ASIAN SEABASS: VALIDATION OF COMMERCIAL GROW-OUT FEEDS CONTAINING OPTIMIZED LEVELS OF SOYBEAN MEAL AND SOY PROTEIN CONCENTRATE (VIETNAM)

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Introduction

NSW DPI Fisheries has been conducting research for the United Soybean Board (USB) on utilization of soybean meal (SBM) and soy protein concentrate (SPC) in aquafeeds for Asian seabass *Lates calcarifer* since 2009. This species is known as Barramundi in Australia. This collaboration has recently culminated in a nine month verification trial which was conducted in Vietnam with the support of a commercial marine fish farmer (Marine Farms Vietnam; MFV) and a commercial aquafeed producer (Invivo Vietnam-Ocialis). In the lead up to the latest trial several experiments were done which have determined the digestibility of SBM, SPC and other major feed ingredients for Asian seabass, their growth response to increasing dietary inclusion of SBM and SPC and effects of soy-based diets on carcass composition. Preliminary trials also investigated the use of feed attractants to overcome palatability issues with feeds containing high levels of SPC. Most of the laboratory work was conducted in Australia, but it was always the intention of the USB and United States Soy Export Council (USSEC) to conduct verification trials on soy-based feeds in South East Asia (SEA), the region with the greatest global production of Asian seabass and therefore the greatest potential for uptake of SBM and SPC in aquafeeds for this species.

The Vietnam trial reported here was established north of Nha Trang at Van Phong Bay and was designed to evaluate two extruded aquafeeds that each contained 30% soy-product; a mixture of about 25% SBM and 5% SPC, respectively. These feeds were also formulated to contain low levels of fishmeal (i.e. either 18 or 28%). As is the norm, the trial feeds were benchmarked against a proprietary aquafeed which was based mostly on fishmeal (45%) but which had similar levels of total fat (11%) and fish oil (6%). All three aquafeeds were produced by Invivo-Ocialis on thier R&D extruder using the same manufacturing techniques. The three aquafeeds were fed to replicate groups of juvenile Asian seabass (100g) from July 2013 to April 2014, until the average weight of fish was close to a marketable size of 800-1000g. At this time the fish were harvested to determine various morphometric and economic indices including survival, growth, feed intake, FCR, body composition, yield and market price. These data were used to assess the nutritional merit of the soy-based feeds as well as the economic cost/benefit of using the soy-based feeds as opposed to the commercial feed. NSW DPI has reported the progress of this verification trial in a previous technical bulletin to the USB (ca. 2014), however, for clarity this report details the results of the completed study in its entirety.

The major objectives of USB Project 1440-512-5261 were to:

1. Complete the grow-out verification trial in Van Phong Bay
2. Complete an economic appraisal of the soy-based formulations compared to a typical commercial Asian seabass diet using least-cost analysis and other economic indicators

Overall Design of Verification Trial

The verification trial was hosted by MFV who hold concessions for 200ha of surface area in Van Phong Bay where they farm cobia, pompano and Asian seabass in Polarcirkel sea cages. The verification trial was conducted in 5m x 5m x 5m depth research cages secured to a rectangular floating raft-system. The raft was tethered in a secure location within the concession (12°34'16" North, 109°24'5" East).

The verification trial was designed to be completed in 2 stages. Stage 1 involved stocking 2500 advanced (100g) fingerlings into each of 6 research cages and tracking weight gain and feed intake of each cage until the average weight of fish reached approximately 400-500g. At this point performance data was collected and analysed using one-way ANOVA (i.e. 3 feeds x 2 replicate cages). Afterwards all fish from each cage were graded into small or large cohorts