Inhibitor	1	Mineral Mix (local)	2	Phytase	3
Poultry By-product Meal 65%	1*¥	Pork Meat Meal (50)	2	RSM (source?)	3
Soy Bean Meal USA	1*¥	Rice Bran	2	Salt	3
Soy Lecithin (paste)	1	Soy Bean Meal ARG	2*¥	Sodium Bicarbonate	3
SPC Low antigen	1*¥	Soy Bean Meal BRA	2*¥	Squid Liver Paste	3
Stay-C 35%	1	Soy Bean Meal IND	2		
Tapioca Pellets / chips	1	Soybean (fermented) Type?	2		
Tapioca Starch	1	Soy oil	2		
Vitamin C-phosphate 35 %	1	Squid Oil	2		
Wheat	1*¥	Vitamin Mix (local)	2		
Wheat flour	1*	Wheat Pollard	2*		
ASA-IM Vitamins Premix	1	shrimp head meal	2		
ASA-IM Mineral Premix	1	Wheat gluten	2*		
Broken Rice	1				
Squid liver powder or squid meal	1				

* Indicates some data on digestibility is available or can be inferred from data collected for Asian seabass on other feed ingredients.
¥ Indicates data on digestibility of proximate and amino acids has been determined.

Impacts of soybean meal and soy protein concentrate on the manufacture of extruded pellets for Asian seabass

Introduction

Extrusion cooking is the predominant method of producing aquafeeds (Bouvier, 1997; Aslaksen et al., 2006; Kraugerud & Svihus, 2011). During extrusion the feed materials go through a range of irreversible physical and chemical changes so that the extrudate that emerges is fundamentally different to the mixture that entered the extruder. This process aids the gelatinization of starches and denatures proteins (Wood, 1987; Young, 2003). Extruded aquafeeds are produced via a combination of high temperature (e.g. 120 -130°C), pressure (20-30 bar) and mechanical shear forces (Marsman et al., 1995) to transform feed ingredients into a viscous melt (dough) before the pellets are shaped in a die. (several authors cited in Sorensen et al., 2010; Kraugerud & Svihus, 2011). High moisture content (25-30%) in combination with a short residence time in the extruder (0.5-2 min) generally ensures that the process is not detrimental to the nutritional value of the feed (Barrows et al., 2007; Singh et al., 2007; Sorensen et al., 2010).

The benefits of extrusion-cooking on aquafeeds for fish and shrimp are varied and include positive effects on digestibility of ingredients, especially carbohydrates, improved pellet quality and durability, control of pellet buoyancy and deactivation of heat labile anti-nutrients to name but a few (Booth et al., 2000; Dominy, 2005; Sørensen et al., 2009; Sorensen et al., 2010). Pellet durability is becoming increasingly important as more farming operations switch to the use of automated feeders which pneumatically blow or pump pellets to the required location (Sørensen et al., 2009). However, in some cases, extrusion cooking can be detrimental to the digestibility of certain feed ingredients such as canola meal that contain little starch (Allan & Booth, 2004). Control of buoyancy is important with respect to the feeding behaviour of the target species or the type of feeding systems used by the farmer. These may be simple systems reliant on hand feeding while observing the reaction of fish on the water surface or sophisticated systems which use underwater cameras or elaborate feed back mechanisms to monitor feed intake of sinking pellets.

Apart from the contribution to the nutritional adequacy of aquafeeds, ingredient selection (i.e. formulation) has a marked impact on the quality of extruded aquafeeds. Ingredients are generally classified into just a few small groups including protein, carbohydrate and lipid (fats and oils) sources and additives. Apart from providing amino acids, protein sources are functionally important because they can influence water absorption, binding and the elasticity of the diet (van Barneveld, 2003) and the formation of glutinous doughs (Anon., 2003). Several qualities of proteins are considered important including nitrogen solubility index (NSI), protein dispersibility index (PDI), hydration capacity and binding capacity. For example the hydration rate of different ingredients within a mixture can affect the quality of the finished product by altering mixing, binding and degree of cook (van Barneveld, 2003). Protein sources such as fishmeal and rendered animal meals generally have low protein solubility and make little contribution to the structural nature of extruded aquatic feeds due to the fact they have already undergone quite a bit of thermal treatment in preparation as a raw material (Anon., 2003; Rokey et al., 2003). On the other hand, many plant based proteins have high protein solubility, excellent water absorption characteristics and contribute greatly to the structural nature of aquatic feeds. SPC has a very low protein dispersibility index but is more easily texturized than solvent extracted SBM as long as higher moisture and mechanical energy inputs are provided (Strahm et al., 2003).

Starch is the primary carbohydrate found in aquatic feeds contributing to binding (cohesion) and expansion in the final product, however, it serves no nutritional purpose for carnivorous species such as Asian seabass. To be useful in aquatic feeds it must be well cooked or gelatinized. Gelatinization refers to the unique ability of starch to lose its natural crystalline structure and become a viscous gel capable of dispersing through and around other structures within a mixture. Starch granules swell, absorb water, lose crystallinity and leach amylose (Rokey et al., 2003; Donald, 2004). Upon cooling the amylose chains link together and form a gel composite aided by the structure of amylopectin (Cornell, 2004). It is well known that inclusion of starch from cereal grains and starch rich legumes such as peas (Allan & Booth, 2004) aids pellet binding and promotes pellet expansion (Kumar et al., 2007), a property particularly useful for controlling pellet buoyancy and post-extrusion vacuum coating of oils or other additives. However, increasing starch content generally decreases pellet bulk density (Anon., 2003). The presence of functional proteins with good expansion and binding characteristics can lower the level of starch required to produce extruded feeds. Binding is also critical to promote pellet durability which reduces fracturing and production of fines. The amylose fraction of starch has greater binding properties than that of amylopectin therefore starches high in amylose are considered better for improving the cohesion of finished feeds (Anon., 2003).

Hydration and heating of vegetable proteins in the pre-conditioner and extruder result in the unravelling of long twisted vegetable protein molecules. During extrusion these molecules realign themselves along “streamline flows” of the extruder screws and dies. Increases in shear temperature (i.e. increased SME) and retention time within the extruder itself causes cross-linking of protein strands which yields a textured product which is highly layered and resistant to disintegration upon rehydration (Little et al., 1997; Strahm et al., 2003). The extent of cross-linking is thought to be related to exposure time, temperature and moisture which all affect the viscosity of the extrudate (Strahm et al., 2003). The structure-forming properties of proteins are believed to result from the denaturation, dissociation, and disulfide cross-linkage of globular protein structures during extrusion, resulting in a network of proteins which stabilize the pellet structure (Morken et al., 2011). In most cases a threshold level of shear is necessary to enhance cross linking of vegetable proteins such as soy, however over-shearing before the cross-linking of protein molecules has begun can result in decreased water holding capacity. The impact of moisture on the rheology of soy proteins is one of the most important process variables to consider during extrusion (Strahm et al., 2003; Aslaksen et al., 2006). Within the food industries unfolded soy proteins are well known for their functional adhesive properties which are brought about by intermolecular electrostatic and covalent disulphide bonding (Aslaksen et al., 2006; Sørensen et al., 2009).

The inclusion of soybean meals, soy protein concentrates and other plant sources invariably introduce non-starch polysaccharides (NSP) and other components which may affect the nutritional quality (Irish & Balnave, 1993), functional and physical characteristics of the finished feed as well as the physical quality of faecal material (Glencross et al., 2011a). In addition, the interaction between SBM, SPC and other feed ingredients may be positive or negative in terms of pellet quality. Inclusion of fibrous material such as wheat middlings above 20% disrupt the continuity of the carbohydrate matrix in catfish feeds resulting in a product with a rough external surface and excessive fines (Anon., 2003). Fibrous ingredients possess hydration and bulk density properties which are usually quite different from traditional ingredients and these will require different extruder configurations' and processing conditions. Fibre such as lignin can strengthen pellets by "melting", however it takes much higher processing temperatures to melt lignin than it does to gelatinized starch or denature proteins. For this reason fibre usually has low to moderate binding ability in feeds. High fibre diets tend to produce more fines than diets high in starch due to the different binding characteristics of either carbohydrate (Rokey et al., 2003)

Earlier research for the USB found that high inclusion levels of SPC affected the pellet characteristics of test feeds manufactured using extrusion technology (Booth et al., 2010a). Semi-buoyant slow sinking feeds were requested, but feedback from the extrusion mill staff indicated that control of bulk density in finished feeds containing 40-60% SPC was difficult. Finished feeds containing SPC also appeared to have smaller pellet diameters compared to control feeds containing higher levels of fishmeal indicating problems with pellet expansion. In addition, feeds containing SPC produced high levels of fines indicating a lack of binding. Increasing the amount of SBM and SPC used in aqua-feed formulations will impact on pellet quality due to the physical and functional properties of these ingredients and their interaction with other feed ingredients, particularly starch sources. However, modern extrusion technology allows the mill operator to select a diverse range of operating parameters and conditions by which to produce a finished feed and these selections ultimately have a major influence on the finished product (Sorensen et al., 2010), regardless of the ingredient interactions. For these reasons, extrusion technology is as much an "art form" as it is a science (Thomas & van der Poel, 1996; Whalen et al., 1997).

The objectives of this activity were to review data on the physical characteristics of the semi-commercial feeds used in 2010 and undertake a brief exploration of the literature to explore relevant publications on the effect of SBM and SPC inclusion on the quality of aquafeeds.

Methods

The pellet characteristics of the experimental feeds used in the 2010 utilisation experiment (see Table 15 for feed formulations) were determined to ascertain if there were correlations between ingredient or nutrient inclusion and the pellet quality of finished feeds. The mean ± SD diameter and length of 20 randomly selected pellets from each diet was measured to 0.01 mm using digital veinier callipers. Pellet expansion (radial expansion) was calculated by measuring the diameter of 20 randomly selected pellets from each of the 10 feeds, calculating the mean pellet diameter per feed and expressing this value relative to the width of the extruder die aperture; in this case 6 mm (Booth et al., 2010a)

Radial expansion ratio = mean width of pellet / die aperture

The mean sinking rate of 20 randomly selected pellets was determined by measuring the time taken in seconds for individual pellets to fall through a 400 mm vertical column of saltwater (i.e. glass measuring cylinder; salinity = 32 g L^{-1}). Pellets were carefully released 40mm above the surface of the water column using tweezers and time to reach the bottom was recorded on a stopwatch. Pellets which did not sink were given a standard sinking time of 300 seconds. Measured pellet characteristics for the extruded test feeds used in 2010 are presented in Table 16. Pellet data, with the exception of data on the commercial feed was compared statistically using one-way ANOVA and Tukeys HSD.

Spearman-rank correlation coefficients were calculated to examine relationships between the characteristics and the ingredient composition of pellets. Non-starch polysaccharide (NSP) content of diets was examined by estimating the NSP content of the test diets based on data taken from Choct et al. (2010) (i.e. NSP content of SBM ≈ 27% and of SPC ≈ 18%) while the NSP content of wheat (≈ 10%) was taken from Glencross et al. (2011a). The contribution of other dietary components to dietary NSP content, being of animal meal origin was considered negligible. Correlation coefficients were calculated using statistical software (NCSS) (Hintze, 2006) and considered significant when resultant *P* values were < 0.05. The resultant Spearman-rank correlation coefficients are presented in Table 17.

A brief review of the literature was undertaken to relate data from the present study to other relevant information on the impacts of SBM and SPC inclusion on pellet quality of aqua-feeds. The search was undertaken using NSW DPI Library access to the ISI Web of Knowledge (Thomson Reuters) search engine. The search was limited to documents generally published after 2000 and focused on peer reviewed literature in more technical and engineering based fields such as *Animal Feed Science & Technology* and *Poultry Science*. Key words included soy and soy protein concentrate combined with words such as extrusion, extruder, non-starch polysaccharide etc. Some articles were obtained from bibliographic archives belonging to Dr Mark Booth. A list of manuscripts and articles pertinent to this topic is included at the end of this report.

Results

There were highly significant differences in the diameter, length, sinking rate and expansion ratio of different diets (Table 16; all ANOVA tests $P<0.0001$). Pellet diameter and radial expansion ratio were significantly higher in the diet composed of 40% fishmeal and 30% SBM (i.e. Diet SBM30; Table 16). Pellet length was also numerically highest in this diet. The fishmeal control diet was buoyant and not surprisingly had the slowest sinking rate. Increasing the dietary level of SBM or SPC at the expense of other ingredients appeared to increase sinking rate (Table 16). The physical appearance of each feed is presented below to give an indication of the shift in the colour of pellets as level of SPC was increased.

Examination of the relationships between the composition of the diets and their pellet characteristics revealed weak to moderately strong correlations between many of the measured variables (Table 17). Please note radial expansion is an index of pellet diameter therefore the correlation values for these two characteristics are almost identical. For this reason we make no further reference to pellet diameter apart from in the discussion. Pellet length was significantly negatively correlated with NSP content whereas sinking rate was significantly positively correlated with NSP content (Table 17). Sinking rate was also negatively correlated with pellet length and bulk density, meaning that longer pellets tended to have a lower bulk density (greater buoyancy) compared to shorter pellets. Radial expansion ratio of pellets was significantly negatively correlated with the total level of dietary soy and the dietary level of SPC, but significantly positively correlated with the level of dietary fishmeal (Table 17). Bulk density of pellets was significantly positively correlated with dietary level of SBM. Although not quite reaching the level of significance there were moderately strong negative correlations between the dietary level of wheat and sinking rate or bulk density of pellets (*P* = 0.054 in both cases) (Table 17.).

Discussion

To date there has been little targeted scientific research on interactions between SBM, SPC and other ingredients on pellet characteristics of extruded aquafeeds. A detailed review on the general nutritional changes that occur during the extrusion process was published several years ago (Singh et al., 2007). Noteworthy publications dealing exclusively with SBM and SPC products in aquafeeds include the following (Chang et al., 2001a; Chang et al., 2001b; Aslaksen et al., 2006; Moure et al., 2006; Sørensen et al., 2009; Glencross et al., 2010a; Draganovic et al., 2011; Glencross et al., 2011b; Jiang et al., 2011; Kraugerud et al., 2011; Kraugerud & Svihus, 2011; Morken et al., 2011; Schaeffer et al., 2011) and there are numerous articles associated with extruded products containing soy products for human consumption (e.g. Faller et al., 1999; de Mesa et al., 2009; Colombo et al., 2011). It is not surprising there is little scientific literature available on the impacts of SPC and SBM on the quality of aquafeeds given the number of differences among extrusion systems, either from research or commercial facilities and the seemingly endless options available to operators in terms of processing parameters and ingredient selection and formulation. There are numerous other factors which can also affect the quality of finished aquafeeds including the skill and experience of the operator and the quality of the feed ingredients. In addition, it is often difficult to predict which of the ingredient-ingredient and ingredient-processing interactions ultimately affect the quality of the end product produced under variable or even steady state conditions (Whalen et al., 1997). Review of the aforementioned articles will highlight the variation in approaches and results obtained by various researchers exploring this topic. From a commercial perspective feed formulators are often dealing with commodity forecasting and supply issues and their impacts on margin as much as the nutritional needs of the fish or shrimp they are formulating aquafeeds for. Thus there are often subtle or even dramatic day to day changes in feed formulation and equipment will be in various states of wear, and with these changes must come inevitable adaptations in extrusion process parameters.

Literature on advances and understanding in feed technology for Asian seabass is also somewhat limited, however Glencross et al. (2011a) recently investigated the effect of different cereals and legumes on the technical quality of pelleted aquafeeds for this species. They found that pellets produced using faba beans and some types of barley tended to increase pellet hardness (measured as compression strength using a texture meter) while pellets manufactured using oats and triticale tended to decrease hardness (pellet hardness). Increasing starch content tended to increase radial expansion (Glencross et al., 2011a). Assessment of the effects of inclusion of different varieties of lupin (which are high in NSP) in simple formulations containing fishmeal and wheat on extruded pellet characteristics (Twin-screw laboratory extruder; Model APV MFP19:25 APV-Baker, Peterborough, UK) found that bulk density, sinking rate and pellet hardness all increased as the dietary inclusion level of the different lupin cultivars increased (Glencross et al., 2010a). In addition, higher levels of lupin resulted in less radial expansion and poorer oil absorption in finished pellets, presumably as a consequence of the higher level of dietary NSP (Glencross et al., 2010a). Like the results from our study their work also found that the bulk density of feeds containing blends of fishmeal, soybean meal and wheat tended to increase in response to increases in the content of SBM. However these authors found the bulk density of feeds containing more than 20% SBM tended to plateau. Moisture retention of dry mash containing SBM or lupin meals (with the exception of *Lupinus luteus;* Wodjil cultivar) also increased as inclusion level of these ingredients increased (Glencross et al., 2010a).

Associated with the same study Glencross et al. (2010a) also explored the pasting characteristics (e.g. gelatinization, peak viscosity) of pre-extruded mash (no oil) containing fishmeal and lupin meals or fishmeal and SBM using rapid viscosity analysis (RVA™ time/temperature program = 2 min at 50°C, ramping to 95°C over 3 min, held at 95°C for 5 min, before reducing to 50°C over 3 min; Whalen et al. 1997) to assess the effects of the extrusion cooking process on different ingredient combinations and concentrations. Compared to a control mash containing fishmeal (70%) and wheat (14%), they found the inclusion of soybean meal in the mash reduced both the rate of gelatinization and the peak viscosity during the RVA analysis. In contrast inclusion of similar amounts of lupin meal such as *L. angustifolius* increased rate of gelatinization and peak viscosity above that of the control mash. Based on these results they hypothesised that inclusion of lupins preferentially lowered the temperature at which the starch within the mash was being gelatinized whereas inclusion of SBM acted to lower the viscosity of the mash and hinder gelatinization. Please note the level of starch (as wheat) was similar across different rates of fishmeal substitution (Glencross et al., 2010a)

Assessment of extruded feeds produced in 2010

The operator of the extrusion facility employed to manufacture the semi-commercial test feeds indicated that initial attempts to pellet the formulations high in SPC failed due to an overly dry melt which resulted in the extruder shutting down, presumably as a consequence of high motor load (Booth et al., 2010a). Increasing the moisture content in the differential diameter conditioner (DDC) seemed to solve these problems and pellets containing high levels of SPC were produced, albeit not of the highest quality. It is possible the addition of more water in the DDC and extruder reduced barrel temperatures to the point where cooking and gelatinization temperatures were reduced, limiting both adhesion and expansion of pellets high in SPC (Botting, 1991). This effect has previously been documented in extrusion studies with SPC where the addition of moisture was tightly controlled (Draganovic et al., 2011). It is well known that the moisture level of the dough or melt can have a significant effect on motor torque, specific mechanical energy (SME) and die pressure (Kraugerud et al., 2011). In general, the higher the moisture content the lower the motor load, SME and die pressure. Interacting with moisture content, the functional quality of some ingredients under high temperature and pressure can increase motor loading while the qualities of other ingredients may decrease it (Sorensen et al., 2010; Draganovic et al., 2011). Like us, other researchers have also documented increased motor torque and die pressure when extruding dough with high levels of SPC (45%) and low levels of fishmeal under low moisture conditions. Subsequently, motor torque and die pressure decreased as moisture and fishmeal content of dough was increased and SPC content was reduced (Draganovic et al., 2011). The positive effects of SPC on motor torque appear to be related to the better water retention properties of SPC compared to products like fishmeal (Draganovic et al., 2011). In our case it should be noted that only 200 kg of each dietary mash was provided to the extrusion facility. As such, modifications to the extruder settings (Wenger X-85) during the pelleting process were governed by the risk of exhausting the mash before enough of the finished feed could be produced. Therefore the pellet characteristics of the feeds used in the 2010 trial may have been different had different DDC and extruder settings been used and had the operator had more material to work with. Moreover, even slightly different combinations of the basic feed ingredients used in the 2010 trial (Table 15) would probably have affected pellet quality. For example, relationships between dependent processing parameters (e.g. SME, die pressure, motor load die temperature) and pellet quality (e.g. hardness, durability, bulk density) of formulations containing fishmeal, or toasted and untoasted SBM have been examined using principal component analysis. This research found that approximately 93% of the variation in pellet quality was explained by just two major components; one attributed to ingredient composition (56%) and the other to processing parameters (35%) (Sørensen et al., 2009). Commercial development of aquafeeds would generally allow for judicious manipulation of raw material concentrations and fine tuning of pre-conditioner, extruder and dryer settings to produce a satisfactory end product. (Botting, 1991). For these reasons, the affects of different ingredients or nutrients on extruded pellet characteristics should ideally be examined under controlled conditions where most extrusion parameters are held constant and ingredient-ingredient interactions are minimised (Draganovic et al., 2011; Kraugerud et al., 2011).

Observations on radial expansion

Apart from the impact of the extruder settings and die selection, the degree of radial expansion is thought to be positively affected by the level and type of starch (Sorensen et al., 2010) and soluble protein (Anon., 2003). Correlation analysis indicated the inclusion level of fishmeal had the greatest positive effect on expansion while that of SPC had the greatest negative affect with respect to our dietary formulations and extrusion conditions. However, it should be noted that the majority of pellets produced under the aforementioned conditions had smaller radial diameters than the 6mm pellet die (Table 16), indicating negligible expansion of the extrudate or shrinkage of the extrudate during cooling (Kumar et al., 2007; Sørensen et al., 2009). In studies exploring use of SPC, fishmeal and wheat gluten in extruded aquafeeds, Draganovic et al. (2011) concluded that fishmeal has unique functional qualities that are not present in either SPC or wheat gluten. Moreover, if high levels of SPC are used in place of fishmeal then far higher moisture levels are required during extrusion to produce a commercially acceptable pellet. It is known that pellet expansion can be critically affected by the number and size of die holes as this governs the pressure on the extrudate leaving the extruder. In our case a die plate consisting of 3 x 6mm holes was used (Booth et al., 2010a). We also recorded greater variability in the standard deviation of pellet length among the different formulations compared to those determined for pellet diameter (Table 16). Pellets of different length, a problem often encountered in extruded aquafeeds, can be the result of low die pressure which causes uneven product flow at the die. In contrast, if the resultant die pressure is to high the extrudate can crack and split resulting in a pellet with poor durability and water stability (Botting, 1991).

There was virtually no correlation between level of dietary wheat and radial expansion, which could be indicative of the narrow range in wheat inclusion (i.e. starch) across all formulations (Table 15; 10-16%). This is somewhat unlikely as commercial aquafeeds for many species such as salmonids (Sorensen et al., 2010) and snapper (Booth et al., 2011) often include as little as 10% total starch in their formulations. Alternatively, lack of expansion could be indicative of lower than necessary die pressure (Sørensen et al., 2009), temperature and moisture conditions affecting the visco-elastic properties of the dough and its water holding characteristics due to the interactions between soy products and other feed constituents such as NSP (see below). Insufficient water particles trapped within the melt prior to reductions in pressure as the extrudate exits the die can limit the "flash-off" of steam and hence the degree of expansion and porosity of pellets (Sørensen et al., 2009; Sorensen et al., 2010). Given cereal grains such as wheat are poorly digested by Asian seabass (see previous sections of this report on digestibility) it will be unlikely increases in wheat content or other starch sources will be appropriate for increasing radial expansion of finished feeds. Recent research has explored the use of additives such as sodium diformate (NaDF) in extruded aquafeeds for salmon and found it significantly increased the expansion ratio, durability, and water stability of pellets containing barley protein concentrates compared to diets formulated without it. The authors speculated this may be due to the NaDF causing a reduction in pH of the melt or the improved structuring capability of proteins in the presence of Na (Morken et al., 2011). Use of additives such as NaDF and potassium diformate (KDF) appear promising and may be worthy of investigation in diets for Asian seabass that containing high levels of plant proteins such as SPC.

The level of dietary NSP contributed by SPC was not strongly correlated with radial expansion, however it is likely the extremely high dietary levels we examined (i.e. 40 to 60% SPC inclusion) have negatively impacted on the finished quality of the pellets in our trial. Based on the correlation coefficients for SBM and total soy, the impact of SBM on radial expansion seems to be somewhat positive. Increases in dietary NSP attributed to plant protein sources such as lupins (Glencross et al., 2010a) and from cellulose (Hansen & Storebakken, 2007; Kraugerud et al., 2011) have been implicated in the poor expansion of extruded aquafeeds, although qualities such as pellet hardness and durability are often improved (Hansen & Storebakken, 2007; Sørensen et al., 2009). Similar to our results, Sorenson et al. (2009) observed higher radial expansion of doughs containing fishmeal as opposed to doughs where a proportion of the fishmeal was replaced by soybean meal. In contrast, Draganovic et al. (2011) found that higher levels of fishmeal actually decreased the radial expansion of extruded pellets compared to formulations which included SPC when manufactured under controlled test conditions. In their trial, lowest radial expansion was obtained from a mash containing an equal amount of fishmeal and SPC at a medium moisture level (26%), while highest radial expansion was recorded from a mash containing an equal amount of wheat gluten and SPC at a moisture level of 32%. Based on mixture model analysis these authors found that radial expansion increased only slightly with increasing level of SPC and was better in diets containing higher levels of wheat gluten. In addition, SPC tended to cause axial (lengthwise) expansion rather than radial expansion. Draganovic et al. (2011) also found that increased moisture led to greater radial expansion of finished pellets containing SPC and pellets containing blends of SPC and wheat gluten. It should be noted that these authors employed melt temperatures within the extrusion barrel of between 120-130°C during their study on SPC inclusion (Draganovic et al., 2011(Draganovic et al., 2011) which appears to far higher than the temperatures used to extrude aquafeeds for the 2010 USB trial.

Observations on pellet durability

The observation of a greater proportion of fines and cracked pellets in the test feeds containing high levels of SPC may be related to dietary NSP. Non starch polysaccharides may have disrupted or interfered with the gelatinization of dietary starch and the functional qualities of the protein-starch matrix formed during extrusion. This has been shown to affect the finished quality of pasta and is thought to be related to the soluble and insoluble components of different fibre sources (Tudorica et al., 2001). This may partly explain the poorer quality of the diets containing low levels of fishmeal and extreme levels of SPC, as SPC has a relatively higher insoluble fraction of NSP (14-16%) as a result of the concentration process compared to solvent extracted SBM (8-15%) (Choct et al., 2010). Increasing additions of NSP's such as cellulose have been shown to reduce pellet expansion in test feeds for rainbow trout (Hansen & Storebakken, 2007). The reduced expansion is thought to be related to the high water-binding capacity of celluloses which may result in competition for water within the extrusion environment and thus reduce the amount of water available for the hydration and thorough gelatinization of starch granules (Hansen & Storebakken, 2007). These relationships are complicated and further research would be needed to clarify which fraction of the carbohydrate in SBM and SPC is implicated, if at all.

Alternatively, the adhesion/binding properties of our pellets may have been affected by the low levels of wheat and or the amylose / amylopectin ratio of the wheat source. Low ratios can result in poor binding qualities and starch from field peas, having a higher ratio and better pasting characteristics, may be a better choice for use with diets high in SPC (and thus high in NSP). Use of SPC in combination with pregelled potato starch may also assist in radial expansion (Sorensen et al., 2010). Others have shown that pellets containing denatured soybean protein exhibited limited cohesive properties and pellet binding and durability only increased in proportion to the percentage of pregelled tapioca starch included in the ration (Wood, 1987).

Effects of protein source and quality

Differences in the finished feed characteristics of the SPC and fishmeal based diets could possibly be related to the native state of the protein sources. Fishmeal is mainly fibrous muscular protein while that of SBM is globular. Globular proteins from soybean meal generally require higher temperatures (≈ 95°C) to be denatured and form covalent cross-linkages (i.e. disulphide bonds) than fibrous proteins such as fishmeals (≈ 50-60°C) (Aslaksen et al., 2006). According to the extrusion report (Booth et al., 2010a), recorded temperature in the various zones on the Wenger X-85 did not exceed 90°C during manufacture of test feeds containing SPC. Thus the formation of adequate cross-linkages in diets containing elevated levels of SPC may have been reduced. Sorensen et al. (2009) has implicated the un-denatured state of soybean meal protein (white flakes) in elevated motor torque, die pressure and SME which to some extent reflects the observations of the mill operator and the poor quality of diets high in SPC. The absence of adequate levels of cysteine and cystine is thought to reduce the potential formation of covalent disulphide bonds during extrusion, potentially leading to a pellet with poor cohesive properties (Øverland et al., 2009; Sørensen et al., 2009; Morken et al., 2011). However the cystine content of all USB test diets was similar (4.3 – 6.8 g kg^{-1}) therefore the concentration of these amino acids is unlikely to have affected the functional quality of the finished feeds.

We did not measure the nitrogen solubility index (NSI) of ingredients used to manufacture our test diets. In general, lower NSI or protein dispersibility index (PDI) values indicate that more heat damage has been done to the protein/s during processing. Commercial solvent extracted SBM has a typical PDI of 50 to 70 but, SPC has a very low PDI generally in the range of 5 to 10 (Strahm et al., 2003; ADM, 2007).

Consequently, if a raw material has a low solubility then more energy is required to texturize it. However, SPC has a low NSI because it is made by the aqueous alcohol process and its low NSI value is not necessarily related to poor functionality. This is because the denaturation mechanism used to produce SPC is different from that used to produce toasted solvent extracted SBM. Although SPC has a low NSI it is easily texturized, but the process requires higher levels of moisture and mechanical energy than for products such as SBM (Strahm et al., 2003).

Observations on bulk density and pellet length

Many of our test diets exhibited little variation in radial expansion, however there was a considerable range in bulk density (697-809 g L^{-1}) and pellet length (Table 16). Inclusion of SBM in our formulations was also positively correlated with bulk density, however there was little effect of total soy or SPC inclusion on this characteristic. Level of NSP was also positively correlated with bulk density and highly correlated with sinking rate indicating the addition of NSP from SBM was most likely responsible for increases in pellet bulk density. In contrast, the content of NSP was also negatively correlated with pellet length, indicating pellets tended to be shorter in length as level of NSP increased.

High levels of NSP are known to reduce pellet expansion and have been implicated in increases in the length of pellets (Lue et al. 1990 cited in Sørensen et al., 2009). In contrast, variation in the inclusion rate of pea protein concentrate which contains between 12-14% NSP was shown to improve overall pellet quality of diets for Atlantic salmon but significantly reduce pellet length compared to diets based predominantly on fishmeal or SBM (Øverland et al., 2009). The floatability (bulk density) of extruded aquafeeds is governed by the volumetric expansion of the product in both the radial and axial direction (Draganovic et al., 2011) which explains the negative correlation between pellet length and bulk density in our study i.e. longer pellets were more buoyant and took longer to sink. This is not evidence of axial expansion in our formulations, however it is clear there is probably a link between the resultant length of pellets in our study and the dietary content of NSP (Table 17). Bulk density of pellets has been inversely related to radial expansion (Draganovic et al., 2011), however there was only a weak negative correlation between bulk density and radial expansion in our examination (Table 17). Chang et al. (2001a) have studied the effects of extrusion parameters on SPC and shown that highest values for axial expansion were found when dietary levels of SPC are maximised and feed moisture is kept relatively low. They found that dietary levels of SPC greater than 25% tended to reduce radial expansion while the specific volume of the feed increased as the SPC level in the blend increased and feed moisture decreased.

The increasing NSP content of test feeds was the result of increases in SBM and SPC content in the different formulations more so than from minor changes in wheat content (e.g. between 10 and 16%). However, wheat inclusion was moderately negatively correlated with sinking rate (-0.65) and bulk density (-0.66) suggesting addition of wheat in each of the formulations where SBM and SPC were present tended to counter their effects on density. As fishmeal content did not appear to be correlated with bulk density, it appears that the bulk density and sinking rate of the diets we examined is related more to the interaction between starch provided from wheat and the level of NSP contributed from SBM and or SPC (Figure 5).

Control of bulk density or buoyancy in aquafeeds can be a difficult process and is affected by more than ingredient concentrations and physical extruder settings. Regardless of formulation, the accuracy and timing of water addition has one of the greatest bearings on the production of acceptable aquafeeds (Draganovic et al., 2011) and there is often only a small difference between the rate of water addition and the production of pellets which will float, those that will be neutrally buoyant and those that will sink (Botting, 1991). In addition the buoyancy of extruded aquafeeds is likely to be different depending on whether they are used to feed animals in fresh or saltwater. As noted earlier the operator of the extruder used to produce feeds in 2010 indicated it was difficult to decide on the appropriate moisture levels for some of the extreme formulations. Consequently diets high in SPC may have been more physically stable had a different moisture profile been used. Extrudates high in protein, low in oil and low in starch such as the extreme formulations we tested are known to disintegrate when exiting the die if water addition is too low (Botting, 1991).

Conclusion

Diets composed of between 40 and 60% SPC in conjunction with low dietary concentrations of wheat (10 to 16%), fishmeal (10 to 16%) and poultry meal (10 to 24%)

produced extruded pellets that tended to have poor cohesion and quality. Diets composed of between 30 and 38% SBM in which the level of fishmeal was relatively high (≈ 40%) but the level of wheat was low (10%) produced better quality pellets. Despite differences in the ingredient composition of the different diets radial expansion was limited and most diets sank quickly. Reasons for the observed pellet characteristics of the aquafeeds we trialed in 2010 were discussed with reference to relevant scientific research on this topic.

Review of the scientific literature has indicated there is a need for further research into the interactions between SBM, SPC and other common feed ingredients subjected to extrusion processing. In addition, more research is required to better understand how the selection of adjustable extrusion parameters such as moisture level, barrel temperature, feeding rate, screw speed and retention time etc. affect dependent variables such as motor torque, die pressure and SME in aquafeed formulations containing SBM and SPC. Ideally these factors should be examined under controlled conditions where most extrusion parameters are held constant and ingredient-ingredient interactions are minimised. This will lead to a far better understanding of ingredient-ingredient interactions and knowledge of the functional value of SBM and SPC in the presence of other important feed proteins.

Table 15. Formulation and measured composition of experimental feeds used in 2010 utilisation experiment.

	Diet code									
	Commercial diet	D2 Fishmeal	D3 SBM 30	D4 SBM 35	D5 SBM 38	D6 SPC 40	D7 SPC 50	D8 SPC 60	D9 SBM/SPC A	D10 SBM/SPC B
Ingredient (%)										
Fish oil	-	10.00	10.00	10.00	10.40	10.00	10.00	10.00	10.72	11.98
Fish meal	-	55.00	40.56	38.31	40.64	10.00	10.00	10.00	14.44	16.11
SBM[1]	-	0.00	30.00	35.45	38.00	0.00	0.00	0.00	25.00	30.00
SPC[2]	-	0.00	0.00	0.00	0.00	40.00	50.00	60.00	25.00	30.00
Wheat	-	14.70	10.00	10.00	10.00	15.93	14.43	12.92	10.00	10.00
Meat meal	-	10.00	9.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Poultry meal	-	10.00	0.00	5.93	0.66	23.57	15.02	6.48	14.36	1.41
L-methionine	-	0.00	0.00	0.00	0.00	0.20	0.25	0.29	0.18	0.20
Vit/min premix	-	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Proximate analysis (%)										
Ash	8.1	13.2	10.9	9.6	9.5	7.2	6.7	7.0	8.5	7.5
Nitrogen	8.55	8.64	7.99	8.31	8.28	8.67	8.8	9.0	8.35	8.21
Crude protein	53.44	54.0	49.94	51.94	51.75	54.19	55.0	56.25	52.19	51.31
Fat	12.7	16.7	17.5	16.0	15.2	14.5	13.5	10.9	13.8	14.5
NFE	25.76	16.1	21.66	22.46	23.55	24.11	24.8	25.85	25.51	26.69
Gross energy (MJ kg^{-1})	22.58	21.87	22.33	22.24	21.92	22.52	22.54	22.39	22.35	22.25
Bulk density (g L^{-1})	659.4	715.4	738.9	802.1	809.0	697.4	747.0	758.1	722.3	777.0
NSP* (%)		1.47	9.10	10.57	11.26	8.79	10.44	12.09	12.25	14.50

[1.] Solvent extracted soybean meal, Argentine.
[2.] ADM Specialty Ingredients Soycomil K.
* Estimated amount of NSP based on contribution from wheat, soybean meal and soy protein concentrate.

Table 16. Mean ± SD of pellet characteristics of experimental diets containing soybean meal and soy protein concentrate (n=20 pellets per diet).

Diet description[1]	Diameter (mm)	Length (mm)	Sink rate (mm sec^{-1})	Radial expansion (ratio)
Commercial[2]	6.05 *0.13*	6.93 *0.33*	1.33 *0.00*	na *na*
Fishmeal	5.06c *0.23*	7.66cd *1.08*	6.26a *8.06*	0.84c *0.04*
SBM30	5.46d *0.36*	9.34e *1.30*	43.83bc *23.24*	0.91d *0.06*
SBM35	4.95bc *0.26*	6.06a *1.06*	61.89de *16.81*	0.83bc *0.04*
SBM38	4.96bc *0.21*	6.81abc *0.53*	73.31ef *11.26*	0.83bc *0.04*
SPC40	4.77ab *0.32*	8.15de *1.10*	38.13b *17.16*	0.80ab *0.05*
SPC50	4.57a *0.36*	7.44bcd *1.51*	53.69cd *8.04*	0.76a *0.06*
SPC60	4.70ab *0.17*	6.66abc *1.26*	64.99def *9.56*	0.78ab *0.03*
SBM/SPC25	4.78ab *0.23*	7.20abcd *1.09*	61.82de *6.17*	0.80ab *0.04*
SBM/SPC30	4.75ab *0.21*	6.38ab *1.63*	76.46f *8.56*	0.79ab *0.03*

[1] Diet formulations are fully described in the FY 2010 final report on Asian seabass (Booth et al., 2010a).
[2.] Data on the commercial diet is presented only for comparison and is not included in statistical tests.

Table 17. Matrix of correlation coefficients relating pellet characteristics to variations in the ingredient composition of test diets.

	Pellet characteristic				
	Diameter (mm)	Length (mm)	Sink rate (mm sec^{-1})	Radial expansion ratio	Bulk density (g L^{-1})
Dietary level of:					
SBM	0.46	-0.43	0.58	0.44	0.73*
SPC	-0.93***	-0.07	0.14	-0.91***	-0.16
Total soy	-0.86**	-0.45	0.61	-0.89**	0.21
Fishmeal	0.86**	0.05	-0.07	0.84**	0.20
Wheat	-0.29	0.47	-0.65	-0.24	-0.66
NSP	-0.41	-0.71*	0.88**	-0.48	0.57
Pellet characteristic:					
Diameter	1	0.35	-0.33	0.99***	-0.08
Length		1	-0.81**	0.35	-0.77*
Sink rate			1	-0.38	0.85**
Radial expansion				1	-0.11
Bulk density					1

* indicates *P*<0.05, ** indicates *P*<0.01, *** indicates *P*<0.001

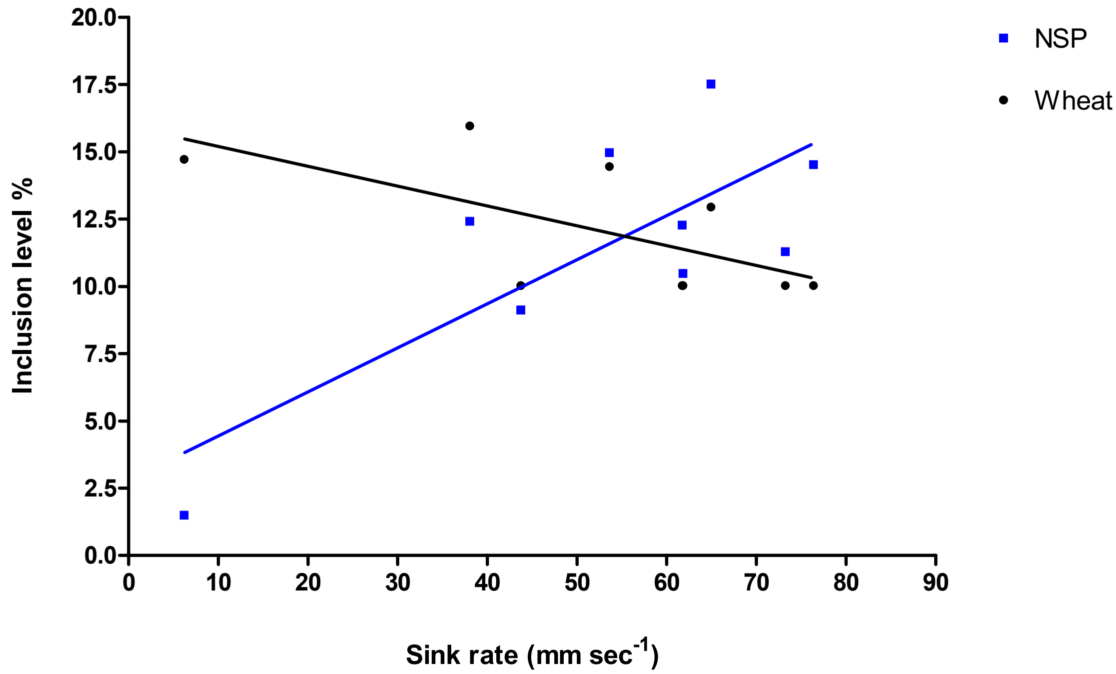


Figure 6. Relationship between sinking rate of feed pellets and dietary inclusion level of wheat or non starch polysaccharides (NSP).

Bibliography

Abery NW, Gunasekera RM, De Silva SS (2002) Growth and nutrient utilization of Murray cod *Maccullochella peelii peellii* (Mitchell) fingerlings fed diets with varying levels of soybean meal and blood meal. *Aquacult. Res.,* **33**, 279-289.

ADM (2007) Soycomil K: an excellent protein source for aquafeed. In: *ADM Speciality Ingredients (Europe) B.V.*, Netherlands.

Allan GL, Booth MA (2004) Effects of extrusion processing on digestibility of peas, lupins, canola meal and soybean meal in silver perch *Bidyanus bidyanus* (Mitchell) diets. *Aquacult. Res.,* **35**, 981-991.

Allan GL, Parkinson S, Booth MA, Stone DAJ, Rowland SJ, Frances J, Warner-Smith R (2000) Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture,* **186**, 293-310.

Allan GL, Stone DAJ, Anderson AJ, booth MA (2003) Carbohydrate metabolism in silver perch and barramundi. *Recent Advances in Animal Nutrition in Australia,* **14**, 171-177.

Amirkolaie AK, Verreth JAJ, Schrama JW (2006) Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (*Oreochromis niloticus* (L.)). *Aquaculture,* **260**, 194-205.

Anderson AJ (2003) Metabolic studies on carbohydrate utilisation by barramundi and tilapia. In: *Aquaculture diet development subprogram: ingredient evaluation. Project 1996 / 391 Final Report to Fisheries Research and Development Corporation (FRDC)* (ed. by Allan GL, Booth MA, Stone DAJ, Anderson AJ). Fisheries Research and Development Corporation (FRDC), Canberra, Australia, pp. 120-134.

Anon. (2003) Aquatic Feed Production - Wenger Process Description PAA-400-GJR. In: *Food and Feed Extrusion Workshop* (eds Forte D, van Doore F, van Barneveld R, Young G). Food Engineering Industry Pty Ltd jointly with FRDC, Adelaide University, Roseworthy Campus, South Australia.

AOAC (2005) *Official methods of analysis of AOAC International,* AOAC International 18th. ed., Gaithersburg, Md.

Aslaksen MA, Romarheim OH, Storebakken T, Skrede A (2006) Evaluation of content and digestibility of disulfide bonds and free thiols in unextruded and extruded diets containing fish meal and soybean protein sources. *Anim. Feed Sci. Technol.,* **128**, 320-330.

Barrows FT, Stone DAJ, Hardy RW (2007) The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (Oncorhynchus mykiss). *Aquaculture,* **265**, 244-252.

Blaufuss P, Trushenski J (2011) Exploring soy-derived alternatives to fish meal: Using soy protein concentrate and soy protein isolate in hybrid striped bass feeds. *N. Am. J. Aquacult.,* **74**, 8-19.

Boonyaratpalin M, Williams K (2002) Asian Sea Bass, *Lates calcarifer*. In: *Nutrient Requirements and Feeding of Finfish for Aquaculture* (ed. by Webster CD, Lim CE). CABI Publishing, New York, pp. 40-50.

Booth MA, Allan GL (2010) Formulation of aqua-feeds for Asian seabass *Lates calcarifer* containing optimal inclusion levels of SBM and SPC. In: *Final Report Submitted to the United Soybean Board (USB) New Uses Committee USB Project FY2009 SB9463*. Industry & Investment NSW Port Stephens Fisheries Institute (PSFI), Taylors Beach, NSW, Australia, pp. 66.

Booth MA, Allan GL, Anderson AJ (2005) Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. *Aquacult. Res.,* **36**, 378-390.

Booth MA, Allan GL, Anderson AJ (2011) Influence of poultry meal, meat meal or soybean meal inclusion on weight gain and production characteristics of Australian snapper *Pagrus auratus*. *Aquacult. Int.,* **DOI 10.1007/s10499-011-9445-9**.

Booth MA, Allan GL, Russell I (2010a) Development of aqua-feeds containing optimal inclusion levels of SBM and SPC for Asian seabass *Lates calcarifer*. In: *Final Report Submitted to the United Soybean Board (USB) New Uses Committee USB Project FY2010 SB0463*. Industry & Investment NSW Port Stephens Fisheries Institute (PSFI), Taylors Beach, NSW, Australia, pp. 59.

Booth MA, Allan GL, Warner-Smith R (2000) Effects of grinding, steam conditioning and extrusion of a practical diet on digestibility and weight gain of silver perch, *Bidyanus bidyanus*. *Aquaculture,* **182**, 287-299.

Booth MA, Anderson AJ, Allan GL (2006) Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider 1801): digestibility of gelatinized wheat starch and clearance of an intra-peritoneal injection of d-glucose. *Aquacult. Res.,* **37**, 975-985.

Booth MA, Pirozzi I, Allan GL, Fielder DS (2010b) Diet Development: temperate species. In: *Aquafin CRC Project 1B5: Feed Technology For Temperate Fish Species; Final Report to Aquafin CRC & FRDC, Project No. 2004/220, Volume 2*. Industry & Investment NSW, Cronulla, Sydney, pp. 376.

Botting CC (1991) Extrusion technology in aquaculture feed processing. In: *Proceedings of the aquaculture feed processing and nutrition workshop September 19-25 1991* (eds Akiyama DM, Tan RKH). American Soybean Association - Singapore, Thailand and Indonesia, pp. 129-137.

Bouvier JM (1997) Generic extrusion processes in the agro-food industries. *Feed Tech,* **1**, 13-15.

Chang YK, Hashimoto JM, Acioli-Moura R, Martinez-Flores HE, Martinez-Bustos F (2001a) Influence of extrusion conditions on cassava starch and soybean protein concentrate blends. *Acta Alimentaria,* **30**, 189-203.

Chang YK, Hashimoto JM, Moura-Alcioli R, Martinez-Bustos F (2001b) Twin-screw extrusion of cassava starch and isolated soybean protein blends. *Nahrung-Food,* **45**, 234-240.

Choct M, Dersjant-Li Y, McLeish J, Peisker M (2010) Soy Oligosaccharides and Soluble Non-starch Polysaccharides: A Review of Digestion, Nutritive and Anti-nutritive Effects in Pigs and Poultry. *Asian-Australasian Journal of Animal Sciences,* **23**, 1386-1389.

Colombo A, León AE, Ribotta PD (2011) Rheological and calorimetric properties of corn-, wheat-, and cassava- starches and soybean protein concentrate composites. *Starch - Stärke,* **63**, 83-95.

Cornell H (2004) The functionality of wheat starch. In: *Starch in food. Structure, function and applications* (ed by Eliasson A-C). Woodhead Publishing Limited, Oxford, pp. 211-240.

De la Higuera M (2001) Effects of nutritional factors and feed characteristics on feed intake. In: *Food Intake in Fish* (ed. by Houlihan D, Boujard T, Jobling M). Blackwell Science, Oxford, pp. 250-268.

de Mesa NJE, Alavi S, Singh N, Shi Y-C, Dogan H, Sang Y (2009) Soy protein-fortified expanded extrudates: Baseline study using normal corn starch. *J. Food Eng.,* **90**, 262-270.

Dhont J, Van Stappen G (2003) Biology, tank production and nutritional value of Artemia. In: *Live feeds in aquaculture* (ed. by Stottrup JG, McEvoy LA). Blackwell Science, Oxford, pp. 65-121.

Dias J, Gomes EF, Kaushik SJ (1997) Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets. *Aquat. Living Resour.,* **10**, 385-389.

Dominy WG (2005) Extrusion cooking and feed ingredients. Aquafeed.com.

Donald AM (2004) Understanding starch structure and functionality. In: *Starch in food. Structure, function and applications* (ed by Eliasson A-C). Woodhead Publishing Limited, Oxford, pp. 156-184.

Draganovic V, van der Goot AJ, Boom R, Jonkers J (2011) Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. *Anim. Feed Sci. Technol.,* **165**, 238-250.

Eusebio PS, Coloso RM (2000) Nutritional evaluation of various plant protein sources in diets for Asian sea bass *Lates calcarifer*. *J. Appl. Ichthyol.,* **16**, 56-60.

Faller JY, Klein BP, Faller JF (1999) Acceptability of extruded corn snacks as affected by inclusion of soy protein. *J. Food Sci.,* **64**, 185-188.

FAO (2011) FISHSTAT Plus Version 2.32. Food & Agricultural Organistion of United Nations, Rome.

Floreto EAT, Brown PB, Bayer RC (2001) The effects of krill hydrolysate-supplemented soya-bean based diets on the growth, colouration, amino and fatty acid profiles of juvenile American lobster, Homarus americanus. *Aquacult. Nutr.,* **7**, 33-43.

Fujita S, Watanabe T, Kitajima C (1980) Nutritional quality of Artemia from different localities as a living feed for marine fish from the viwepoint of essential fatty acids. In: *Proceedings of the International Symposium on the brine shrimp Artemia salina* (eds Persoone G, Sorgeloos p, Roels O, Jaspers E). Universa Press, Corpus Christi, Texas, USA, pp. 277-290.

Gaber MMA (2005) The effect of different levels of krill meal supplementation of soybean-based diets on feed intake, digestibility, and chemical composition of juvenile Nile tilapia *Oreochromis niloticus*, L. *J. World Aquacult. Soc.,* **36**, 346-353.

Gatlin DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW, Herman E, Hu GS, Krogdahl A, Nelson R, Overturf K, Rust M, Sealey W, Skonberg D, Souza EJ, Stone D, Wilson R, Wurtele E (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res.,* **38**, 551-579.

Glencross B (2001) Feeding lupins to fish : A review of the nutritional and biological value of lupins in aquaculture feeds. Department of Fisheries – Research Division Government of Western Australia, PO Box 20, North Beach, WA 6160, Australia
pp. 117.

Glencross B (2011) A comparison of the digestibility of diets and ingredients fed to rainbow trout (*Oncorhynchus mykiss*) or barramundi (*Lates calcarifer*) – the potential for inference of digestibility values among species. *Aquacult. Nutr.,* **17**, e207-e215.

Glencross B, Blyth D, Tabrett S, Bourne N, Irvin S, Anderson M, Fox-Smith T, Smullen R (2011a) An assessment of cereal grains and other starch sources in diets for barramundi (*Lates calcarifer*) – implications for nutritional and functional qualities of extruded feeds. *Aquacult. Nutr.*, 10.1111/j.1365-2095.2011.00903.x.

Glencross B, Hawkins W, Evans D, Rutherford N, McCafferty P, Dods K, Hauler R (2011b) A comparison of the effect of diet extrusion or screw-press pelleting on

the digestibility of grain protein products when fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **312**, 154-161.
Glencross B, Hawkins W, Maas R, Karopoulos M, Hauler R (2010a) Evaluation of the influence of different species and cultivars of lupin kernel meal on the extrusion process, pellet properties and viscosity parameters of salmonid feeds. *Aquacult. Nutr.,* **16**, 13-24.
Glencross BD (2008) A factorial growth and feed utilization model for barramundi, *Lates calcarifer* based on Australian production conditions. *Aquacult. Nutr.,* **14**, 360-373.
Glencross BD, Booth M, Allan GL (2007) A feed is only as good as its ingredients - a review of ingredient evaluation strategies for aquaculture feeds. *Aquacult. Nutr.,* **13**, 17-34.
Glencross BD, Rutherford N, Jones B (2010b) Evaluating options for fishmeal replacement in diets for juvenile barramundi (*Lates calcarifer*). *Aquacult. Nutr.,* **TBA**, TBA.
Hansen JØ, Storebakken T (2007) Effects of dietary cellulose level on pellet quality and nutrient digestibilities in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **272**, 458-465.
Harris TA (2006) The effectiveness of dietary supplementation with feed attractants on the feed intake and growth performance of juvenile mulloway (*Argyrosomus japonicus*) (Pisces: Sciaenidae). In: *A thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours), School of Environment and Life Sciences*. University of Newcastle, Newcastle, New South Wales, Australia, pp. 77.
Hintze J (2006) NCSS, PASS and GESS. *NCSS, Kaysville, Utah, USA.*, www.ncss.com.
Irish GG, Balnave D (1993) Non-Starch Polysaccharides and Broiler Performance on Diets containing Soyabean Meal as the Sole Protein Concentrate. *Aust. J. Agric. Res.,* **44**, 1483-1499.
Jiang N, Zhang AZ, Yang RQ, Zhang YC (2011) An experimental approach to optimize several processing conditions when extruding soybeans. *Anim. Feed Sci. Technol.,* **170**, 277-283.
Jirsa D, Davis A, Stuart K, Drawbridge M (2011) Development of a practical soy-based diet for California yellowtail, *Seriola lalandi*. *Aquacult. Nutr.,* **17**, e869-e874.
Jobling M, Gomes E, Dias J (2001) Feed types, manufacture and ingredients. In: *Food intake in fish* (ed. by Houlihan D, Boujard T, Jobling M). Blackwell Science, Oxford, pp. 25-48.
Kolkovski S, Czesny S, Dabrowski K (2000) Use of Krill Hydrolysate as a Feed Attractant for Fish Larvae and Juveniles. *J. World Aquacult. Soc.,* **31**, 81-88.
Koskela J, Pirhonen J, Virtanen E (1993) Effect of attractants on feed choice of rainbow trout, *Oncorhynchus mykiss*. *Fish Nutrition in Practice*, 419-427.
Kraugerud OF, Jørgensen HY, Svihus B (2011) Physical properties of extruded fish feed with inclusion of different plant (legumes, oilseeds, or cereals) meals. *Anim. Feed Sci. Technol.,* **163**, 244-254.
Kraugerud OF, Svihus B (2011) Effects of online pretreatment of plant ingredients on processing responses and physical properties in extruded fish feed. *Anim. Feed Sci. Technol.,* **168**, 250-256.
Kumar A, Ganjyal GM, Jones DD, Hanna MA (2007) Experimental determination of longitudinal expansion during extrusion of starches. *Cereal Chemistry,* **84**, 480-484.

Laining A, Rachmansyah, Ahmad T, Williams K (2003) Apparent digestibility of selected feed ingredients for humpback grouper, *Cromileptes altivelis*. *Aquaculture,* **218**, 529-538.
Lamb CF (2001) Gustation and feeding behaviour. In: *Food intake in fish* (ed. by Houlihan D, Boujard T, Jobling M). Blackwell Science, Oxford, pp. 108-130.
Lian P, Lee CM, Bengtson DA (2008) Development of a squid-hydrolysate-based larval diet and its feeding performance on summer flounder, *Paralichthys dentatus*, larvae. *J. World Aquacult. Soc.,* **39**, 196-204.
Little C, Maguilere J, Morales A, Kokoni J (1997) Changes in Soy Protein during Heating Analyzed by Pressure Rheometry. *Food Sci. Technol. Int.,* **3**, 130-133.
Lupatsch I, Kissil GW, Sklan D, Pfeffer E (1997) Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. *Aquacult. Nutr.,* **3**, 81-89.
Marsman GJP, Gruppen H, Van der Poel AFB, Resink JW, Verstegen MWA, Voragen AGJ (1995) The effect of shear forces and addition of a mixture of a protease and a hemicellulase on chemical, physical and physiological parameters during extrusion of soybean meal. *Anim. Feed Sci. Technol.,* **56**, 21-35.
Masumoto T, Ruchimat T, Ito Y, Hosokawa H, Shimeno S (1996) Amino acid availability values for several protein sources for yellowtail (*Seriola quinqueradiata*). *Aquaculture,* **146**, 109-119.
McMeniman NP (1998) The apparent digestibility of feed ingredients based on stripping methods. In: *Replacement of fishmeal in aquaculture diets sub-program 93/120; Fishmeal replacement in aquaculture feeds for barramundi project 93/120-4. Final Report to Fisheries Research and Development Corporation (FRDC)* (ed by Wiiliams K). Fisheries Research and Development Corporation (FRDC), Canberra, Australia, pp. 34-38.
McMeniman NP (2003) Digestibility and utilisation of starch by barramundi. In: *Aquaculture diet development subprogram: ingredient evaluation. Project 1996 / 391 Final Report to Fisheries Research and Development Corporation (FRDC)* (ed. by Allan GL, Booth MA, Stone DAJ, Anderson AJ). Fisheries Research and Development Corporation (FRDC), Canberra, Australia, pp. 135-139.
Michael RJ, Partridge GJ, Hofmann T, Caro ED (2010) Evaluation of mosquito fish *Gambusia holbrooki* (Girard) as a partial and complete pellet replacement for juvenile barramundi Lates calcarifer (Bloch). *Aquacult. Res.,* **41**, e788-e794.
Morken T, Kraugerud OF, Barrows FT, Sørensen M, Storebakken T, Øverland M (2011) Sodium diformate and extrusion temperature affect nutrient digestibility and physical quality of diets with fish meal and barley protein concentrate for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **317**, 138-145.
Moure A, Sineiro J, Dominguez H, Parajo JC (2006) Functionality of oilseed protein products: A review. *Food Res. Int.,* **39**, 945-963.
Nengas I, Alexis MN, Davies SJ, Petichakis G (1995) Investigation to determine digestibility coefficients of various raw materials in diets for gilthead sea bream, *Sparus auratus* (L.). *Aquacult. Res.,* **26**, 185-194.
Nunes AJP, Sa MVV, Andriola-Neto FF, Lemos D (2006) Behavioral response to selected feed attractants and stimulants in Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture,* **260**, 244-254.
Oikawa CK, March BE (1997) A method for assessment of the efficacy of feed attractants for fish. *Prog. Fish-Cult.,* **59**, 213-217.
Øverland M, Sørensen M, Storebakken T, Penn M, Krogdahl Å, Skrede A (2009) Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*)—Effect on growth performance, nutrient digestibility,

carcass composition, gut health, and physical feed quality. *Aquaculture,* **288**, 305-311.
Papatryphon E, Soares Jr JH (2000) Identification of feeding stimulants for striped bass, *Morone saxatilis*. *Aquaculture,* **185**, 339-352.
Papatryphon E, Soares Jr JH (2001) Optimizing the levels of feeding stimulants for use in high-fish meal and plant feedstuff-based diets for striped bass, *Morone saxatilis*. *Aquaculture,* **202**, 279-288.
Rokey G, Strathman R, Plattner B (2003) Pelleted livestock feed production - Wenger Process Description. In: *Food and Feed Extrusion Workshop* (ed. by Forte D, van Doore F, van Barneveld R, Young G). Food Engineering Industry Pty Ltd jointly with FRDC, Adelaide University, Roseworthy Campus, South Australia.
Schaeffer TW, Brown ML, Rosentrater KA (2011) Effects of Dietary Distillers Dried Grains with Solubles and Soybean Meal on Extruded Pellet Characteristics and Growth Responses of Juvenile Yellow Perch. *N. Am. J. Aquacult.,* **73**, 270-278.
Singh RK, Balange AK, Khandagale PA, Chavan SL (2006) Evaluation of fish meals as natural feed stimulants on the feeding behaviour of fry and juveniles of *Lates calcarifer* (Bloch). *Asian Fish. Sci.,* **19**, 97-106.
Singh RK, Vartak V, Balange A (2005) Effect of stimulants on feeding response, feeding behavior, and growth of fry of sea bass, Lates calcarifer (Bloch, 1790). *Israeli Journal of Aquaculture-Bamidgeh,* **57**, 32-38.
Singh S, Gamlath S, Wakeling L (2007) Nutritional aspects of food extrusion: a review. *Int. J. Food Sci. Tech.,* **42**, 916-929.
Smith DM, Tabrett SJ, Barclay MC, Irvin SJ (2005) The efficacy of ingredients included in shrimp feeds to stimulate intake. *Aquacult. Nutr.,* **11**, 263-272.
Sorensen M, Nguyen G, Storebakken T, Overland M (2010) Starch source, screw configuration and injection of steam into the barrel affect the physical quality of extruded fish feed. *Aquacult. Res.,* **41**, 419-432.
Sørensen M, Stjepanovic N, Romarheim OH, Krekling T, Storebakken T (2009) Soybean meal improves the physical quality of extruded fish feed. *Anim. Feed Sci. Technol.,* **149**, 149-161.
Strahm B, Rokey G, Thomas C (2003) Textured vegetable proteins - Wenger Process Description. In: *Food and Feed Extrusion Workshop* (ed. by Forte D, van Doore F, van Barneveld R, Young G). Food Engineering Industry Pty Ltd jointly with FRDC, Adelaide University, Roseworthy Campus, South Australia.
Thomas M, van der Poel AFB (1996) Physical quality of pelleted animal feed 1. Criteria for pellet quality. *Anim. Feed Sci. Technol.,* **61**, 89-112.
Trushenski J, Laporte J, Lewis H, Schwarz M, Delbos B, Takeuchi R, Sampaio LA (2011) Fish Meal Replacement with Soy-derived Protein in Feeds for Juvenile Cobia: Influence of Replacement Level and Attractant Supplementation. *J. World Aquacult. Soc.,* **42**, 435-443.
Tudorica CM, Kuri V, Brennan CS (2001) Nutritional and Physicochemical Characteristics of Dietary Fiber Enriched Pasta. *J. Agric. Food Chem.,* **50**, 347-356.
van Barneveld R (2003) Aquafeed ingredient selection - what needs to be considered? In: *Food and Feed Extrusion Workshop* (eds Forte D, van Doore F, van Barneveld R, Young G). Food Engineering Industry Pty Ltd jointly with FRDC, Adelaide University, Roseworthy Campus, South Australia.
Webster CD, Lovell RT (1991) Lipid composition of three geographical sources of brine shrimp nauplii (Artemia sp.). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,* **100**, 555-559.

Whalen PJ, Bason ML, Booth RI, Walker CE, Williams PJ (1997) Measurement of extrusion effects by viscosity profile using the rapid viscoanalyser. *Cereal Foods World,* **42**, 469-475.
Williams KC (1998a) Comparison of dissection and sedimentation procedures for determining the apparent digestibility of protein feeds. In: *Replacement of fishmeal in aquaculture diets sub-program 93/120; Fishmeal replacement in aquaculture feeds for barramundi project 93/120-4. Final Report to Fisheries Research and Development Corporation (FRDC)* (ed by Wiiliams K). Fisheries Research and Development Corporation (FRDC), Canberra, Australia*,* pp. 25-30.
Williams KC (1998b) Fishmeal replacement in aquaculture feeds fo barramundi (*Lates calcarifer*) Project 93 /120-04. *Replacement of fishmeal in aquaculture diets sub-program 93/120. Final Report to Fisheries Research and Development Corporation (FRDC)*, pp 89.
Williams KC, Barlow C (1998) Dietary requirements and optimal feeding practices for barramundi (*Lates calcarifer*) Project 92/63. *Replacement of fishmeal in aquaculture diets sub-program 93/120. Final Report to Fisheries Research and Development Corporation (FRDC)*, pp 97.
Williams KC, Barlow CG, Rodgers LJ, Ruscoe I (2003) Potential of meat meal to replace fish meal in extruded dry diets for barramundi, Lates calcarifer (Bloch). 1. Growth performance. *Aquacult. Res.,* **34**, 23-32.
Wood JF (1987) The functional properties of feed raw materials and their effect on the production and quality of feed pellets. *Anim. Feed Sci. Technol.,* **18**, 1-17.
Young G (2003) Overview of the extrusion process. In: *Food and Feed Extrusion Workshop* (eds Forte D, van Doore F, van Barneveld R, Young G). Food Engineering Industry Pty Ltd jointly with FRDC, Adelaide University, Roseworthy Campus, South Australia.

Project description and budget

Please fax approval to (314) 579-1599.

SUB-CONTRACT APPROVAL REQUEST

TO: **Bob Haselwood**

FROM: **Lisa Childs**

DATE: **February 11, 2011**

RE: **USB approval of a New Uses Sub-Contract Funded by Project Number 1463, "Soy in Aquaculture Research"**

CC: **Wayne Watkinson**

CONTRACT DESCRIPTION:

Approval is hereby requested for the attached subcontract between SmithBucklin and **Industry & Investment NSW** to carry out the activities described in the project identified above and approved by USB.

The agreement is effective as of **January 1, 2011** with your signature below.

A copy of the contract has been sent to Wayne Watkinson, USB attorney. If you have any questions on the legal document or project description, please call Wayne prior to returning this signed document. SmithBucklin will proceed with this subcontract upon receipt of your approval. Thank you.

Approved by: Bob Haselwood
Bob Haselwood, Chairman

Date: 2-11-11

End Result Contract

Article 1. *Parties*

SmithBucklin Corporation
16305 Swingley Ridge Road, Suite 120
Chesterfield, MO 63017 USA
(Hereinafter referred to as SmithBucklin)

Industry & Investment NSW for and on behalf of the State of New South Wales
Locked Bag 21, 161 Kite Street
Orange, New South Wales
Australia 2800
(Hereinafter referred to as Contractor)

Article 2. *Scope of Services to be Rendered*

Contractor will perform the services described in the following project proposal(s) (the Project(s)) attached as Attachment A:

USB Project #	***Title***	***Performance Period***	***Amount Funded***
1463	Asian Seabass: Validation of Commercial Grown-Out Fees Containing Optimal Levels of SBM and SPC	January 1, 2011 to December 31, 2011	$171,746

Article 3. *Terms of Agreement*

Payment terms are as follows:

1) Contractor may invoice SmithBucklin twice monthly, but no less than bi-monthly, for all fees, direct costs and reasonable travel and out-of-pocket expenses. Invoices are due on or about the 5th and 20th of each month and SmithBucklin must pay them within 30 days of receipt. SmithBucklin may withhold payment until Contractor submits any report that Contractor has failed to submit in the time set forth in this Agreement.

2) Contractor shall submit an invoice to SmithBucklin for all expenses incurred on or before September 30 not later than the October 31 immediately following. Failure of the Contractor to submit expenses or invoices by this deadline may result in ineligibility for reimbursement. All invoices should be sent to:

SmithBucklin Corporation
16305 Swingley Ridge Road, Suite 120
Chesterfield, MO 63017

3) Fees and direct costs must include costs related to suppliers and contract employees. Contractor must bill travel and out-of-pocket expenses at net cost. SmithBucklin will pay fees, direct cost and expenses only to the extent that they comply with this Agreement and Attachment B.

4) Budget line transfers in excess of ten percent (10%) of any one general budget category or any transfers involving travel expense cannot be made without a written request from the Contractor and the written consent of SmithBucklin.

Article 4. <u>Reports</u>

(1) Contractor must submit to SmithBucklin written reports on the 15th of December, March, June and September that:

(1) describe the progress toward the performance measures as stated in the Project and the relevant Constraint or Opportunity as stated in the FY2011 Action Plan,

(2) provide any additional information requested by USB or SmithBucklin.

(b) Within 30 days after the end of the Project performance period stated in Article 2, Contractor must submit a final report summarizing the work performed, results obtained relative to the Project and Action Plan, conclusions reached, and recommendations for further action. The report must disclose any new discoveries. SmithBucklin may withhold any final payment due under this Agreement until Contractor submits the final report.

(c) Within 30 days after the end of the Project performance period stated in Article 2, contractor must submit a final accounting of project expenses. This requirement will be met by returning to SmithBucklin a signed reconciliation letter supplied by SmithBucklin.

Article 5. <u>*Relationship of the Parties*</u>

(a) Contractor is deemed to be an independent contractor and not an employee, agent or legal representative of SmithBucklin for any purpose. SmithBucklin's sole obligation to Contractor shall be pursuant to this Contract.

(b) Contractor agrees to serve under the coordination of the Director of SmithBucklin responsible for the administration of the program for which services are rendered under this Contract.

Article 6. <u>*Confidentiality and Ownership*</u>

(a) Contractor may not discuss or disclose any information relating to this Contract to any other parties without prior consent of SmithBucklin, except to meet government reporting requirements.

(b) SmithBucklin shall retain the exclusive right to use, distribute, disseminate or publish materials produced pursuant to this Contract in any manner it so desires in perpetuity and for no additional fee or royalty.

(c) Any and all intellectual property in the reports provided to SmithBucklin under this Contract is hereby deemed "Work for Hire" and is the exclusive property of SmithBucklin and shall be immediately surrendered to SmithBucklin upon written request.

Article 7. <u>*Governing Laws*</u>

(a) Contractor's services may be rendered in several jurisdictions and since the laws of said jurisdictions may vary one from the other and from those of the United States, it is hereby agreed that the provisions of this Contract and any claim or action arising therefrom or having as a basis the subject matter of this Contract shall be governed by the laws of the State of Missouri, United States and any such claim or action may be brought only in the courts of applicable jurisdiction in such state.

(b) If a provision of this Contract does not comply with a law or regulation, the law and regulation will prevail. If any term or provision of the Contract is held to be illegal or unenforceable, the validity or enforceability of the remainder of the Contract will not be affected.

Article 8. <u>*Assignments and Termination*</u>

(a) Contractor shall not assign or otherwise transfer rights or obligations under this Contract.

(b) SmithBucklin may terminate this Contract in the event of Contractor's death or inability to perform the services set forth in Article 2 herein.

(c) In the event of any criminal or civil action brought against Contractor, which adversely affects the ability of Contractor to complete the assignment or creates a negative image of SmithBucklin and/or its programs, including, without limitation, breach of the representations and warranties of Article 9 of this Contract, SmithBucklin may terminate the Contract immediately and will pay the Contractor for all services provided up to and including the date of termination, and expenses incurred.

(d) SmithBucklin reserves the right to cancel this Contract at any time prior to the start of the performance period due to, including but not limited to, acts of God, government-issued restrictions, bio-security concerns or any and all other such causes that are beyond the control of SmithBucklin. In this event, SmithBucklin's sole liability, financial or otherwise, is limited to payment for all services up to and including the date of cancellation, and expenses incurred.

Article 9. <u>*Compliance*</u>

(a) Contractor acknowledges that it is the policy of SmithBucklin to use only ethical business practices while conducting business activities. Contractor should not seek to influence sales or other business by illegal payments, bribes, kickbacks or other questionable inducements. As specifically mandated by the Foreign Corrupt Practices Act, 15 U.S.C. §§ 78dd-1, *et seq.*, Contractor may not, and represents and warrants that he or she shall not, make, or offer to make, payments of money or anything of value, directly or indirectly, to government officials, political parties or candidates for foreign political office for the purpose of conducting business. Contractor shall maintain books, records and accounts, which, in reasonable detail, accurately and fairly reflect the transactions and dispositions of payments provided by SmithBucklin to Contractor pursuant to this Contract.

(b) Contractor represents and warrants that to the best of Contractor's knowledge and belief, that Contractor:

(i) is not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any U.S. Federal department or agency;

(ii) has not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a U.S. public (Federal, State or Local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(iii) is not indicted or otherwise criminally or civilly charged by a U.S. Government entity (Federal, State or Local) with commission of any of the offenses enumerated in Article 8(b)(ii) of this Contract; and

(iv) has not within a three-year period preceding the date of this Contract, had one or more U.S. public transactions (Federal, State or Local) terminated for cause or default.

Article 10. *<u>Miscellaneous</u>*

(a) Signature of this Contract by Contractor constitutes acceptance of all conditions and terms. This Contract supersedes all prior arrangements between SmithBucklin and Contractor on this subject matter, if any, and shall continue for the duration of this Contract. This Contract shall be the entire agreement of the parties and any modification must be made in writing and signed by both parties.

(b) The headings contained in this Contract have been inserted for convenience of reference only and shall not be deemed to be a part of this Contract and shall in no way affect the interpretation of this Contract.

(c) Affirmative Action Notice: SmithBucklin is an Equal Opportunity Employer M/F/D/V.

Additional Articles – End Result Contract

Article 11 - <u>Logo</u>

Notwithstanding the terms of Article 6, SmithBucklin must not and must not allow any other party, to use the name Industry & Investment NSW or any other derivative thereof, or any trademark or logo of Industry & Investment NSW.

Article 12 – Goods and Services Tax

a. Unless otherwise defined in this Article, words or expressions used in this Article have the same meaning as defined in the *A New Tax System (Goods and Services Tax) Act 1999* of Australia ("GST law").
b. Unless otherwise indicated all amounts referred to in this contract are inclusive of GST.

[SIGNATURES ON FOLLOWING PAGE]

IN WITNESS WHEREOF, THE PARTIES HERETO HAVE EXECUTED THIS CONTRACT ON THE DATES SHOWN BELOW.

SMITHBUCKLIN CORPORATION
16305 Swingley Ridge Road, Suite 120
Chesterfield, MO 63017

By:

Mark Winkle
Senior Director of Domestic Programs

Date: 2/11/11

Industry & Investment NSW
Locked Bag 21, 161 Kite Street
Orange, New South Wales
Australia 2800

By:

A/ED APISR
Alison Bowman.

Date: 14.1.11

ND: 4815-5990-2210, Ver 1

ATTACHMENT A

Approved Project

New Uses FY2011

Target Area: Animal Utilization

Project Title: Asian seabass: validation of commercial grow-out feeds containing optimal levels of SBM and SPC and impacts of feed attractants

Project Number: 1463

Contractor Name: Industry & Investment NSW

Contact Name: Dr Mark Booth / Dr Geoff Allan

Contact Address: Locked Bag 1, Nelson Bay, NSW 2315, Australia

Contact Phone Number: +61 2 4916 3816

Contact E-mail: Mark.Booth@industry.nsw.gov.au

Project Overview:

Introduction

The New Uses Committee of the United Soybean Board has invited Industry & Investment NSW (I&I NSW) to submit a funding proposal for conducting applied research on Asian Sea Bass beginning on January 1, 2011.

Specific research objectives requested by USB include:

- Expand the current formulation data base developed for this species in USB projects 9463 and 0463 by identifying potential ingredients available for feed manufacturing in key target markets of South East Asia.
- Evaluate the digestibility and & utilisation of key feed ingredients identified from SEA.
- Understand the impacts of high dietary inclusion levels of SBM and/or SPC on the production and quality of modern extruded aquafeeds for Asian seabass
- Improve the understanding of formulation flexibility for feeds with high contents of SBM/SPC.
- Investigate the use of feed attractants to stimulate feed intake in diets containing high levels of SBM or SPC (particularly for SPC).

- Conduct a commercial grow-out verification trial with optimum SBM & SPC formulation/s.

- Conduct an economic feasibility analysis of alternative soy based diets compared to the traditional commercial Asian seabass diet.

These new objectives are designed to build on successful research conducted on Asian seabass for the USB by I&I NSW in FY 2009 (Project # 9463) and FY 2010 (Project # 0463). The aforementioned research objectives represent a large quantum of work which I&I NSW feels is best suited to a sustained approach over a two year period targeting ingredient review and digestibility plus attractants in the first year (FY 2011; activities 1-3) and utilization and commercial verification trials in the following year (FY 2012; activities 4-6). We propose dividing the research into the following basic components:

1) Desk-top research related to expanding the current feed ingredient formulation data base for Asian seabass and improving knowledge of the impacts of high inclusion levels of SBM and or SPC on the manufacture of extruded pellets

2) Determine digestibility of key complimentary feed ingredients from ASA International Marketing (IM) identified target markets in South East Asia such as Thailand, Philippines, Indonesia, Vietnam or Malaysia.

3) Identification and evaluation of suitable feed attractants to improve feed intake of diets with high levels of SBM or SPC by Asian seabass.

4) Conduct a laboratory based utilisation trial to examine reliability of new apparent digestibility coefficients in formulating aquafeeds for Asian seabass on a digestible protein and digestible energy basis

5) Conduct a grow-out verification trial at a commercial farm to evaluate the production and economic performance of Asian seabass reared on commercially manufactured diets with optimised levels of SBM and SPC.

Please note:

- I&I NSW has recommended a programme of research that it believes is the most appropriate strategy for achieving the aims of USB. However I&I NSW recognise that the USB fund research on an annual basis and that our proposal to conduct research over a two year time frame may not match the strategic needs of the USB. For this reason I&I NSW is prepared to conduct different components of the proposed research according to the priorities of USB. As such the estimated cost of each component is presented separately in the budget. There is some scope to reduce costs for some components (e.g. by reducing number of ingredients for which digestibility is determined). All budgeted items are maximum values and USB will be charged on basis of actual costs up to this amount.
- This proposal has been submitted but remains to be approved by Industry & Investment NSW
- This proposal has been submitted based on an exchange rate of $AUD 1.00 = $US 0.98 on 22.10.2010. At each billing period the amount invoiced (billed) will be based on costs incurred in Australian dollars and converted to the equivalent

amount in US dollars at that time. Significant further appreciation of the Australian dollar against the US dollar will require either additional funds or a reduction in the scope of the work proposed.

- I&I NSW have contacted Lukas Manomaitis (Technical Director Aquaculture, American Soybean Association International Marketing Program, Bangkok, Thailand) for advice on conducting verification trials in the Philippines. The indicative cost of these trials has been estimated, but has not been included in the current submission. For more detail please refer to the section subtitled ***Potential of conducting verification trials with Asian seabass in the Philippines.*

Project Start Date:	January 1, 2011
Project End Date:	Year 1 - December 31, 2011; Year 2 – December 31, 2012
Project Funding Request:	FY11 - $171,746 FY12 - $182,293
Other Cooperators:	Mr Nick Arena (Tailor Made Fish Farm, Bob's Farm, NSW, Australia) Dr Richard Smullen (Ridley Aquafeed Pty Ltd, Narangba, Qld, Australia) Lukas Manomaitis (Technical Director Aquaculture, American Soybean Association International Marketing Program, Bangkok, Thailand)

Performance Measures:

- I&I NSW will identify potential ingredients available for aquafeed manufacturing in key target markets of South East Asia and evaluate the digestibility and utilisation of priority ingredients.
- I&I NSW will expand the current formulation data base for Asian seabass by including new data on the apparent digestibility from additional feed ingredients and where available from any new data in peer reviewed literature.
- I&I NSW will provide advice on the potential impacts / benefits of high dietary inclusion of SBM and/or SPC on the production and quality of modern extruded aquafeeds which will improve understanding of formulation flexibility in feeds designed for Asian seabass.
- I&I NSW will investigate the use of several commercial feed attractants and provide recommendations on the most suitable attractants for use in diets containing high levels of SBM or SPC (particularly for SPC).
- I&I NSW will conduct and report on a commercial grow-out verification trial with diet/s containing optimum levels of SBM & SPC.
- I&I NSW will report on the economic feasibility of alternative soy based diets compared to traditional commercial diets for Asian seabass.

Activities:

Background on USB research conducted by I&I NSW in FY 2009 and FY 2010

Industry & Investment NSW has conducted applied nutritional research with Asian seabass for the United Soybean Board (USB) New Uses Committee in FY 2009 and FY 2010. Research finalised in FY 2009 provided USB with key information on the digestibility of SBM and SPC at two dietary inclusion levels (30% and 50%) as well as the digestibility of key ingredients such as fishmeal (Table 1).

In addition, I&I NSW collated a comprehensive ingredient formulation data base for Asian seabass from peer reviewed literature which provided invaluable material for commercial diet formulation. This new data base was used by I&I NSW in FY 2010 to formulate and manufacture 8 extruded feeds containing high levels of SBM and SPC, each formulated on a digestible protein and energy basis to meet the theoretical protein and energy requirements of Asian seabass weighing between 100 and 500g. These test feeds, as well as a control feed based on fishmeal and a commercially manufactured diet were fed to Asian seabass reared in saltwater tanks for a period of 8 weeks with the aim of comparing weight gain, feed intake, feed conversion ratio (FCR), protein and energy retention and impacts on gut health (Table 2).

Table 1. Reproduced from "Formulation of aqua-feeds for Asian seabass *Lates calcarifer* containing optimal inclusion levels of SBM and SPC USB Project FY2009 SB9463"

Apparent digestibility coefficients (ADC) of nutrients, amino acids and gross energy of test ingredients fed to Asian seabass (%).

Test diet	SBM-30%	SBM-50%	SPC-30%	SPC-50%	Fishmeal-30%
Dry matter	60.1±0.4^{b}	45.0±2.6^{a}	74.4±1.0^{c}	74.9±2.1^{c}	98.2±4.2^{d}
Protein	88.9±2.3^{ab}	83.2±1.2^{a}	95.9±0.7^{b}	95.6±0.4^{b}	95.6±2.6^{b}
Energy	71.7±3.1^{b}	62.8±1.4^{a}	81.3±1.1^{c}	83.4±0.9^{c}	105.0±3.3^{d}
Total fat	79.8±12.6^{a}	67.8±4.4^{a}	na	na	98.9±1.7^{a}
Alanine	92.9±1.6	83.3±3.2	100.1±2.3	97.5±0.5	104.0±2.8
Arginine	97.1±0.4	96.5±1.2	102.9±1.6	98.2±0.1	99.7±1.8
AsparticAcid	92.9±0.1	85.0±1.8	94.0±3.2	90.0±0.2	100.1±3.3
Cystine	91.2±0.9	78.0±0.0	99.5	98.4	97.9±5.1
GlutamicAcid	96.7±0.3	87.5±4.4	96.5±1.2	96.2±0.4	101.2±2.3
Glycine	88.9±0.3	81.6±2.7	98.3±3.4	95.4±0.3	100.1±2.2
Histidine	87.5±1.3	84.6±2.5	103.2±1.5	97.7±0.8	100.4±2.0
isoLeucine	90.8±0.1	90.3±0.4	94.6±1.4	96.2±0.1	98.8±1.5
Leucine	93.6±1.1	84.5±2.0	96.7±2.0	101.1±0.1	104.8±3.1
Lysine	92.3±1.2	88.7±2.2	95.6±1.6	97.0±0.2	101.2±2.0
Methionine	101.1±2.6	101.8±0.1	96.4	98.5	99.4±0.7
Phenylalanine	93.1±0.6	87.7±1.7	99.0±1.4	101.7±0.2	105.5±3.1
Proline	96.5±0.6	88.6±3.2	97.7±2.6	100.1±0.1	103.5±2.7
Serine	87.8±0.4	85.1±1.8	95.0±2.4	96.3±0.2	101.6±3.4
Threonine	89.1±1.2	84.0±1.6	96.5±2.9	97.0±0.3	101.6±2.8
Tryptophan	83.0±0.4	82.5	77.7	-	-
Tyrosine	99.0±0.2	87.6±1.5	93.0±1.7	101.4±0.0	99.1±1.9
Valine	88.1±1.1	81.9±3.0	97.8±3.0	100.0±0.2	104.3±3.5
Average of AA's	92.3	86.6	96.4	97.8	101.4

For proximate data, different superscript letters indicate a significant difference between mean ADC's within each row (One-way *ANOVA*; SNK).

At the time of this application I&I NSW has completed the growth assay and submitted carcass samples for proximate analysis and tissue samples for histological examination. Data on weight gain, feed intake and feed conversion indicate that the 8 test feeds we formulated performed extremely well (Table 3). Although there was some reduction in feed intake of seabass fed diets with high inclusion levels of SPC, there was in fact little variation in the FCR of these feeds compared to feeds promoting the highest weight gains. This indicated that the utilisation of all feeds by fish was similar and that low feed intake of diets high in SPC was the major driver of reduced weight gain in these treatments. This result demonstrated there were palatability issues with diets high in SPC and low in fishmeal and that if increased intake could be promoted similar weight gains could be expected.

The results of research conducted in FY2009 and FY2010 have confirmed that digestibility coefficients for SBM, SPC, fishmeal and other ingredients can be used to successfully formulate feeds for Asian seabass designed to test the upper inclusion levels of SBM and SPC under a similar plane of nutrition. The similarity in feed utilisation demonstrates that our strategy of formulating feeds on a digestible protein and energy basis to match the specific growth stage we investigated was successful and underpins the premise that once the digestibility of ingredients are known the risk of undersupplying nutrient requirements in formulated feeds is reduced. Greater insight into these results will be available once protein and energy retention data are available.

Table 2. Reproduced from "Formulation of aqua-feeds for Asian seabass *Lates calcarifer* containing optimal inclusion levels of SBM and SPC USB Project FY2009 SB9463"

Diet formulations used to test extreme levels of soybean meal (SBM), soy protein concentrate (SPC) or blends of SBM and SPC on performance of Asian seabass.

Ingredient	Fish Meal	SBM	SBM	SBM	SPC	SPC	SPC	SBM+SPC	SBM+SPC
%	Control	30	36	38	40	50	60	25/25 Blend	30/30 Blend
Fish oil	10.0	10.0	10.0	10.4	10.0	10.0	10.0	10.7	11.9
Fishmeal	55.0	40.6	38.3	40.6	10.0	10.0	10.0	14.4	16.0
SBM	-	30.0	35.5	38.0	-	-	-	25.0	30.0
SPC	-	-	-	-	40.0	50.0	60.0	25.0	30.0
Wheat	14.7	10.0	10.0	10.0	15.9	14.4	12.9	10.0	10.0
Meat meal	10.0	9.1	-	-	-	-	-	-	-
Poultry meal	10.0	-	5.9	0.7	23.6	15.0	6.5	14.4	1.4
L-meth.	0	-	-	-	0.2	0.3	0.3	0.2	0.2
Vit/min	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Est. analysis (as is basis)									
DP %	45	44.7	44.2	44.2	45.4	47.3	49.3	44.2	44.2
DE MJ/kg	18	17.2	17.0	17.0	17.0	17.0	17.0	17.0	17.0

The outcomes of FY2010 research conducted for USB New Uses by I&I NSW were presented to a joint meeting of the U.S Export Council (USB; USSEC; ASA IM) at the Global Soy in Aquaculture Stakeholders Meeting Aug 30- Sept 10 in Campbell River, British Columbia. At this meeting a representative of I&I NSW also made recommendations on further research that would enhance the inclusion of SBM and SPC in commercial diets for Asian seabass. These recommendations and objectives are detailed in the project overview. The activities required to address the specific objectives are detailed below.

Table 3. FY2010 Performance of Asian seabass reared in saltwater systems and fed extruded feeds formulated as per Table 3 for a period of eight weeks.

Diet type	Stock weight (g)	Harvest weight (g)	SGR (%/d)	Daily gain (g/d)	Daily intake (g/d)	FCR	Feed efficiency (%)

Commercial	117.9	324.9	1.81	3.70	4.03	1.09	91.7
sem	1.1	6.4	0.0	0.1	0.1	0.0	1.1
Fishmeal	121.2	379.7	2.04	4.62	4.22	0.92	109.4
sem	2.2	11.0	0.0	0.2	0.1	0.0	1.7
SBM 30	117.7	361.4	2.00	4.35	4.16	0.96	104.7
sem	1.7	9.4	0.0	0.1	0.1	0.0	0.8
SBM 35	116.6	364.0	2.03	4.42	4.37	0.99	101.0
sem	1.8	6.6	0.0	0.1	0.1	0.0	0.9
SBM 38	120.4	350.6	1.91	4.11	4.15	1.01	99.0
sem	1.4	1.9	0.0	0.0	0.0	0.0	0.4
SPC 40	117.3	304.4	1.70	3.34	3.46	1.04	96.6
sem	1.5	5.2	0.0	0.1	0.1	0.0	0.6
SPC 50	116.6	285.7	1.60	3.02	3.34	1.11	90.2
sem	1.8	11.4	0.1	0.2	0.1	0.0	2.0
SPC 60	118.2	279.3	1.54	2.88	3.31	1.15	86.8
sem	1.3	2.6	0.0	0.1	0.1	0.0	0.1
SBM:SPC 25	116.9	318.4	1.79	3.60	3.81	1.06	94.5
sem	3.3	8.6	0.0	0.1	0.1	0.0	0.7
SBM:SPC 30	116.0	294.7	1.66	3.19	3.65	1.15	87.2
sem	2.6	7.9	0.0	0.1	0.1	0.0	1.1

Proposed FY 2011 Research

I&I NSW propose to divide the requested research into 6 activities with each activity achieved through a mix of practical applied research and detailed desk-top review. In this way new peer reviewed literature on SBM and SPC as well as research on Asian seabass will be captured and the research effort applied to practical studies can be maximised.

Activity 1) Desk-top research related to expanding the current feed ingredient formulation data base for Asian seabass and improving knowledge of the impacts of high inclusion levels of SBM and or SPC on the manufacture of extruded pellets

In order to increase the use of SBM and SPC in diets for Asian seabass more information is required on the digestibility and utilisation of the range of feed ingredients for this species. In earlier work for USB, I&I NSW identified there was a lack of reliable data on the digestibility of alternative feed ingredients, especially with regard to carbohydrate sources and amino acid availability of protein sources. To maximise use of soybean products, knowledge of digestibility and utilisation of other components in the diet must also be known so formulations can match requirements. Expansion of the current feed ingredient data base to include data on digestible amino acids will greatly improve the usefulness of this resource and greatly increase the probability of formulation success.

Work will be undertaken to thoroughly investigate the latest peer reviewed literature on SBM and SPC and review nutrition work undertaken on Asian seabass by other agencies and universities with links to I&I NSW. Reliable data will be included in the expanded ingredient formulation data base providing greater flexibility for aquafeed manufacturers using SBM and SPC as staple feed ingredients in their aquafeeds.

High inclusion levels of SPC affected the pellet characteristics of test diets manufactured using extrusion technology in FY2010 project #0463. Feedback from the mill staff indicated that control of bulk density was difficult. SPC diets also had small pellet diameters compared to control feeds indicating problems with expansion. In addition, SPC diets produced a high level of fines indicating a lack of binding. Increasing the amount of SBM and SPC used in aquafeed formulations will impact on pellet quality due to the functional properties of these ingredients and their interaction with other feed ingredients, particularly starch sources. In addition, the physical qualities of the finished product are affected by the diverse range of operating conditions available to or selected by the extrusion manager.

A literature review will be undertaken to investigate the impacts of high SBM and SPC inclusion on pellet quality of aquafeeds and to help understand how these ingredients work and interact with other feed ingredients. The outcomes of this work will provide invaluable information on the most suitable complimentary feed ingredients for use with SBM and SPC in diets for Asian seabass. This information may be suitable for use in targeted workshop presentations to feed formulators and aquafeed manufacturers seeking to increase use of SBM and SPC in aquafeeds.

Activity 2) Evaluation of key complimentary feed ingredients from ASA International Marketing (IM) identified target markets in South East Asia such as Thailand, Philippines, Indonesia, Thailand, Vietnam or Malaysia.

- *Digestibility of complimentary ingredients from SEA*

I&I NSW successfully determined the digestibility of SBM and SPC in previous work for USB (FY2009 SB9463). Through links with ASA IM contract staff in SEA (e.g. Lukas Manomaitis; Levy Manalac), key SEA feed ingredients, quality and quantities will be identified. Key SEA ingredients will be selected and imported from SEA to Australia (subject to Australian quarantine laws) for evaluation in a digestibility trial. The number of key ingredients to be evaluated is yet to be determined, but we envisage a list of approximately 7-10 ingredients is likely (N.B. should importation of key SEA ingredients be prohibited ingredients with similar composition will be obtained within Australia). The digestibility of key SEA ingredients will be determined using similar indirect techniques to that used in earlier work which relies on feeding test diets which contain a mixture of a reference diet, the test ingredient and an inert marker (e.g. chromium; ytterbium). These diets are then fed to fish for an appropriate period before faecal material is collected from the distal intestine using stripping or dissection techniques. Appropriate chemical analyses of ingredients, diets and faecal material followed by application of suitable calculations will allow determination of diet and ingredient digestibility coefficients. Results from this strategy / study will enhance the current feed formulation data base and provide data and results directly applicable to the SEA region as well as increase formulation options and certainty.

Experimental methods will be similar to those employed in FY2009 USB research. In short, digestibility trials will be conducted with Asian seabass weighing approximately 200-500g. Fish will be obtained from a commercial farm, size graded and stocked into experiment tanks containing saltwater at the appropriate density. Water quality (temperature, salinity, pH, dissolved oxygen, total ammonia-nitrogen etc.) will be recorded daily and adjusted to ensure the range of each parameter is suitable for fish culture. An ambient photoperiod will be adopted. A single batch of commercial Asian seabass feed will be used as the reference diet. After grinding, the reference mash will be thoroughly dry mixed with the marker. This mixture will then be combined with the test ingredients, thoroughly mixed and formed into pellets. Test diets will be fed to duplicate / triplicate groups of Asian seabass for a minimum of 14 days prior to collection of faecal material. After this time fish will be anaesthetized and digested material will be recovered from the distal portion of the intestine using stripping techniques. Faecal material will be collected from individual fish and pooled within tank (tank as replicate) until sufficient material for is obtained analysis. Material will be immediately chilled and stored frozen at < -15C prior to moderate oven drying.

All diets, ingredients and collected faecal material will be outsourced for chemical analyses (outsourced to DEEDI; formerly QDPI&F, Queensland, Australia). Samples will be analyzed for moisture, gross energy (bomb calorimetry), crude protein (Nx6.25), lipid, ash and amino acids (providing enough faecal material can be collected). The additive nature of ADC's for ingredients will be ascertained using modern calculations that account for the nutritive value of the ingredients as well as the reference diet.

Activity 3) Evaluation of suitable feed attractants to improve feed intake of diets high in SPC by Asian seabass.

The utilisation of feeds that contained high levels of SPC (e.g. 30, 40 or 50%) by Asian seasbass was similar to test diets containing SBM, a fishmeal control or a commercial Asian seabass feed. However, weight gain was reduced due to concomitant decreases in feed intake. This indicated a reduction in the palatability of these feeds, most likely due to low levels of fishmeal (10%) and extreme levels of SPC. The use of feed attractants may overcome problems with palatability and induce increased feed intake of diets high in SPC.

I&I NSW will obtain a selection of 3-5 commercially available feed attractants and incorporate these attractants on experimental feeds fed to Asian seabass. Test diets high in SPC and low in fishmeal will be prepared so they have similar formulations to those presented in Table 2. Half the manufactured quantity of each test diet will be top-coated with a feed attractant and the remaining half will be unaltered. Diet pairs (e.g. with and without attractant) will be fed to triplicate groups of 100g Asian seabass sourced from the same supplier, stocked into the same experimental system and reared using the same procedures as those described in Activities Section 2.

Key performance measures such as weight gain, FCR and feed intake will be determined at the conclusion of this trial which will allow identification of the most appropriate feed attractants for use with Asian seabass. Chemical analysis of up to 10 ingredients and 10 experimental diets will be undertaken, but comparative slaughter analyses will not be required for this experiment.

Proposed FY 2012 Research

Activity 4) Evaluation of key complimentary feed ingredients from ASA International Marketing (IM) identified target markets in South East Asia such as Thailand, Philippines, Indonesia, Thailand, Vietnam or Malaysia.

- *Utilisation of key SEA ingredients; reduction or elimination of fishmeal in high SBM diets*

Research undertaken in FY2010 showed that Asian seabass will tolerate high dietary levels of SBM (35%) in diets that contain relatively high amounts of fishmeal (40%). A better understanding of the nutritional quality of alternative ingredients will result in significant reductions in fishmeal level while allowing high dietary levels of SBM and SPC to be maintained. This objective meets ASA IM's goal of sustainable aquaculture production through increased use of soy.

Data from the digestibility experiment on key SEA ingredients will be used to design and formulate a series of diets to test the effect of reducing fishmeal content while holding the inclusion level of SBM at levels tested in FY2010 project 0463. Fishmeal will be replaced on a digestible nutrient basis with suitable SEA ingredients (or similar ingredients available in Australia) tested in the digestibility trial.

As in previous research, our approach will be to formulate (on a digestible nutrient basis) a basal diet based predominantly on fishmeal that meets the digestible protein and energy requirements of 100-500g fish. This diet will be trialled against diets with similar formulations to those tested in FY2010 project#0463, but where fishmeal content is reduced or eliminated in favour of SEA ingredients. In this way, the dietary level of SBM can be maintained and the level of fishmeal reduced while maintaining a single plane of nutrition. The overall design would include 8-10 diets including controls and a commercial feed. The most successful attractant from Component 3 (above) may be included is a limited number of diets to help discriminate ingredient impacts of utilisation from feed intake.

Following formulation, test diets for the growth trial will be mixed and batched (e.g. 200 kg of each diet) then transported to an experimental extrusion facility for manufacture into suitable pellets (e.g. outsourced to SARDI - Australasian Experimental Stockfeed Extrusion Facility, South Australia or similar). The intention is to make test diets that closely resemble the physical quality of commercial Asian seabass feeds. The 8-10 dietary treatments will be fed to replicate groups of Asian seabass (e.g. initial weight of 120 g) for about 8-10 weeks to assess the utilization of experimental feeds. A minimum of 3 replicate tanks will be provided for each dietary treatment.

Fish will be sourced from a commercial Asian seabass farm (Tailor Made), carefully size graded and stocked into experiment tanks (e.g. 200L). A representative sample of initial fish will be euthanased and frozen (<-15C) for chemical composition.

Fish will be carefully hand fed experimental diets to apparent satiation twice daily at predetermined times (e.g. 0900h and 1500h). The trial will be run for approximately 8-10 weeks. Fish will be weighed at stocking, after 4 weeks and at harvest to assess changes in weight gain, feed intake, feed conversion and condition factor. Fish will be starved 24h prior to these procedures. At the conclusion of the trial, a representative sample of fish from each treatment will be euthanized and frozen for chemical analyses.

Water quality (temperature, salinity, pH, dissolved oxygen, total ammonia-nitrogen etc.) will be recorded daily and adjusted to ensure the range of each parameter is suitable for fish culture. Experiment tanks will be cleaned and siphoned regularly to maintain tank hygiene. An ambient photoperiod will be adopted.

All ingredients and diets will be prepared and outsourced for chemical analyses. Samples will be analysed for moisture, gross energy (bomb calorimeter), crude protein (Nx6.25), lipid, ash and amino acids. Carcass samples will be analysed only for proximate composition.

Activity 5) Conduct a grow-out verification trial at a commercial farm to evaluate the performance of Asian seabass reared on commercially manufactured diet/s with optimised levels of SBM and SPC.

I&I NSW will formulate 1-2 diets that contain optimized levels of soybean meal, SPC and other key ingredients identified in the earlier stages of this project for use in an on-farm verification trial. These diets will be formulated in consultation with representatives from USB, Ridley Aquafeed Pty Ltd. and SEA. The "optimized feeds" feed/s will be validated against commercially available feeds for Asian seabass (n.b. diet yet to be determined).

A suitable quantity of the new formulation/s will be batched and manufactured at either the SARDI - Australasian Experimental Stockfeed Extrusion Facility, South Australia or Ridley Aquafeed's Narangba Plant, Qld (or in SEA). The dietary treatments will be fed to replicate groups of Asian seabass (initial weight of 50-100g) for a period of approximately 3 months to assess standard performance indices including weight gain, feed conversion ratio, feed intake, and survival. Changes in carcass composition of Asian seabass will be assessed by evaluating the proximate composition of fish at stocking against carcass samples taken at or near harvest (e.g. comparative slaughter).

Asian seabass will sourced from a commercial Asian seabass farm (e.g. Tailor Made, Bobs Farm, NSW Australia; farm in SEA), carefully size graded and stocked into large replicate commercial tanks or cages (number of tanks and stocking density to be decided after consultation with commercial farm; minimum of 2 replicates per diet).

Fish will be handled and fed by farm staff according to typical on-farm procedures in order to ensure results are based on those generated from a commercial facility. However, scientists from I&I NSW (or there representative, e.g. SEA group) will conduct regular and ongoing supervision of progress and review of data to ensure validity and rigueur of results. This will be via regular visits and liaison with the farm managers and their technical staff. I&I NSW staff will supervise and participate in stocking, weight check and harvest procedures. As the envisaged field trial will be conducted at a site remote from I&I NSW research premises additional funds will be required to employ a suitably qualified technician on a part-time basis to manage the day-to-day experimental duties related to this experiment (not if trial in SEA).

Fish will be weighed individually at stocking and then every 4-6 weeks (sub sampled) to monitor progress. Fish will be starved 24h prior to these procedures. Water quality will vary according to day-to-day operations at the commercial farm, however, temperature, salinity, pH, dissolved oxygen and total ammonia-nitrogen will be recorded at regular intervals throughout the trial. An ambient photoperiod will be adopted.

All ingredients, diets and carcass samples will be collected, prepared and outsourced for chemical analyses I&I NSW staff. Samples will be analyzed for moisture, gross energy (bomb calorimeter), crude protein (Nx6.25), lipid, ash and amino acids.

Special considerations for on-farm validation trials in Australia

Conducting validation / verification field trials in a commercial facility requires the support of the farms proprietor (e.g. Mr Nic Arena; other if SEA) and the use of his grow-out facilities and staff. Normally, the farm proprietor agrees to commit a proportion of his facilities and available fish stocks to new diet trials on the understanding that these trials will not expose him to significant economic loss. For example, if fish grown on a new diet formulation perform poorly compared to fish reared on benchmark diets or that use of his facilities and staff place increased pressure on the overall day-to-day operation of his farm. These risks represent an "opportunity cost" to the participating farm that is usually underwritten by the research provider to ensure the commercial operation is not penalized financially for participating in the research. The value of this lost opportunity, if in fact it is materialized, is normally arrived at by calculating the difference between the overall income received for fish reared on a standard industry diet and those reared on the new formulation. Consequently we have included $US10,000 as a contingency fee. This will not be charged if, as expected, the fish fed the new soy based formulation during the trial perform as well as those fed the existing commercial diets used by the farmer.

*****Potential of conducting verification trial with Asian seabass in the Philippines***

Prior to submitting this proposal to USB, I&I NSW was able to contact Mr Lukas Manomaitis (Technical Director Aquaculture, American Soybean Association International Marketing Program, Bangkok, Thailand; phone contact on the *21.10.2010*) to discuss opportunities for running commercial verification trials in SEA. Through his links in the Philippines, Mr Manomaitis was able to identify a potential farmer collaborator that may be interested in participating in an Asian seabass verification trial. The name of the company is Oversea Corporation, a company which has suitable facilities to run a cage based verification trial (up to 16 research cages in saltwater ; each 3m x 3m x 3m). This company has previously been involved with USB research on pompano. In addition, Oversea has an integrated extrusion mill capable of producing custom aquafeeds of reasonable quality (Lukas Manomaitis; *pers communication 21.10.2010*). Successful research at the Oversea facility may also provide a suitable platform for USB to demonstrate extrusion and feeding of diets containing SBM and SPC to Pilipino farmers (subject to the agreement of Oversea).

Based on the advice of Mr Manomaitis, the likely cost of running a verification trial at the Oversea facility would be close to $US1500 per production run for the hire of 3 research cages. These costs include farm labor. Based on the design detailed above and the production of 4-5 research diets each replicated 3 times, the cost would be approximately %US7500 for the hire and use of the research cages. There is a possibility that the owners of Oversea would produce research diets at no-cost due to their interest in furthering their own knowledge of extrusion and commercial production of Asian seabass.

While this amount is significantly lower than for running a similar trial in Australia, it does not include costs associated with chemical analysis, travel and oversight by collaborators listed on this proposal and other as yet, unforeseen costs. Oversight and rigueur of the verification trial may be achieved through use of a good local technical manager liaising with I&I NSW researchers (Lukas Manomaitis; *pers communication 21.10.2010*).

***Please note the indicative costs of running a verification trial in the Philippines have not been included in the current proposal.*

Activity 6) Conduct an economic feasibility analysis of alternative soy based diets compared to typical commercial Asian seabass diet

I&I NSW will collate data from previous USB research and the research proposed in this document to conduct an economic feasibility analysis of soy based diets for Asian seabass. Primarily this will be done by conducting a series of hypothetical least-cost diet formulations using the expanded formulation data base and combining it with production data (growth rates, FCR, feed cost, sale price), obtained from the verification trial or other suitable information. The outcome of this component will link the nutritional information derived from USB research on Asian seabass to the specific cost of different feed ingredients, allowing derivation of specific economic indicators (e.g. cost per kilogram of fish produced). These indicators can be used by formulators and farm managers to assess the economic cost / benefit of feeding diets high in soy compared to diets formulated with more traditional feed ingredients such as fishmeal.

Reporting and analyses

I&I NSW staff will undertake the statistical design and evaluation of all experiments. They will also be responsible for liaison with industry supporting the research, purchase and sourcing of ingredients, preparing, stocking, weight check and harvest procedures related to the controlled feeding experiments conducted at PSFI. Additional responsibilities include sample preparation, dispatch and review etc. Statistical analyses of the results will ultimately depend on the final design of the experiment, but we envisage that ANOVA or multi-factor ANOVA will be used to compare / assess dietary treatments. Industry & Investment NSW will outsource all chemical analyses of ingredients, diets and whole fish to commercial or government agencies specializing in the analyses of these materials. A report will be prepared that thoroughly details the background, materials and methods and results of experiments. This report will be prepared and provided in confidence to USB.

Risk assessment

Perceived risks associated with this proposal include:

1) failure in supply of fish 2) sudden death of experimental animals due to unforeseen physical or biological factors prior to the completion of experiment 3) problems with biochemical analyses 4) difficulties associated with procurement of SBM, SPC or other key ingredients in Australia 5) problems associated with extrusion of experimental feeds.

Contingencies:

1) Fish will be sourced from a reputable hatchery / nursery. There have been no problems in supply of Asian seabass over the last five years.

2) Careful attention will be paid to fish health and husbandry during the trial. No significant disease issues or unexplained mortalities with Asian seabass have been experienced over the last five years.

3) Biochemical analyses will be outsourced to reputable nationally accredited laboratories that have been used extensively for similar analyses by Industry & Investment NSW in the past.

4) SBM, SPC and other key ingredients (origin to be decided) will be sourced from existing importers in Australia in consultation with USB. If importation of key SEA ingredients is prohibited, similar ingredients will be sourced from within Australia where possible.

5) Batching and extrusion of experimental feeds will be outsourced to a pilot scale extrusion facility (SARDI) or to a commercial mill (Ridley Aquafeed Pty Ltd). To minimize risks with inaccurate blending and mixing of ingredients after formulation, preparation, production and traceability of experimental diets, Industry & Investment NSW will supervise all aspects of diet manufacture.

FY11 Budget $US

Total Project Budget

$171,746	**DETAILED DESCRIPTION**
Personnel/Contractual Services $111,128	**Billing rates and qualifications of personnel*.**

Industry & Investment NSW Research Leader = $270.73/hour (PhD qualified 25y experience, extensive publication list, world recognized industry leader and consultant, contract liaison with Smith-Bucklin and USB, Departmental liaison, contract approval, supervision of research scientists, host representatives of USB, experimental design etc.).

Industry & Investment NSW Research Scientist Level 6 = $165.47/hour (PhD qualified, well published in field of expertise, highly experienced in experimental design and interpretation, plan lead and supervise research programs and projects, supervise staff, supervise students etc, liaison with collaborators, liaison with Smith-Bucklin and USB).

Industry & Investment NSW Fisheries Technician Grade 2 = $94.70/hour (Bachelor of Science (degree) qualification with at least 5 years experience in all aspects of fish husbandry, water quality, maintenance and construction of small scale laboratories, record keeping, ability to work unsupervised and lead others, data management, ordering and purchasing, logistics etc. Ability to write technical and non-technical reports).

Tailor Made Farm Proprietor = $165.47/hour
Owner operator of registered 60-90 metric tonne per annum Asian seabass (barramundi) recirculating aquaculture enterprise, Bobs Farm NSW, Australia

Tailor Made Fisheries Technician = $45/hour
(Trade or higher qualification, experienced in daily management and husbandry of commercial scale Asian seabass farm, work unsupervised, lead and supervise subordinate staff, highly developed skills in maintenance, repair and construction of aquaculture systems, record keeping, purchasing, sales).

* Please note: Salary rates for Industry & Investment NSW cover all facility costs, infrastructure costs (e.g. power, telephone), and all administration (i.e. financial services, legal and insurance, staff management, etc).

<u>Proposed FY 2011 Research</u>

Activity 1) Desk-top research - expanding the current feed ingredient formulation data base and improving knowledge of the impacts of high inclusion levels of SBM and or SPC on the manufacture of extruded pellets

Salaries for 2 staff ; 2 x 3 week blocks for Res. Scientist and approximately 3 days for Res. Leader.

Salary for I&I NSW Research Leader for 24 hours = $6,498
Salary for I&I NSW Research Scientist for 210 hours = $34,749

Activity 2) Evaluation of key complimentary feed ingredients from SEA – Digestibility trial

1 week set up, 2 week acclimation, 4 week faecal collections, 1 week clean up. Testing of up to 8 ingredients, 9 diets, 3 reps per treatment = 27 tanks. Salaries for 3 staff; Approximately 60% Fish Tech, 40% Res. Scientist & 2% Res. Leader.

Salary for I&I NSW Research Leader for 6 hours = $1,624
Salary for I&I NSW Research Scientist for 112 hours = $18,533
Salary for I&I NSW Fisheries Technician 168 hours = $15,910

Activity 3) Evaluation of suitable feed attractants to improve feed intake of diets high in SPC

1 week set up, 6 week experiment, 1 week clean up. Up to 10 treatments, 3 reps per treatment = 30 tanks. Salaries for 3 staff; Approximately 70% Fish tech, 30% Res. Scientist & 2% Res. Leader.

Salary for I&I NSW Research Leader for 5 hours = $1,354
Salary for I&I NSW Research Scientist for 84 hours = $13,899
Salary for I&I NSW Fisheries Technician 196 hours = $18,561

FY11 Total - $111,128

Subcontractors

$25,355

Chemical analyses of ingredients, diets, faeces or whole fish carcass will be required for **Activities 2, 3, 4 & 5**. Chemical analysis will be outsourced to specialist providers used in previous USB projects. The following table lists indicative costs for individual chemical analyses.

TEST	COST $US
Grinding	11.52
Freeze drying	26.18
Dry matter	6.39
Crude protein	28.59
Gross energy	42.94
Fat (ether)	22.52
Ash	6.39
Handling fee	2.09
Amino acids	312.12
Marker (e.g. chromium)	36.66

Thus:

Proximate analysis on a per sample basis (includes handling fee) = $146.63

Amino acid analysis on a per sample basis = $312.12
Marker analysis on a per sample basis = $36.66

Proposed FY 2011 Research

Activity 2) Evaluation of key complimentary feed ingredients from SEA – Digestibility trial

Chemical testing of up to 8 ingredients, 9 diets, 3 reps per treatment = 27 tanks.

Proximate analysis ($146.63 per sample)
10 x ingredient samples = $1,466.30
9 x diet samples = $1,319.67
27 x faecal samples = $3,959.01

Sub-total for 46 samples = $6744.98

Amino acid analysis ($312.12 per sample)
10 x ingredient samples = $3121.20
9 x diet samples = $2,809.08
27 x faecal samples = $,8427.24

Sub-total for 46 samples = $14,357.52

Marker analysis ($36.66 per sample)
9 x diet samples = $329.94
27 x faecal samples = $989.82

Sub-total for 36 samples = $1,319.76

Total chemical costs for Activity 2 = $22,422.26

Activity 3) Evaluation of suitable feed attractants to improve feed intake of diets high in SPC

Chemical testing of up to 10 feed ingredients (including attractants) and 10 diets used in attractant study.

Proximate analysis ($146.63 per sample)
10 x ingredient samples = $1,466.30
10 x diet samples = $1,466.30

Sub-total for 20 samples = $2,932.60

Total chemical costs for Activity 3 = $2,932.60

FY11 Total - $25,355

Meetings & Conferences $0	Not applicable
Travel	***Proposed FY 2011 Research***
$4,000	**Activity 2) Evaluation of key complimentary feed ingredients from SEA – Digestibility trial**

	Travel / airfares and accommodation for I&I NSW Res. Scientist to SEA to liaise with ASA IM contractor and ingredient suppliers to identify and source potential feed ingredients for Asian seabass etc. $4,000. **FY11 Total - $4,000**
Printing $0	Not applicable
Postage $0	Not applicable
Equipment Rental $0	Not applicable
Other Operating Expenses	***Proposed FY 2011 Research***
$31,263	**Activity 2) Evaluation of key complimentary feed ingredients from SEA – Digestibility trial** Purchase of 200 sub-adult fish = $800.00 Purchase of feed ingredients and markers for diet making = $3,000.00 Purchase of commercial feed = $250.00 Freight and or shipping costs associated with movement of new research ingredients from South East Asia or within Australia = $5,000.00 Miscellaneous costs associated with purchase and use of chlorine, oxygen, sample containers and sample bags, equipment failure etc. = $1,500.00 **Activity 3) Evaluation of suitable feed attractants to improve feed intake of diets high in SPC** Purchase of 320 sub-adult fish = $1,280.00 Purchase of feed attractants = $1,000.00 Purchase of ingredients for diet manufacture = $1,000.00 Freight and or shipping costs associated with movement of feeds / diets within Australia = $1,000.00 Miscellaneous costs associated with purchase and use of chlorine, oxygen, sample containers and sample bags, breakdown of equipment etc. = $820.00 ***Australian Goods and Services Tax (GST), 10% of total proposed project budget (i.e 10/100 x $156,133=$15,613)*** **FY11 Total - $31,263**
Institutional Overhead $0	Not applicable

FY12 Budget $US

Total Project Budget

$182,293

DETAILED DESCRIPTION

Personnel/Contractual Services

$106,078

Billing rates and qualifications of personnel*.

Industry & Investment NSW Research Leader = $270.73/hour
(PhD qualified 25y experience, extensive publication list, world recognized industry leader and consultant, contract liaison with Smith-Bucklin and USB, Departmental liaison, contract approval, supervision of research scientists, host representatives of USB, experimental design etc.).

Industry & Investment NSW Research Scientist Level 6 = $165.47/hour
(PhD qualified, well published in field of expertise, highly experienced in experimental design and interpretation, plan lead and supervise research programs and projects, supervise staff, supervise students etc, liaison with collaborators, liaison with Smith-Bucklin and USB).

Industry & Investment NSW Fisheries Technician Grade 2 = $94.70/hour (Bachelor of Science (degree) qualification with at least 5 years experience in all aspects of fish husbandry, water quality, maintenance and construction of small scale laboratories, record keeping, ability to work unsupervised and lead others, data management, ordering and purchasing, logistics etc. Ability to write technical and non-technical reports).

Tailor Made Farm Proprietor = $165.47/hour
Owner operator of registered 60-90 metric tonne per annum Asian seabass (barramundi) recirculating aquaculture enterprise, Bobs Farm NSW, Australia

Tailor Made Fisheries Technician = $45/hour
(Trade or higher qualification, experienced in daily management and husbandry of commercial scale Asian seabass farm, work unsupervised, lead and supervise subordinate staff, highly developed skills in maintenance, repair and construction of aquaculture systems, record keeping, purchasing, sales).

* Please note: Salary rates for Industry & Investment NSW cover all facility costs, infrastructure costs (e.g. power, telephone), and all administration (i.e. financial services, legal and insurance, staff management, etc).

Proposed FY 2012 Research

Activity 4) Evaluation of key complimentary feed ingredients from SEA - Utilisation trial

1 week set up, 8 week experiment, 1 week clean up. Up to 10 treatments, 3 reps per treatment = 30 tanks. Salaries for 3 staff;

Approximately 70% Fish Tech, 30% res. Scientist & 2% Res. Leader.

Salary for I&I NSW Research Leader for 10 hours = $2,707
Salary for I&I NSW Research Scientist for 105 hours = $17,374
Salary for I&I NSW Fisheries Technician 245 hours = $23,202

Activity 5) Conduct a grow-out verification trial at a commercial farm to evaluate the performance of commercially manufactured diet/s with optimised levels of SBM and SPC.

1 week set up, 12 week experiment, 1 week clean up, Up to 4 treatments , 4 reps per treatment = 16 tank. Salaries for 5 staff; Approximately 30% Fish Tech, 20% Res. Scientist, 2% Res. Leader, 4.5% Tailor Made proprietor & 50% Tailor Made Technician.

Salary for I&I NSW Research Leader for 15 hours = $4,061
Salary for I&I NSW Research Scientist for 98 hours = $16,216
Salary for I&I NSW Fisheries Technician 147 hours = $13,921
Salary for Tailor Made Farm Proprietor 35 hours = $5,791
Salary for tailor Made Technician 336 hours = $15,120

Activity 6) Conduct an economic feasibility analysis of alternative soy based diets compared to typical commercial Asian seabass diet

Salaries for 2 staff ; 1 week block for Res. Scientist and approximately 1 day for Res. Leader.

Salary for I&I NSW Research Leader for 7 hours = $1,895
Salary for I&I NSW Research Scientist for 35 hours = $5,791

General duties for staff include but are not limited to literature review and reporting, design of experiments, supervision, liaison with contractors, procurement of ingredients diets and fish, importation of ingredients from SEA or interstate Australia, preparation and manufacture of experimental feeds, stocking and harvesting procedures and movement of fish, sample collection and preparation, preparation and clean up of laboratories, daily feeding and husbandry duties (including weekend work) such as tank cleaning and water quality analysis, interpretation of data, preparation of reports, fish grading, breakdown, maintenance of equipment, dispatch of samples, preparation of invoices and timesheets, order consumables, emergency on-call allowances etc.

FY12 Total - $106,078

Subcontractors

$27,942

Chemical analyses of ingredients, diets, faeces or whole fish carcass will be required for **Activities 2, 3, 4 & 5**. Chemical analysis will be outsourced to specialist providers used in previous USB projects. The following table lists indicative costs for individual chemical analyses.

TEST	COST $US
Grinding	11.52
Freeze drying	26.18
Dry matter	6.39

Crude protein	28.59
Gross energy	42.94
Fat (ether)	22.52
Ash	6.39
Handling fee	2.09
Amino acids	312.12
Marker (e.g. chromium)	36.66

Thus:

Proximate analysis on a per sample basis (includes handling fee) = $146.63
Amino acid analysis on a per sample basis = $312.12
Marker analysis on a per sample basis = $36.66

Proposed FY 2012 Research

Activity 4) Evaluation of key complimentary feed ingredients from SEA - Utilisation trial

Chemical testing of up to 10 ingredients, 10 diets, 3 carcass reps per dietary treatment = 30 carcass samples plus initial carcass sample.

Proximate analysis ($146.63 per sample)
10 x ingredient samples = $1,466.30
10 x diet samples = $1,466.30
31 x carcass samples = $4,545.53

Sub-total for 51 samples = $7,478.13

Amino acid analysis ($312.12 per sample)
10 x ingredient samples = $3,121.20
10 x diet samples = $,3121.20

Sub-total for 20 samples = $6,242.40

Total chemical costs for Activity 4 = $13,720.53

5) Conduct a grow-out verification trial at a commercial farm to evaluate the performance of commercially manufactured diet/s with optimised levels of SBM and SPC.

Chemical analysis of up to 4 diets and 4 reps per dietary treatment = 16 carcass samples plus initial fish sample.

Proximate analysis ($146.63 per sample)
10 x ingredient samples = $1,466.30
4 x diet samples = $586.52
17 x carcass samples = $2,492.71

Sub-total for 31 samples = $4,545.53

Amino acid analysis ($312.12 per sample)
10 x ingredient samples = $3,121.20

4 x diet samples = $1,248.48
17 x carcass samples = $,5306.04

Sub-total for 31 samples = $9,675.72

Total chemical costs for Activity 5 = $14,221.25

FY12 Total - $27,942

Meetings & Conferences $0 — Not applicable

Travel

$2,750

Proposed FY 2012 Research

Activity 4) Evaluation of key complimentary feed ingredients from SEA - Utilisation trial

Travel / airfares and accommodation for I&I NSW Res. Scientist to AESEC extrusion facility to supervise manufacture of experimental diets for Activity 4 = $1,000.

Activity 5) Conduct a grow-out verification trial at a commercial farm to evaluate the performance of commercially manufactured diet/s with optimised levels of SBM and SPC.

Travel / airfares and accommodation for I&I NSW Res. Scientist to AESEC extrusion facility to supervise manufacture of experimental diets for Activity 5 = $1,000.

Activities 2-5 (general)

Travel associated with miscellaneous vehicles (cars, trucks) used to support Activities 2, 3, 4 and 5 = $750.00 (e.g. movement of staff, equipment, ingredients, diets and fish, transport to and from commercial farm for data collection and supervision etc.)

FY12 Total - $2,750

Printing $0 — Not applicable

Postage $0 — Not applicable

Equipment Rental $0 — Not applicable

Other Operating Expenses

$46,523

Proposed FY 2012 Research

Activity 4) Evaluation of key complimentary feed ingredients from SEA - Utilisation trial

Purchase of 320 sub-adult fish = $1,280.00
Purchase of ingredients for diet manufacture = $1,000.00
Extrusion of 10 experimental feeds for use in Activity 4 at AESEC or similar facility = $6,000.00
Freight and or shipping costs associated with movement of feeds / diets within Australia = $1,000.00
Miscellaneous costs associated with purchase and use of chlorine,

oxygen, sample containers and sample bags, breakdown of equipment etc. = $1,200

Activity 5) Conduct a grow-out verification trial at a commercial farm to evaluate the performance of commercially manufactured diet/s with optimised levels of SBM and SPC.

Purchase of approximately 100 adult fish for carcass samples at conclusion of verification trial = $1,280.00
Purchase of ingredients for diet manufacture = $2,500.00
Extrusion of 2-3 experimental feeds for use in Activity 5 at AESEC or similar facility = $2,300.00
Freight and or shipping costs associated with movement of feeds / diets within Australia = $1,500.00
Miscellaneous costs associated with purchase of sample containers and sample bags, breakdown of equipment etc. = $1,800.00

Contingency fee for validation / verification field trial = $10,000

FY12 Total - $29,860

Australian Goods and Services Tax (GST), 10% of total proposed project budget (i.e 10/100 x $166,630=$16,663)

Institutional Overhead $0	Not applicable

ATTACHMENT B

Compensation and Expense Reimbursement Policies

SMITHBUCKLIN CORPORATION
COMPENSATION AND EXPENSE REIMBURSEMENT POLICY

This agreement calls for payment of time charges and reimbursement of documented out-of-pocket expenses budgeted in connection with the project as approved by USB. No reimbursements can be made without appropriate documentation.

I. Reimbursement of Time Charges

SmithBucklin agrees to pay for time spent by the Contractor for Project services. The services provided by the Contractor are to be billed at the Contractor's hourly rate as set forth in Attachment A, or at rates approved in writing by SmithBucklin. Detailed time sheets shall show an hourly rate and information as to what work was done, when it was done, and by whom the services were provided, and copies shall be included with the invoice submitted to SmithBucklin for reimbursement.

II. Reimbursement for Materials, Postage and Telephone Expenses

1. The Contractor will be reimbursed for reasonable, ordinary and necessary direct out-of-pocket expenses incurred in carrying out the Project, which shall be reimbursed at net cost. These direct out-of-pocket expenses shall include, but not be limited to, supplies, telephone, postage charges, and travel-related expenses.

2. All billings rendered to SmithBucklin by the Contractor shall be supported by adequate information so the charge can be identified to the Project. Billing documentation shall include invoices, copies of advertising, and other support materials requested by SmithBucklin and shall describe the services provided to SmithBucklin and direct expenses incurred on an itemized basis. Copies of all invoices or receipts in excess of $25.00 rendered to the Contractor verifying costs shall be provided to SmithBucklin.

3. Equipment is not reimbursed unless specified in the budget, and is subject to return to USB at the end of the project. For purposes of this agreement, "Equipment" is defined as any tangible property with a value of $2,000.00 or more and a useful life of more than one year.

III. Reimbursement for Travel Expenses

1. Contractor shall evaluate whether the contemplated trips are reasonable and necessary and if the matters can be handled over the telephone and/or by fax. All personnel will fly coach, on the most economical fare basis, unless only higher air travel fare space is available on the day of necessary travel. Any charges for flight changes need to be substantiated with a receipt and notation as to the reason the flight was changed. A daily limit of $50.00 shall be placed on personal out-of-town meal costs and tips unless approved by SmithBucklin. If a meal is purchased for Contractor officials as a group, then the per capita cost of that meal shall be charged against each individual's $50.00 daily limit. The Contractor shall retain original receipts for any expenses in excess of $25.00.

   No international travel will be approved unless it is specified in the budget approved by USB and unless Contractor informs the Foreign Agriculture Service (FAS) Attaché/Counselor in the destination country far enough in advance to enable the Attaché/Counselor to disapprove the trip in the event of security or other concerns. Evidence of approval from FAS Attaché/Counselor shall be supplied to USB in advance of departure.

2. Expenses will be reimbursed from the time the individual departs from home or office until return, assuming direct travel to and from. If the trip is combined with other travel, costs are to be prorated based on time spent on USB projects.

3. Itemized receipts are required for all commercial travel, lodging and any individual expenses in excess of $25.00. Credit card statements are not acceptable unless no other documentation is available.

4. Guidelines for reimbursement of travel expenses are as follows:

   a. Mileage: IRS official rate.

   b. Commercial Travel: Reimbursement will be made for domestic airfare by the most economical route and class, transportation to and from airport as well as parking. Attach a ticket receipt for substantiation.

   c. Lodging/Meals: Reimbursement is limited to single room rate, or 1/2 of the double room rate if sharing a room with another person entitled to reimbursement, plus applicable taxes. Room service is allowed, but not bar, lounge or hotel room mini-bar costs for any items.

      For domestic travel, hotel receipt and up to $50 per day meals/incidentals with receipts will apply. Alcohol purchased only with a meal or meal function will be reimbursed.

      For international travel, the Federal Travel Regulations maximums for the specific city of the overnight stay will apply for lodging, meals and incidentals expense.

   d. Telephone: USB business calls only. For calls over $10, indicate person called and purpose. One personal call home daily, if reasonable, is allowed.

   e. Personal Expenses: No expenses of a personal nature will be approved, i.e., movies, liquor, tobacco, magazines, books, laundry (unless traveler is in a continuous travel status for more than five days), etc.

   f. Private Auto: If an individual elects to travel via private auto, the total reimbursement authorized for the period of physical travel for mileage, meals and lodging shall not exceed the equivalent of commercial airfare.

5. No expenses will be reimbursed for a spouse or other companion.

6. Travel time will not be billed.

IV. Other Reimbursement Requirements

1. Contractor shall not use outside suppliers or subcontractors for services or supplies valued in excess of $2,500 without SmithBucklin's prior written approval of such suppliers or subcontractors and their rates. SmithBucklin shall reimburse Contractor for all of Contractor's costs that are incurred on SmithBucklin's behalf with previously approved outside suppliers, after submission of invoices to substantiate such costs.

2. Contractor will not use contract employees without the prior written approval of SmithBucklin of such contract employees and their rates. Contract employees used to perform services on specific projects will be billed at the Contractor's cost for such employees after submission of invoices to substantiate such costs. The Contractor shall be responsible for directing and compensating all consultants, suppliers and contract employees. The Contractor shall be responsible for all tax withholdings and any medical, pension or other benefits applicable to its employees and subcontracted employees.

3. All sub-contracts must include terms comparable to or more restrictive than terms of this Agreement. Sub-contracts valued at $50,000 or more must be approved in writing by SmithBucklin.

4. Except as specifically disclosed in writing to SmithBucklin, the Contractor warrants that it shall receive no commission or discounts from any supplier and that its only remuneration shall be the fees agreed upon with SmithBucklin.

5. The benefit of all special rate and volume discount arrangements and all other benefits that the Contractor obtains or that enable the Contractor to buy, on behalf of SmithBucklin, at rates better than those published in their current rate cards or published rates, will accrue to the benefit of SmithBucklin. The Contractor shall advise SmithBucklin of all available discounts, rebates and any other benefits received by Contractor.

Activity report #1

United Soybean Board Domestic Programs
Report Form

Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report.

Project # and Title	Project # 1463 Asian seabass: validation of commercial grow-out feeds containing optimal levels of SBM and SPC and impacts of feed attractants
Organization & Project Leader	Industry & Investment NSW Dr Mark Booth
Reporting Period	15th March 2011

Progress: Statement of quantifiable progress objective(s) achieved during this reporting period.

The following activities have been undertaken since January 1st 2011:

- FY 2011 contracts were signed and exchanged.
- Contact was made with Lukas Manomaitis (SEA) about access to SEA ingredients for use in upcoming digestibility trials with Asian seabass in Australia.
- Biosecurity protocols for use of Asian seabass at PSFI were updated and approved.
- The laboratory at PSFI was prepared for the 1st Asian seabass experiment (i.e. use of feed attractants on diets high in SBM and SPC).
- 530x 120g Asian seabass were sourced and transported from Infinity Fisheries Pty Ltd (NSW) to PSFI in readiness for the 1st Asian seabass experiment.
- Asian sebass have been stocked into experiment tanks and the attractant trial has commenced.

Plans: Activities planned between now and the next reporting period.

The following activities are planned during the next reporting period:

- Complete attractant trial with Asian seabass and report interim results to USB (John Campen).
- Investigate options for importing ingredients from SEA into Australia for assessment of digestibility.
- Commence importation of small quantities of SEA ingredients to Australia
- Commence desk-top study on expanding ingredient data base for use in formulation of aquafeeds for Asian seabass.

Changes: Problems, obstacles, new developments or market/industry/research changes that impacted or may impact the completion date, cost or scope of the project.

Importation of rendered animal meals from SEA could be problematic. Enquiries with AQIS (Australian Quarantine & Inspection Service) are underway.

Messages: Message, questions, comments or requests.

NA.

Activity report #2

United Soybean Board Domestic Programs
Report Form

Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report.

Project # and Title	Project # 1463 Asian seabass: validation of commercial grow-out feeds containing optimal levels of SBM and SPC and impacts of feed attractants
Organization & Project Leader	Industry & Investment NSW Dr Mark Booth
Reporting Period	15th March 2011

Progress: Statement of quantifiable progress objective(s) achieved during this reporting period.

The following activities have been undertaken since January 1st 2011:

- FY 2011 contracts were signed and exchanged.
- Contact was made with Lukas Manomaitis (SEA) about access to SEA ingredients for use in upcoming digestibility trials with Asian seabass in Australia.
- Biosecurity protocols for use of Asian seabass at PSFI were updated and approved.
- The laboratory at PSFI was prepared for the 1st Asian seabass experiment (i.e. use of feed attractants on diets high in SBM and SPC).
- 530x 120g Asian seabass were sourced and transported from Infinity Fisheries Pty Ltd (NSW) to PSFI in readiness for the 1st Asian seabass experiment.
- Asian sebass have been stocked into experiment tanks and the attractant trial has commenced.

Plans: Activities planned between now and the next reporting period.

The following activities are planned during the next reporting period:

- Complete attractant trial with Asian seabass and report interim results to USB (John Campen).
- Investigate options for importing ingredients from SEA into Australia for assessment of digestibility.
- Commence importation of small quantities of SEA ingredients to Australia
- Commence desk-top study on expanding ingredient data base for use in formulation of aquafeeds for Asian seabass.

Changes: Problems, obstacles, new developments or market/industry/research changes that impacted or may impact the completion date, cost or scope of the project.

Importation of rendered animal meals from SEA could be problematic. Enquiries with AQIS (Australian Quarantine & Inspection Service) are underway.

Messages: Message, questions, comments or requests.

NA.

Activity report #3

United Soybean Board Domestic Programs
Report Form

Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report.

Project # and Title	Project # 1463 Asian seabass: validation of commercial grow-out feeds containing optimal levels of SBM and SPC and impacts of feed attractants
Organization & Project Leader	Industry & Investment NSW Dr Mark Booth
Reporting Period	15th September 2011

Progress: Statement of quantifiable progress objective(s) achieved during this reporting period.

The following activities have been undertaken since June 15 2011:

- Experimental ingredients were procured and test feeds for digestibility experiment with Asian seabass were made.
- The laboratory at PSFI was prepared for the digestibility experiment with Asian seabass
- 300 x 300g Asian seabass were purchased and shipped to PSFI for use in digestibility experiment.
- Asian seabass were stocked into experiment tanks and acclimated to test feeds for 1 week after which collection of faecal material commenced.
- Continue desk-top study on expanding ingredient data base for use in formulation of aquafeeds for Asian seabass.
- Dr Mark Booth travelled to Kona Hawaii to attend USB USSEC Stakeholders meeting and present update of research on Asian seabass participate in USB research plans for 2012 and 2013.

Plans: Activities planned between now and the next reporting period.

The following activities are planned during the next reporting period December 15^{th} 2011:

- Complete digestibility experiment and submit ingredient, diet and faecal samples for chemical analysis of proximate composition and amino acids
- Continue desk-top study on expanding ingredient data base for use in formulation of aquafeeds for Asian seabass.
- Prepare and submit draft final report on 2011 research for USB.

- Submit a proposal for conducting applied research on Asian Seabass beginning on January 1, 2012 with the following research objectives:
  1) Evaluation of key complimentary feed ingredients from ASA International Marketing (IM) identified target markets in Southeast Asia Utilization of key SEA ingredients; reduction or elimination of fishmeal in high SBM diets
  2) Conduct a grow-out verification trial at a commercial farm (SEA or Australia)
  3) Conduct an economic feasibility analysis of alternative soy based diets compared to typical commercial Asian seabass diet (least-cost analysis)
  4) Bring researchers from Australia to SEA both at the outset and at the conclusion of the work to allow them to see what challenges we are facing and to bring the research to audiences in SEA. The latter should include travel to 3-5 nations for 2-3 days each.

Changes: Problems, obstacles, new developments or market/industry/research changes that impacted or may impact the completion date, cost or scope of the project.

The experiment to determine the digestibility of key ingredients by Asian seabass commenced at the beginning of September. Collection of faecal material from Asian seabass is done by striping techniques which is time consuming and difficult. At present the experiment is on-time and we plan to finish the practical component in mid October 2011. Following this, samples will need to be prepared and sent for chemical analyses. Analysis of samples (approx. 45) can take as long as 8-10 weeks to complete. If analyses take longer than this then it is possible that submission of the final report may be delayed till January 2012. At this stage we do not envisage major problems.

Messages: Message, questions, comments or requests.

Activity report #4

United Soybean Board Domestic Programs
Report Form

Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report.

Project # and Title	Project # 1463 Asian seabass: validation of commercial grow-out feeds containing optimal levels of SBM and SPC and impacts of feed attractants
Organization & Project Leader	Industry & Investment NSW Dr Mark Booth
Reporting Period	15th December 2011

Progress: Statement of quantifiable progress objective(s) achieved during this reporting period.

The following activities have been undertaken since September 15th 2011:

- Experiment to investigate the digestibility of several feed ingredients for use in diets of Asian seabass was successfully completed.
- All fish were euthanized at the end of the experiment in line with biosecurity protocols.
- The laboratory was cleaned and disinfected in line with biosecurity protocols.
- Samples of faecal material, diets and ingredients were submitted to NSW DPI Analytical Laboratory for proximate and amino acid analysis.
- Samples were submitted to Ecoteam (University of Sunshine Coast) for analysis of chromium oxide.
- Dr Booth continued desk-top study on expanding ingredient data base for use in formulation of aquafeeds for Asian seabass and information on effects of SBM and SPC on pellet quality.
- Dr Booth commenced collating results from analytical tests, evaluating data and preparing final report for USB.
- NSW DPI sent invoices to Smith-Bucklin for project costs and for travel to 2011 Stakeholders Meeting in Kona, Hawaii.
- NSW DPI provided Smith Bucklin with updates of project spending up to the 30th September 2011.
- At the request of the USB, NSW DPI submitted a new research proposal to Smith-Bucklin for conducting applied research on Asian Seabass beginning January 1, 2012.

Qualified approval for the 2012 research has been given.

Plans: Activities planned between now and the next reporting period.

The following activities are planned for completion before the 31 January 2012

- Submit final report to USB on project 1463 Asian seabass.
- Submit final invoice to Smith-Bucklin for project 1463 Asian seabass.
- Complete contract arrangements for FY 2012 research on Asian seabass.

.

Changes: Problems, obstacles, new developments or market/industry/research changes that impacted or may impact the completion date, cost or scope of the project.

NSW DPI plans to submit the final report on project 1463 Asian seabass before the end of December 2012. However we are still waiting on several outstanding invoices from contractors related to chemical analysis (as detailed in previous activity report). It is hoped these invoices will arrive prior to the end of January 31 2012 to allow NSW DPI to submit its final invoice to Smith-Bucklin. Should any issues regarding the final invoice or the final report be forthcoming, NSW DPI will contact Smith-Bucklin (Lisa Childs, Rick Scott and John Campen) to discuss.

Messages: Message, questions, comments or requests.

Peer reviewed literature on the nutrition of Asian seabass.

2011 References

Alhazzaa R, Bridle AR, Nichols PD, Carter CG (2011) Replacing dietary fish oil with Echium oil enriched barramundi with C18 PUFA rather than long-chain PUFA. *Aquaculture,* **312**, 162-171.

Biswas G, Thirunavukkarasu AR, Sundaray JK, Kailasam M (2011) Culture of Asian seabass *Lates calcarifer* (Bloch) in brackishwater tide-fed ponds: growth and condition factor based on length and weight under two feeding systems. *Indian J. Fish.,* **58**, 53-57.

Etzion A, Gillor O, Harpaz S, Pasternak Z, Zilberg D (2011) Dietary supplementation of probiotic bacteria: effect on immune function and resistance to bacterial infection in Barramundi, *Lates calcarifer* (Bloch, 1790). *Israeli Journal of Aquaculture-Bamidgeh,* **63**, 1-9.

Fraser MR, de Nys R (2011) A quantitative determination of deformities in barramundi (*Lates calcarifer*; Bloch) fed a vitamin deficient diet. *Aquacult. Nutr.,* **17**, 235-243.

Glencross B (2011) A comparison of the digestibility of diets and ingredients fed to rainbow trout (*Oncorhynchus mykiss*) or barramundi (*Lates calcarifer*) – the potential for inference of digestibility values among species. *Aquacult. Nutr.,* **17**, e207-e215.

Glencross B, Blyth D, Tabrett S, Bourne N, Irvin S, Anderson M, Fox-Smith T, Smullen R (2011) An assessment of cereal grains and other starch sources in diets for barramundi (*Lates calcarifer*) – implications for nutritional and functional qualities of extruded feeds. *Aquacult. Nutr.*, doi: 10.1111/j.1365-2095.2011.00903.x.

Glencross B, Rutherford N (2011) A determination of the quantitative requirements for docosahexaenoic acid for juvenile barramundi (*Lates calcarifer*). *Aquacult. Nutr.,* **17**, E536-E548.

Glencross BD, Bermudes M (2011) The effect of high water temperatures on the allometric scaling effects of energy and protein starvation losses in juvenile barramundi, *Lates calcarifer*. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology,* **159**, 167-174.

Glencross BD, Bermudes M (2011) Adapting bioenergetic factorial modelling to understand the implications of heat stress on barramundi (Lates calcarifer) growth, feed utilisation and optimal protein and energy requirements – potential strategies for dealing with climate change? Aquacult. Nutr., 10.1111/j.1365-2095.2011.00913.x

Sapkale PH, Singh RK (2011) Dietary zinc and cobalt requirements of fry of Seabass (*Lates calcarifer*) and Catfish (Clarias batrachus). *Israeli Journal of Aquaculture-Bamidgeh,* **63**.

2010 References

Arockiaraj AJ, Appelbaum S (2010) Dietary salt requirement for barramundi Asian Seabass (*Lates calcarifer*, Bloch 1970) fingerlings reared in freshwater recirculation units. *Israeli Journal of Aquaculture-Bamidgeh,* **62**, 245-250.

Bermudes M, Glencross B, Austen K, Hawkins W (2010) The effects of temperature and size on the growth, energy budget and waste outputs of barramundi (*Lates calcarifer*). *Aquaculture,* **306**, 160-166.

Biswas G, Thirunavukkarasu AR, Sundaray JK, Kailasam M (2010) Optimization of feeding frequency of Asian seabass (*Lates calcarifer*) fry reared in net cages under brackishwater environment. *Aquaculture,* **305**, 26-31.

Glencross B, Rutherford N (2010) Dietary strategies to improve the growth and feed utilization of barramundi, *Lates calcarifer* under high water temperature conditions. *Aquacult. Nutr.,* **16**, 343-350.

Glencross BD, Rutherford N, Jones B (2010) Evaluating options for fishmeal replacement in diets for juvenile barramundi (*Lates calcarifer*). *Aquacult. Nutr.,* **TBA**, TBA.

Katersky RS, Carter CG (2010) The effect of temperature on post-prandial protein synthesis in juvenile barramundi, *Lates calcarifer*. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology,* **156**, 529-536.

Michael RJ, Partridge GJ, Hofmann T, Caro ED (2010) Evaluation of mosquito fish Gambusia holbrooki (Girard) as a partial and complete pellet replacement for juvenile barramundi *Lates calcarifer* (Bloch). *Aquacult. Res.,* **41**, e788-e794.
Mohd-Yusof NY, Monroig O, Mohd-Adnan A, Wan KL, Tocher DR (2010) Investigation of highly unsaturated fatty acid metabolism in the Asian sea bass, *Lates calcarifer*. *Fish Physiol. Biochem.,* **36**, 827-843.

2009 References

Katersky RS, Carter CG (2009) Growth and protein synthesis of barramundi, *Lates calcarifer*, fed lupin as a partial protein replacement. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology,* **152**, 513-517.
Nankervis L, Southgate PC (2009) Enzyme and acid treatment of fish meal for incorporation into formulated microbound diets for barramundi (*Lates calcarifer*) larvae. *Aquacult. Nutr.,* **15**, 135-143.
Vartak VR, Singh RK (2009) Effects of live food organisms and formulated diets on growth, survival, and body protein of Asian Sea Bass fry (*Lates calcarifer*, Bloch). *Israeli Journal of Aquaculture-Bamidgeh,* **61**, 63-67.

2008 References

Glencross B, Michael R, Austen K, Hauler R (2008) Productivity, carcass composition, waste output and sensory characteristics of large barramundi *Lates calcarifer* fed high-nutrient density diets. *Aquaculture,* **284**, 167-173.
Glencross BD (2008) A factorial growth and feed utilization model for barramundi, *Lates calcarifer* based on Australian production conditions. *Aquacult. Nutr.,* **14**, 360-373.
Partridge GJ, Lymbery AJ (2008) The effect of salinity on the requirement for potassium by barramundi (*Lates calcarifer*) in saline groundwater. *Aquaculture,* **278**, 164-170.
Salama AJ (2008) Effects of different feeding frequency on the growth, survival and feed conversion ratio of the Asian sea bass *Lates calcarifer* juveniles reared under hypersaline seawater of the Red Sea. *Aquacult. Res.,* **39**, 561-567.

2007 References

Bowater RO, Burren B (2007) Lateral muscular myopathy associated with vitamin E deficiency in farmed barramundi, *Lates calcarifer* (Bloch). *J. Fish Dis.,* **30**, 117-121.
Kailasam M, Thirunavukkarasu AR, Selvaraj S, Stalin P (2007) Effect of delayed initial feeding on growth and survival of Asian sea bass *Lates calcarifer* (Bloch) larvae. *Aquaculture,* **271**, 298-306.
Katersky RS, Carter CG (2007) High growth efficiency occurs over a wide temperature range for juvenile barramundi *Lates calcarifer* fed a balanced diet. *Aquaculture,* **272**, 444-450.
Katersky RS, Carter CG (2007) A preliminary study on growth and protein synthesis of juvenile barramundi, *Lates calcarifer* at different temperatures. *Aquaculture,* **267**, 157-164.
Schipp G, Bosmans J, Humphrey J (2007) Northern Territory barramundi farming handbook. Department of Primary Industry, Fisheries and Mines - Northern Territory Government, Darwin, Australia, pp. 71.

2006 References

Curnow J, King J, Partridge G, Kolkovski S (2006) Effects of two commercial microdiets on growth and survival of barramundi (*Lates calcarifer* Bloch) larvae within various early weaning protocols. *Aquacult. Nutr.,* **12**, 247-255.
Glencross B (2006) The nutritional management of barramundi, *Lates calcarifer* - a review. *Aquacult. Nutr.,* **12**, 291-309.

Nankervis L, Southgate PC (2006) The influence of dietary protein and energy levels on growth, survival and thyroid hormone (T3 and T4) composition of *Lates calcarifer* larvae. *Aquacult. Nutr.,* **12**, 219-226.
Nankervis L, Southgate PC (2006) An integrated assessment of gross marine protein sources used in formulated microbound diets for barramundi (*Lates calcarifer*) larvae. *Aquaculture,* **257**, 453-464.
Singh RK, Balange AK, Khandagale PA, Chavan SL (2006) Evaluation of fish meals as natural feed stimulants on the feeding behaviour of fry and juveniles of *Lates calcarifer* (Bloch). *Asian Fish. Sci.,* **19**, 97-106.
Williams KC, Barlow CG, Rodgers L, Agcopra C (2006) Dietary composition manipulation to enhance the performance of juvenile barramundi (*Lates calcarifer* Bloch) reared in cool water. *Aquacult. Res.,* **37**, 914-927.

2005 References

Harpaz S, Hakim Y, Barki A, Karplus I, Slosman T, Tufan Eroldogan O (2005) Effects of different feeding levels during day and/or night on growth and brush-border enzyme activity in juvenile *Lates calcarifer* reared in freshwater re-circulating tanks. *Aquaculture,* **248**, 325-335.
Harpaz S, Hakim Y, Slosman T, Eroldogan OT (2005) Effects of adding salt to the diet of Asian sea bass *Lates calcarifer* reared in fresh or salt water recirculating tanks, on growth and brush border enzyme activity. *Aquaculture,* **248**, 315-324.
Singh RK, Vartak V, Balange A (2005) Effect of stimulants on feeding response, feeding behavior, and growth of fry of sea bass, *Lates calcarifer* (Bloch, 1790). *Israeli Journal of Aquaculture-Bamidgeh,* **57**, 32-38.
Tantikitti C, Sangpong W, Chiavareesajja S (2005) Effects of defatted soybean protein levels on growth performance and nitrogen and phosphorus excretion in Asian seabass (*Lates calcarifer*). *Aquaculture,* **248**, 41-50.

2004 References

Coloso RM, Murillo-Gurrea DP, Borlongan IG, Catacutan MR (2004) Tryptophan requirement of juvenile Asian sea bass *Lates calcarifer*. *J. Appl. Ichthyol.,* **20**, 43-47.

2003 References

Allan GL, Stone DAJ, Anderson AJ, booth MA (2003) Carbohydrate metabolism in silver perch and barramundi. *Recent Advances in Animal Nutrition in Australia,* **14**, 171-177.
McMeniman NP (2003) Digestibility and utilisation of starch by barramundi. In: *Aquaculture diet development subprogram: ingredient evaluation. Project 1996 / 391 Final Report to Fisheries Research and Development Corporation (FRDC)* (ed. by Allan GL, Booth MA, Stone DAJ, Anderson AJ). Fisheries Research and Development Corporation (FRDC), Canberra, Australia, pp. 135-139.
Raso S, Anderson TA (2003) Effects of dietary fish oil replacement on growth and carcass proximate composition of juvenile barramundi (*Lates calcarifer*). *Aquacult. Res.,* **34**, 813-819.
Rimmer M (2003) Barramundi. In: *Aquaculture Farming Aquatic Animals and Plants* (ed. by Lucas JS, Southgate PC). Blackwell Publishing, Oxford UK, pp. 364-381.
Williams KC, Barlow CG, Rodgers L, Hockings I, Agcopra C, Ruscoe I (2003) Asian seabass *Lates calcarifer* perform well when fed pelleted diets high in protein and lipid. *Aquaculture,* **225**, 191-206.
Williams KC, Barlow CG, Rodgers LJ, Ruscoe I (2003) Potential of meat meal to replace fish meal in extruded dry diets for barramundi, *Lates calcarifer* (Bloch). 1. Growth performance. *Aquacult. Res.,* **34**, 23-32.

Williams KC, Paterson BD, Barlow CG, Ford A, Roberts R (2003) Potential of meat meal to replace fish meal in extruded dry diets for barramundi, *Lates calcarifer* (Bloch). II. Organoleptic characteristics and fatty acid composition. *Aquacult. Res.,* **34**, 33-42.

2002 References

Boonyaratpalin M, Williams K (2002) Asian Sea Bass, *Lates calcarifer*. In: *Nutrient Requirements and Feeding of Finfish for Aquaculture* (ed. by Webster CD, Lim CE). CABI Publishing, New York, pp. 40-50.

Eusebio PS, Coloso RM (2002) Proteolytic enzyme activity of juvenile Asian sea bass, *Lates calcarifer* (Bloch), is increased with protein intake. *Aquacult. Res.,* **33**, 569-574.

2001 References

Murillo-Gurrea DP, Coloso RM, Borlongan IG, Serrano AE (2001) Lysine and arginine requirements of juvenile Asian sea bass (*Lates calcarifer*). *J. Appl. Ichthyol.,* **17**, 49-53.

Williams K, Barlow C, Rodgers L (2001) Efficacy of crystalline and protein-bound amino acids for amino acid enrichment of diets for barramundi/Asian seabass (*Lates calcarifer* Bloch). *Aquacult. Res.,* **32**, 415-429.

2000 References

Eusebio PS, Coloso RM (2000) Nutritional evaluation of various plant protein sources in diets for Asian sea bass *Lates calcarifer*. *J. Appl. Ichthyol.,* **16**, 56-60.

Nankervis L, Matthews SJ, Appleford P (2000) Effect of dietary non-protein energy source on growth, nutrient retention and circulating insulin-like growth factor I and triiodothyronine levels in juvenile barramundi, *Lates calcarifer*. *Aquaculture,* **191**, 323-335.

References prior to 2000

Barlow C, Williams K (1997) Nutritional research lowers the cost of barramundi feed. *Queensl. Aquac. News,* **May**, 7-9.

Barlow CG, Rodgers LJ, Palmer PJ, Longhurst CJ (1993) Feeding habits of hatchery-reared barramundi *Lates calcarifer* (Bloch) fry. *Aquaculture,* **109**, 131-144.

Boonyaratpalin M (1991) Asian seabass, *Lates calcarifer*. In: *Handbook of Nutrient Requirements of Finfish* (ed by Wilson RP). CRC Press Inc., Boca Raton, Florida, pp. 5-11.

Boonyaratpalin M, Suraneiranat P, Tunpibal T (1998) Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. *Aquaculture,* **161**, 67-78.

Catacutan MR, Coloso RM (1995) Effect of dietary-protein to energy ratios on growth, survival and body composition of juvenile Asian seabass *Lates calcarifer*. *Aquaculture,* **131**, 125-133.

Catacutan MR, Coloso RM (1997) Growth of juvenile Asian seabass, *Lates calcarifer*, fed varying carbohydrate and lipid levels. *Aquaculture,* **149**, 137-144.

Coloso RM, Murillo-Gurrea DP, Borlongan IG, Catacutan MR (1999) Sulphur amino acid requirement of juvenile Asian sea bass *Lates calcarifer*. *Journal of Applied Ichthyology-Zeitschrift Fur Angewandte Ichthyologie,* **15**, 54-58.

Partridge GJ, Southgate PC (1999) The effect of binder composition on ingestion and assimilation of microbound diets (MBD) by barramundi *Lates calcarifer* Bloch larvae. *Aquacult. Res.,* **30**, 879-886.

Phromkunthong W, Boonyaratpalin M, Storch V (1997) Different concentrations of ascorbyl-2-monophosphate-magnesium as dietary sources of vitamin C for seabass, *Lates calcarifer*. *Aquaculture,* **151**, 225-243.

Tucker JW, Mackinnon MR, Russell DJ, Obrien JJ, Cazzola E (1988) Growth of juvenile barramundi (*Lates calcarifer*) on dry feeds.. *Prog. Fish-Cult.,* **50**, 81-85.

Walford J, Lam TJ (1993) Development of digestive tract and proteolytic enzyme activity in seabass (*Lates calcarifer*) larvae and juveniles. *Aquaculture,* **109**, 187-205.
Walford J, Lim TM, Lam TJ (1991) Replacing live foods with microencapsulated diets in the rearing of seabass (*Lates calcarifer*) larvae: do the larvae ingest and digest protein-membrane microcapsules? *Aquaculture,* **92**, 225-235.

Selected literature on the impacts of SBM, SPC and alternative feed ingredients on pellet quality and characteristics.

2011 References

Blaufuss P, Trushenski J (2011) Exploring Soy-Derived Alternatives to Fish Meal: Using Soy Protein Concentrate and Soy Protein Isolate in Hybrid Striped Bass Feeds. N. Am. J. Aquacult., 74, 8-19.

Colombo A, León AE, Ribotta PD (2011) Rheological and calorimetric properties of corn-, wheat-, and cassava- starches and soybean protein concentrate composites. Starch - Stärke, 63, 83-95.

Draganovic V, van der Goot AJ, Boom R, Jonkers J (2011) Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. Anim. Feed Sci. Technol., 165, 238-250.

Glencross B, Hawkins W, Evans D, Rutherford N, McCafferty P, Dods K, Hauler R (2011) A comparison of the effect of diet extrusion or screw-press pelleting on the digestibility of grain protein products when fed to rainbow trout (Oncorhynchus mykiss). Aquaculture, 312, 154-161.

Jiang N, Zhang AZ, Yang RQ, Zhang YC (2011) An experimental approach to optimize several processing conditions when extruding soybeans. Anim. Feed Sci. Technol., 170, 277-283.

Kraugerud OF, Jørgensen HY, Svihus B (2011) Physical properties of extruded fish feed with inclusion of different plant (legumes, oilseeds, or cereals) meals. Anim. Feed Sci. Technol., 163, 244-254.

Kraugerud OF, Svihus B (2011) Effects of online pretreatment of plant ingredients on processing responses and physical properties in extruded fish feed. Anim. Feed Sci. Technol., 168, 250-256.

Morken T, Kraugerud OF, Barrows FT, Sørensen M, Storebakken T, Øverland M (2011) Sodium diformate and extrusion temperature affect nutrient digestibility and physical quality of diets with fish meal and barley protein concentrate for rainbow trout (Oncorhynchus mykiss). Aquaculture, 317, 138-145.

Schaeffer TW, Brown ML, Rosentrater KA (2011) Effects of Dietary Distillers Dried Grains with Solubles and Soybean Meal on Extruded Pellet Characteristics and Growth Responses of Juvenile Yellow Perch. N. Am. J. Aquacult., 73, 270-278.

Sinha AK, Kumar V, Makkar HPS, De Boeck G, Becker K (2011) Non-starch polysaccharides and their role in fish nutrition - A review. Food Chem., 127, 1409-1426.

2010 References

Glencross B, Hawkins W, Maas R, Karopoulos M, Hauler R (2010) Evaluation of the influence of different species and cultivars of lupin kernel meal on the extrusion process, pellet properties and viscosity parameters of salmonid feeds. Aquacult. Nutr., 16, 13-24.

Kannadhason S, Rosentrater KA, Muthukumarappan K, Brown ML (2010) Twin Screw Extrusion of DDGS-Based Aquaculture Feeds1. J. World Aquacult. Soc., 41, 1-15.

Pinto Da Costa PF, Marcondes Ferraz MB, Ros-Polski V, Quast E, Collares Queiroz FP, Steel CJ (2010) Functional extruded snacks with lycopene and soy protein. Ciencia E Tecnologia De Alimentos, 30, 143-151.

Reddy M, Mohanty AK, Misra M (2010) Thermoplastics from Soy Protein: A Review on Processing, Blends and Composites. Journal of Biobased Materials and Bioenergy, 4, 298-316.

Silva ACC, Areas EPG, Silva MA, Areas JAG (2010) Effects of Extrusion on the Emulsifying Properties of Rumen and Soy Protein. Food Biophysics, 5, 94-102.

Sorensen M, Nguyen G, Storebakken T, Overland M (2010) Starch source, screw configuration and injection of steam into the barrel affect the physical quality of extruded fish feed. Aquacult. Res., 41, 419-432.

Zhu L-J, Shukri R, de Mesa-Stonestreet NJ, Alavi S, Dogan H, Shi Y-C (2010) Mechanical and microstructural properties of soy protein - high amylose corn starch extrudates in relation to physiochemical changes of starch during extrusion. J. Food Eng., 100, 232-238.

2009 References

Buchanan NP, Moritz JS (2009) Main effects and interactions of varying formulation protein, fiber, and moisture on feed manufacture and pellet quality. Journal of Applied Poultry Research, 18, 274-283.

Chevanan N, Muthukumarappan K, Rosentrater KA (2009) Extrusion Studies of Aquaculture Feed using Distillers Dried Grains with Solubles and Whey. Food and Bioprocess Technology, 2, 177-185.

de Mesa NJE, Alavi S, Singh N, Shi Y-C, Dogan H, Sang Y (2009) Soy protein-fortified expanded extrudates: Baseline study using normal corn starch. J. Food Eng., 90, 262-270.

Jung S, Mahfuz AA (2009) Low temperature dry extrusion and high-pressure processing prior to enzyme-assisted aqueous extraction of full fat soybean flakes. Food Chem., 114, 947-954.

MacDonald RS, Pryzbyszewski J, Hsieh F-H (2009) Soy Protein Isolate Extruded with High Moisture Retains High Nutritional Quality. J. Agric. Food Chem., 57, 3550-3555.

Øverland M, Sørensen M, Storebakken T, Penn M, Krogdahl Å, Skrede A (2009) Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (Salmo salar)—Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. Aquaculture, 288, 305-311.

Sørensen M, Stjepanovic N, Romarheim OH, Krekling T, Storebakken T (2009) Soybean meal improves the physical quality of extruded fish feed. Anim. Feed Sci. Technol., 149, 149-161.

2008 References

Froidmont E, Bonnet M, Oger R, Decruyenaere V, Romnée JM, Beckers Y, Bartiaux-Thill N (2008) Influence of the grinding level and extrusion on the nutritional value of lupin seed (Lupinus albus) for cattle in the context of the Dutch protein evaluation system. Anim. Feed Sci. Technol., 142, 59-73.

Pérez AA, Drago SR, Carrara CR, De Greef DM, Torres RL, González RJ (2008) Extrusion cooking of a maize/soybean mixture: Factors affecting expanded product characteristics and flour dispersion viscosity. J. Food Eng., 87, 333-340.

Ramírez Ortiz ME, San Martín-Martínez E, Martínez Padilla LP (2008) Rheological and Thermal Properties of Extruded Mixtures of Rice Starch and Isolated Soy Protein. Starch - Stärke, 60, 577-587.Froidmont E, Bonnet M, Oger R, Decruyenaere V, Romnée JM, Beckers Y, Bartiaux-Thill N (2008) Influence of the grinding level and extrusion on the nutritional value of lupin seed (Lupinus albus) for cattle in the context of the Dutch protein evaluation system. *Anim. Feed Sci. Technol.,* 142, 59-73.

2007 References

Barrows FT, Stone DAJ, Hardy RW (2007) The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (Oncorhynchus mykiss). Aquaculture, 265, 244-252.

Hansen JØ, Storebakken T (2007) Effects of dietary cellulose level on pellet quality and nutrient digestibilities in rainbow trout (Oncorhynchus mykiss). Aquaculture, 272, 458-465.

Kraugerud OF, Penn M, Storebakken T, Refstie S, Krogdahl Å, Svihus B (2007) Nutrient digestibilities and gut function in Atlantic salmon (Salmo salar) fed diets with cellulose or non-starch polysaccharides from soy. Aquaculture, 273, 96-107.

Kumar A, Ganjyal GM, Jones DD, Hanna MA (2007) Experimental determination of longitudinal expansion during extrusion of starches. Cereal Chemistry, 84, 480-484.
Ribotta PD, Colombo A, Leon AE, Anon MC (2007) Effects of soy protein on physical and rheological properties of wheat starch. Starch-Starke, 59, 614-623.
Singh S, Gamlath S, Wakeling L (2007) Nutritional aspects of food extrusion: a review. Int. J. Food Sci. Tech., 42, 916-929.

2006 References

Aslaksen MA, Romarheim OH, Storebakken T, Skrede A (2006) Evaluation of content and digestibility of disulfide bonds and free thiols in unextruded and extruded diets containing fish meal and soybean protein sources. Anim. Feed Sci. Technol., 128, 320-330.

Buchanan NP, Hott JM, Cutlip SE, Moritz JS (2006) The Effects of Cellulose and Soy Protein Isolate on Pellet Manufacture Variables and Quality. In: 2006 Annual Meeting on Poultry Science (PSA 2006).
Knudsen D, Ron O, Baardsen G, Smedsgaard J, Koppe W, Froklaer H (2006) Soyasaponins resist extrusion cooking and are not degraded during gut passage in Atlantic salmon (Salmo salar L.). J. Agric. Food Chem., 54, 6428-6435.
Moure A, Sineiro J, Dominguez H, Parajo JC (2006) Functionality of oilseed protein products: A review. Food Res. Int., 39, 945-963.
Venou B, Alexis MN, Fountoulaki E, Haralabous J (2006) Effects of extrusion and inclusion level of soybean meal on diet digestibility, performance and nutrient utilization of gilthead sea bream (Sparus aurata). Aquaculture, 261, 343-356.

2005 References

Dominy WG (2005) Extrusion cooking and feed ingredients. Aquafeed.com.
Jung S, Murphy PA, Johnson LA (2005) Physicochemical and functional properties of soy protein substrates modified by low levels of protease hydrolysis. J. Food Sci., 70, C180-C187.
Lee KA, Brennand CP (2005) Physico-chemical, textural and sensory properties of a fried cookie system containing soy protein isolate. Int. J. Food Sci. Technol., 40, 501-508.
Seker M (2005) Selected properties of native or modified maize starch/soy protein mixtures extruded at varying screw speed. J. Sci. Food Agric., 85, 1161-1165.

2004 References

Allan GL, Booth MA (2004) Effects of extrusion processing on digestibility of peas, lupins, canola meal and soybean meal in silver perch Bidyanus bidyanus (Mitchell) diets. Aquacult. Res., 35, 981-991.
Donald AM (2004) Understanding starch structure and functionality. In: Starch in food. Structure, function and applications (ed by Eliasson A-C). Woodhead Publishing Limited, Oxford, pp. 156-184.
Jung S, Roussel-Philippe C, Briggs JL, Murphy PA, Johnson LA (2004) Limited hydrolysis of soy proteins with endo- and exoproteases. Journal of the American Oil Chemists Society, 81, 953-960.
Parmer EL, Wang B, Aglan HA, Mortley D (2004) Physicochemical properties of texturized meat analog made from peanut flour and soy protein isolate with a single-screw extruder. Journal of Texture Studies, 35, 371-382.
Rueda J, Kil-Chang Y, Martinez-Bustos F (2004) Functional characteristics of texturized defatted soy flour. Agrociencia, 38, 63-73.

2003 References

Anon. (2003) Aquatic Feed Production - Wenger Process Description PAA-400-GJR. In: Food and Feed Extrusion Workshop (eds Forte D, van Doore F, van Barneveld R, Young G).

Food Engineering Industry Pty Ltd jointly with FRDC, Adelaide University, Roseworthy Campus, South Australia.

Cheng ZJ, Hardy RW (2003) Effects of extrusion and expelling processing, and microbial phytase supplementation on apparent digestibility coefficients of nutrients in full-fat soybeans for rainbow trout (Oncorhynchus mykiss). Aquaculture, 218, 501-514.

Cheng ZJ, Hardy RW (2003) Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (Oncorhynchus mykiss). Aquacult. Nutr., 9, 77-83.

O'Keefe T (2003) Plant protein ingredients for aquaculture feeds: use considerations and quality standards. American Soybean Association.

Venou B, Alexis MN, Fountoulaki E, Nengas I, Apostolopoulou M, Castritsi-Cathariou I (2003) Effect of extrusion of wheat and corn on gilthead sea bream (Sparus aurata) growth, nutrient utilization efficiency, rates of gastric evacuation and digestive enzyme activities. Aquaculture, 225, 207-223.

2002 References

Booth MA, Allan GL, Evans AJ, Gleeson VP (2002) Effects of steam pelleting or extrusion on digestibility and performance of silver perch *Bidyanus bidyanus*. *Aquacult. Res.,* **33**, 1163-1173.

Satoh S, Takanezawa M, Akimoto A, Kiron V, Watanabe T (2002) Changes of phosphorus absorption from several feed ingredients in rainbow trout during growing stages and effect of extrusion of soybean meal. *Fish. Sci.,* **68**, 325-331.

2001 References

Chang YK, Hashimoto JM, Acioli-Moura R, Martinez-Flores HE, Martinez-Bustos F (2001) Influence of extrusion conditions on cassava starch and soybean protein concentrate blends. Acta Alimentaria, 30, 189-203.

Chang YK, Hashimoto JM, Moura-Alcioli R, Martinez-Bustos F (2001) Twin-screw extrusion of cassava starch and isolated soybean protein blends. Nahrung-Food, 45, 234-240.

Del Rosario OM, Garcia VV, Del Rosario RR (2001) Physico-chemical properties of extrusion-texturized soybean. Philippine Agricultural Scientist, 84, 201-210.

Tudorica CM, Kuri V, Brennan CS (2001) Nutritional and Physicochemical Characteristics of Dietary Fiber Enriched Pasta. J. Agric. Food Chem., 50, 347-356.

Wang CY, Johnson LA (2001) Functional properties of hydrothermally cooked soy protein products. Journal of the American Oil Chemists Society, 78, 189-195.

2000 References

Allan GL, Booth MA, Stone DAJ (2000) Effects of extrusion on digestibility of field peas, lupins, soybean meal and canola meal fed to silver perch. In: AQUA 2000 Conference: Responsible Aquaculture in the New Millenium, 2 - 6 May 2000. European Aquaculture Society (EAS) Special Publication No. 28, Nice, France, pp. 17.

Booth MA, Allan GL, Warner-Smith R (2000) Effects of grinding, steam conditioning and extrusion of a practical diet on digestibility and weight gain of silver perch, Bidyanus bidyanus. Aquaculture, 182, 287-299.

Lin S, Huff HE, Hsieh F (2000) Texture and chemical characteristics of soy protein meat analog extruded at high moisture. J. Food Sci., 65, 264-269.

References prior to 2000

Alvarezmartinez L, Kondury KP, Harper JM (1988) A GENERAL-MODEL FOR EXPANSION OF EXTRUDED PRODUCTS. J. Food Sci., 53, 609-615.

Bangoula D, Parent JP, Vellas F (1993) Valeur alimentaire du lupin blanc (Lupinus albus var Lutop) chez la truite arc-en-ciel (Oncorhynchus mykiss). Effet de la cuisson-extrusion. Reprod. Nutr. Dev., 33, 325-334.

Booth M, Allan GL, Warner-Smith R (1998) Effects of grinding, steam conditioning and extrusion of a practical diet on the digestibility and growth of silver perch, Bidyanus bidyanus. In: Fish Meal Replacement in Aquaculture Diets for Silver Perch, Project 93/120-03. Final Report to FRDC (ed. by Allan GL, Rowland SJ). NSW DPI Fisheries Final Report Series - Report No. 6, pp. 181-198.

Botting CC (1991) Extrusion technology in aquaculture feed processing. In: Proceedings of the aquaculture feed processing and nutrition workshop September 19-25 1991 (eds Akiyama DM, Tan RKH). American Soybean Association - Singapore, Thailand and Indonesia, pp. 129-137.

Bouvier JM (1997) Generic extrusion processes in the agro-food industries. Feed Tech, 1, 13-15.

Camire ME, Camire A, Krumhar K (1990) CHEMICAL AND NUTRITIONAL CHANGES IN FOODS DURING EXTRUSION. Crit. Rev. Food Sci. Nutr., 29, 35-57.

Chauhan GS, Bains GS (1985) EFFECT OF DEFATTED SOY FLOUR ON THE PHYSICOCHEMICAL CHARACTERISTICS OF EXTRUDED RICE PRODUCTS. Journal of Food Science and Technology-Mysore, 22, 115-118.

Davis DA, Arnold CR (1995) Effects of two extrusion processing conditions on the digestibility of four cereal grains for Penaeus vannamei. AQUACULTURE 1995 vol. 133, no. 3-4, pp. 287-294.

Faller JY, Klein BP, Faller JF (1999) Acceptability of extruded corn snacks as affected by inclusion of soy protein. J. Food Sci., 64, 185-188.

Finkowski JW, Peleg M (1981) SOME RHEOLOGICAL CHARACTERISTICS OF SOY EXTRUDATES IN TENSION. J. Food Sci., 46, 207-211.

Ghorpade VM, Bhatnagar S, Hanna MA (1997) Structural characteristics of corn starches extruded with soy protein isolate or wheat gluten. Plant Foods for Human Nutrition, 51, 109-124.

Hager DF (1984) EFFECTS OF EXTRUSION UPON SOY CONCENTRATE SOLUBILITY. J. Agric. Food Chem., 32, 293-296.

Harris PL, Cuppett SL, Lee KW (1988) A SCANNING ELECTRON-MICROSCOPE STUDY OF MAIZE GLUTEN MEAL AND SOY COEXTRUDATES. Cereal Chemistry, 65, 228-232.

Harris PL, Cuppett SL, Rupnow JH, Walker CE, Mumm RF, Hanna MA (1988) OPTIMIZATION OF INITIAL MOISTURE, PH, AND EXTRUSION TEMPERATURE OF AN ACETONE-EXTRACTED MAIZE GLUTEN MEAL AND SOY EXTRUDATE FOR USE IN PET FOODS. Cereal Chemistry, 65, 267-270.

Hilton JW, Cho CY, Slinger SJ (1981) Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (Salmo gairdneri R.). Aquaculture, 25, 185-194.

Hu L, Huff HE, Heymann H, Hsieh F (1996) Effects of emulsifier and soy fiber addition on sensory properties of corn meal extrudate. J. Food Qual., 19, 57-77.

Hua YF, DeNi P, Gu WY, Shen BY (1996) Mechanism of physical modification of insoluble soy protein concentrate. Journal of the American Oil Chemists Society, 73, 1067-1070.

Irish GG, Balnave D (1993) Non-Starch Polysaccharides and Broiler Performance on Diets containing Soyabean Meal as the Sole Protein Concentrate. Aust. J. Agric. Res., 44, 1483-1499.

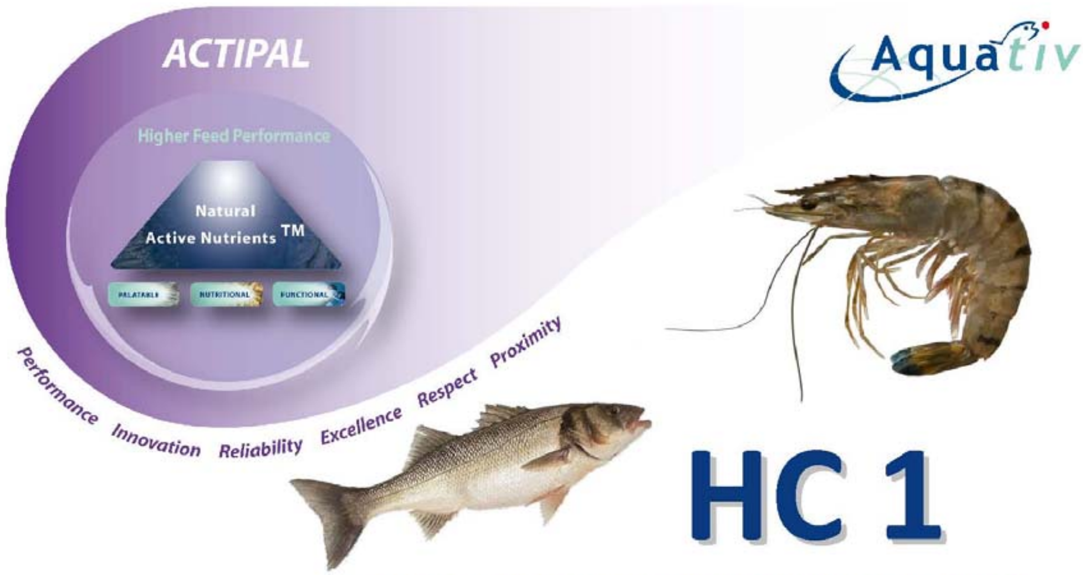
Ito T, Murata H, Tsuda T, Yamada T, Yamauchi K, Ukawa M, Yamaguchi T, Yoshida T, Sakai T (1999) Effects of Alpha-Tocopherol Levels in Extrusion Pellets on in Vivo Lipid Peroxidation Levels and Antioxidant Activities in Cultured Yellowtail Seriola Quinqueradiata Injected With the Causative Bacteria of Fish Jaundice. Fish. Sci., 65, 679-683.

Iwe MO (1998) Effects of extrusion cooking on functional properties of mixtures of full-fat soy and sweet potato. Plant Foods for Human Nutrition, 53, 37-46.

Iwe MO, Ngoddy PO (1998) Proximate composition and some functional properties of extrusion cooked soybean and sweet potato blends. Plant Foods for Human Nutrition, 53, 121-132.

Jeong K-S, Takeuchi T, Watanabe T (1991) Impvement of nutritional quality of carbohydrate ingredients by extrusion process in diets of red sea bream. Nippon Suisan Gakkaishi, 57, 1543-1549.
Jin Z, Hsieh F, Huff HE (1994) EXTRUSION-COOKING OF CORN MEAL WITH SOY FIBER, SALT, AND SUGAR. Cereal Chemistry, 71, 227-234.
Little C, Maguilere J, Morales A, Kokoni J (1997) Changes in Soy Protein during Heating Analyzed by Pressure Rheometry. Food Sci. Technol. Int., 3, 130-133.
Lusas EW, Riaz MN (1995) Soy Protein Products: Processing and use. J. Nutr., 573-580.
Marsman GJP, Gruppen H, Van der Poel AFB, Resink JW, Verstegen MWA, Voragen AGJ (1995) The effect of shear forces and addition of a mixture of a protease and a hemicellulase on chemical, physical and physiological parameters during extrusion of soybean meal. Anim. Feed Sci. Technol., 56, 21-35.
Park J, Rhee KS, Kim BK, Rhee KC (1993) SINGLE-SCREW EXTRUSION OF DEFATTED SOY FLOUR, CORN STARCH AND RAW BEEF BLENDS. J. Food Sci., 58, 9-20.
Pfeffer E, Beckmann-Toussaint J, Henrichfreise B, Jansen HD (1991) Effect of extrusion on efficiency of utilization of maize starch by rainbow trout (Oncorhynchus mykiss). Aquaculture, 96, 293-303.
Pongmaneerat J, Watanabe T (1993) Effect of extrusion processing on the utilization of soybean meal diets for rainbow trout. Nippon Suisan Gakkaishi, 59, 1407-1414.
PrudÊNcio-Ferreira SH, ArÊAs JG (1993) Protein-Protein Interactions in the Extrusion of Soya at Various Temperatures and Moisture Contents. J. Food Sci., 58, 378-381.
Robinson EH, Miller JK, Vergara VM, Ducharme GA (1985) Evaluation of Dry Extrusion-Cooked Protein Mixes as Replacements for Soybean Meal and Fish Meal in Catfish Diets. The Progressive Fish-Culturist, 47, 102-109.
Satoh S, Higgs DA, Dosanjh BS, Hardy RW, Eales JG, Deacon G (1998) Effect of extrusion processing on the nutritive value of canola meal for chinook salmon (Oncorhynchus tshawytscha) in seawater. Aquacult. Nutr., 4, 115-122.
Schmidt GR, Means WJ, Herriot DF, Miller BF (1983) THE FUNCTIONALITY OF SOY PROTEIN-CONCENTRATE IN CANNED LUNCHEON MEAT. Lebensmittel-Wissenschaft & Technologie, 16, 55-58.
Smith J, Mitchell JR, Ledward DA (1982) EFFECT OF THE INCLUSION OF POLYSACCHARIDES ON SOYA EXTRUSION. Progress in Food and Nutrition Science, 6, 139-147.
Taranto MV, Kuo CM, Rhee KC (1981) POSSIBLE ROLE OF THE CRUDE FIBER OF SOY FLOUR IN TEXTURE FORMATION DURING NON-EXTRUSION TEXTURIZATION PROCESSING. J. Food Sci., 46, 1470-1477.
Thiebaud M, Dumay E, Cheftel JC (1996) Influence of process variables on the characteristics of a high moisture fish soy protein mix texturized by extrusion cooking. Food Science and Technology-Lebensmittel-Wissenschaft & Technologie, 29, 526-535.
Thomas M, van der Poel AFB (1996) Physical quality of pelleted animal feed 1. Criteria for pellet quality. Anim. Feed Sci. Technol., 61, 89-112.
Thomas M, van Vliet T, van der Poel AFB (1998) Physical quality of pelleted animal feed 3. Contribution of feedstuff components. Anim. Feed Sci. Technol., 70, 59-78.
Thomas M, van Zuilichem DJ, van der Poel AFB (1997) Physical quality of pelleted animal feed. 2. contribution of processes and its conditions. Anim. Feed Sci. Technol., 64, 173-192.
Vens-Cappell B (1984) The effects of extrusion and pelleting of feed for trout on the digestibility of protein, amino acids and energy and on feed conversion. Aquacult. Eng., 3, 71-89.
Whalen PJ, Bason ML, Booth RI, Walker CE, Williams PJ (1997) Measurement of extrusion effects by viscosity profile using the rapid viscoanalyser. Cereal Foods World, 42, 469-475.
Wood JF (1987) The functional properties of feed raw materials and their effect on the production and quality of feed pellets. Anim. Feed Sci. Technol., 18, 1-17.

Proprietary brochure on Actipal HC1 – tuna hydrolysate



NATURAL ACTIVE NUTRIENTS FOR FISH AND SHRIMP

PRESENTATION

ACTIPAL HC 1 is a premium **functional hydrolysate concentrate** designed for use in **aqua feed**.

This product is manufactured from **tuna co-products** carefully selected for their unique nutritional profile and superior attractiveness and whose freshness is guaranteed by a strict local supply chain controls and a fast local production processing.

These raw materials are then submitted to our core hydrolysis process that guarantees adequate level of **Natural Active Nutrients** such as **free amino acids** and **peptides** to ensure exceptional product **performance** and **feed efficiency** improvement.

Through these **Natural Active Nutrients, ACTIPAL HC 1** will improve your feed formula by increasing:

- Feed **Palatability** → *Higher Feed Intake*
- Feed **Nutritional** quality → *Better FCR, Faster Growth*
- Feed **Bioactivity** properties → *Higher survival, Better Animal Health*

According to your particular problematic, you might take advantage of **ACTIPAL HC 1** benefits:

- As a **cost effective alternative solution** to substitute specific raw material like fishmeal
- As a **performance booster** in your premium feed range

RECOMMENDED USE

ACTIPAL HC 1 should be used in inclusion from 1 to 5% concentration in feed.
In order to optimize handling, **ACTIPAL HC 1** should be applied at a minimum temperature of 45°C after 15 min agitation (15-30 rpm).

ACTIPAL HC 1 should be stored:

- at ambient temperature
- away from sunlight
- for up to 6 months following the date of manufacture

HC 1

NATURAL ACTIVE NUTRIENT FOR FISH AND SHRIMP

ANALYSIS

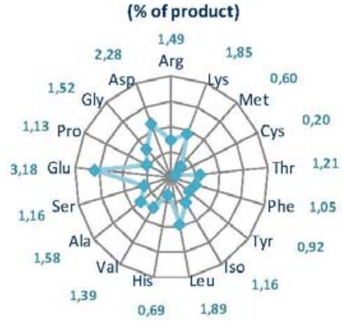
Proximal Analysis

- Moisture:	≤ 57 %	(typical = 56%)
- Protein:	≥ 27 %	(typical = 29%)
- Fat:	≤ 8 %	(typical = 7%)
- Ash:	≤ 9 %	(typical = 6%)
- pH:	3.8-4.2	

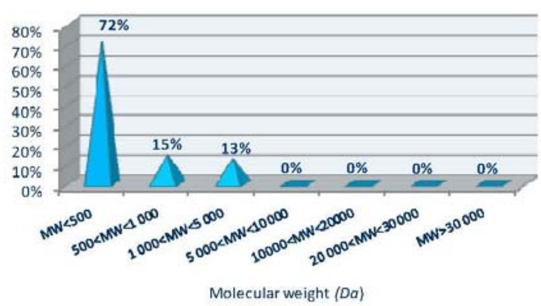
Bacteriological Analysis

- Salmonella:	abs/25g
- Enterobacteria:	< 10 CFU/g

Main Amino acids
(% of product)



Molecular weight repartition
(% of peptides)



INGREDIENTS - ADDITIVES

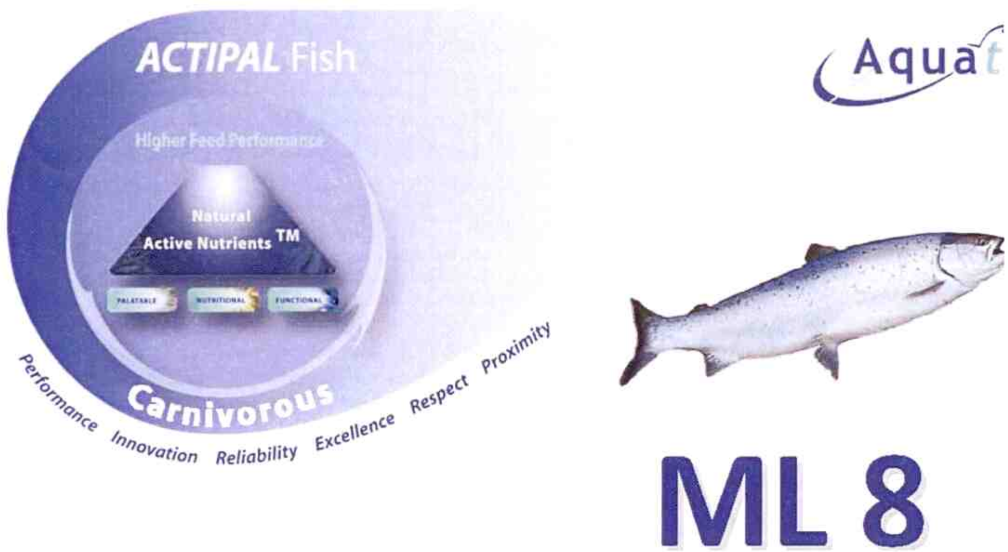
Ingredients: tuna hydrolysate
Additives: phosphoric acid (E338), citric acid (E330), ascorbic acid (E300), ethoxyquin (E324), potassium sorbate (E202)

For more information, please contact your local representative: Email: contact@aquativ-diana.com

Product for fish and shrimp feed only – This data sheet was created to assist users. The data listed is based on general use only and is not intended to establish a binding contractual relationship

Date: 15/03/2011 – Ref: HC 1 1180242 V6 Cancels and replaces the former version

Proprietary brochure on Actipal ML8 – poultry hydrolysate



TM
NATURAL ACTIVE NUTRIENTS ™ FOR FISH

PRESENTATION

ACTIPAL Fish ML 8 is a premium liquid functional hydrolysate designed for use in carnivorous fish feed.

Our product is manufactured from poultry raw materials carefully selected for their unique nutritional profile and superior attractiveness and whose freshness is guaranteed by our control of the local supply chain and our rapid local production processes.

These raw materials are then submitted to our core hydrolysis process that guarantees adequate level of Natural Active Nutrients™ such as free amino acids and peptides to ensure exceptional product performance and feed efficiency improvement, making ACTIPAL Fish ML 8 a unique natural growth factor.

Through these Natural Active Nutrients™, ACTIPAL Fish ML 8 improves the feed formula allowing the fish farmer to boost the productivity of his operation:

- Increased feed intake
- Accelerated fish growth
- Improved Feed efficiency resulting in enhanced FCR

In addition, the high attractiveness of ACTIPAL Fish ML 8 will help formulation team to optimize formula cost by permitting the substitution of high-efficiency ingredients for fish meal.

RECOMMENDED USE

ACTIPAL Fish ML 8 can be used in inclusion at 2 to 5% concentration. For an optimal palatability performance, we
can be used in inclusion at 2 to 5% concentration. For an optimal palatability
performance, we recommend to apply the product in coating after fish oil at 1 to 3% concentration. In order to optimize handling, ACTIPAL Fish ML 8 should be applied at a minimum temperature of 45°C after 15 min agitation (15-30 rpm).

The product should be stored:

- at ambient temperature (preferably under 30°C),
- away from sunlight,
- for up to 12 months following the date of manufacture

Packaging: 1000l containers or 1000l bags in box

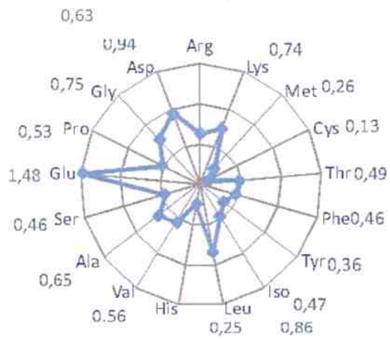
ANALYSIS

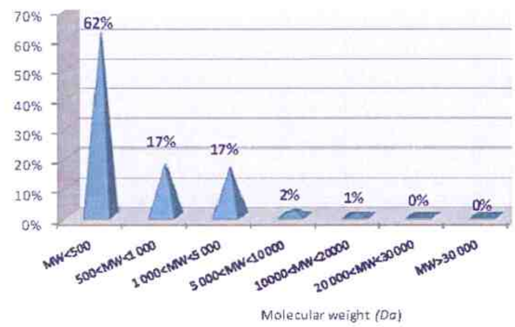
Proximal Analysis Bacteriological Analysis

-Moisture: ≤ 72 %
-Protein: ≥ 9 % -Salmonella: abs/25g
-Fat: ≤ 20 % -Enterobacteria: < 10 CFU/g

-Ash: ≤ 5 %
-pH: 2.7-3.1

Main Amino acids Molecular weight repartition (% of product) (% of peptides)





INGREDIENTS - ADDITIVES

Ingredients: poultry hydrolysate, sensory additives Additives: phosphoric acid (E338), BHT (E321)

For more information, please contact your local representative : Email : contact@aquativ-diana.com

Product for fish feed only – This data sheet was created to assist users. The data listed is based on general use only and is not intended to establish a binding contractual relationship

Date: 30/04/2010 – Ref: ML8 0390204 V4 Cancels and replaces the former version

SPF
Australia
ABN 26 093 990 759

Dr Mark Booth
Port Stephens Research Institute
Taylors Beach Road
TAYLORS BEACH NSW 2316

To Dr Mark Booth

Ref. : SAM887

16 February, 2011

Dear Mark

Please find enclosed sample of the following product:

NATURAL ACTIVE NUTRIENTS FOR FISH

Product name: ML8 0390204
Quantity: 1ltr
Batch no: 110214A

We will not fail to contact you soon to provide you with the necessary technical assistance and advice you may require.

Meanwhile, we hope our parcel will reach you in good condition and we look forward to hearing from you at your earliest convenience.

Best regards

JDaley

Jodie Daley
Sales Assistant
SPF Australia

LET001

NEWCASTLE PLANT Hawthorne St, Beresfield - PO Box 191 - NSW 2322 - Ph: 61 (0)2 4964 4399 - Fax: 61 (0)2 4964 4155
e-mail: contact@spfaus.com - www.spf-diana.com

Proprietary brochure on Artemia hydrolysate

Nutrattract®

– the Aroma for Growth

Grow out diets

Fishmeal replacement diets are currently being developed and tested around the world. In some cases, plant-based proteins are already replacing substantial amounts of fishmeal (e.g. salmon diets). However, marine finfish diets are still, almost solely based on fishmeal as a protein source. One of the impediments for the use of plant-based proteins is their low attractability, resulting in low ingestion.

Larvae weaning microdiets

Larvae weaning diets are still far from being optimised. Early weaning of marine fish larvae is impeded by the low acceptance of formulated diets. Although, in the past decade, the use of live food and specifically Artemia reduced significantly, it is still in most cases, hard to match performances of formulated weaning diets with live food. Acceptance and ingestion of the diets are usually one of the problems.

THE SOLUTION – better attractability

A new solution is now available to improve the attractability and acceptability of any finfish diets (as well as other marine organisms), whether it is weaning or growout diets.

Nutrattract® is a new, innovative (patent pending) attractant, 100% natural and based on Artemia (cultured under unique conditions). It is manufactured using a proprietary and unique process, enabling the preservation of all the natural nutrients that make Artemia such an attractive feed for marine organisms.

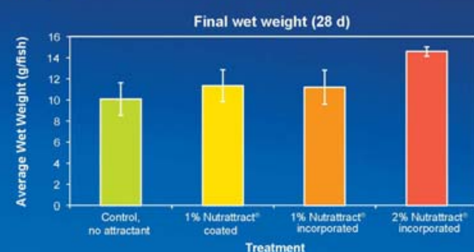
Nutrattract® can be coated, vacuum-infused or incorporated into any food particle, pelleted or extruded (usually at 1-3% inclusion). It will not change the diet shelf life. It is available in liquid or powder forms.

Plant-based diets with Nutrattract®

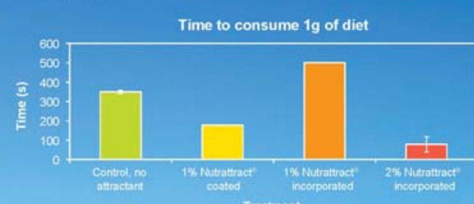
Nutrattract® was added at a range of inclusion levels, to a lupin-based growout diet (100% lupin protein as a fish meal replacement) for barramundi (*Lates calcarifer*). Nutrattract® was incorporated or vacuum-infused into the diet during the preparation using standard pellet extruder.

Each treatment was tested in three replicates (20 fish, 6.7±2.1 gr in each tank) for 28 days. Fish fed to apparent satiety.

Fish fed diet with 2% inclusion of Nutrattract® had 140% higher growth (as final weight) compared to fish fed with only (100%) lupin-based diet (14.6g vs. 10.1g respectively).

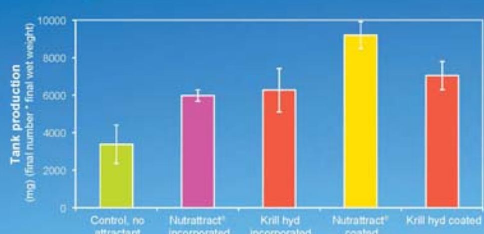


This was due to higher ingestion rates. Inclusion of Nutrattract® in the diet seems to have dose response in terms of growth rate, where 1% attractant inclusion resulted in final weight of 11.2g and 2% included were 14.6g. It is possible that higher Nutrattract® percentage coating the diet surface would result in even higher intake and growth as proved to be the case with fish larvae diets.



Weaning diets with different attractants

Yellowtail kingfish (*Seriola lalandi*) larvae were reared from 14 days post hatch (dph) to 29 dph using one of six experimental microdiets with 4 replicates (3000 larvae in each tank). A single protocol that progressively excluded live feeds (rotifers and *Artemia*) was used to wean the larvae onto microdiets. Two attractants were compared in two incorporation methods; krill hydrolysate and Nutrattract® incorporated into the diet or coated over the diet particles. Larvae received Nutrattract® coated microdiet demonstrated significantly higher biomass due to higher survival rates (Kolkovski et al., 2009).



Nutra-Kol
NUTRITION SOLUTIONS

Western Australia, Australia www.nutrakol.com
Email: info@nutrakol.com Tel/fax. +61-8-9403 2287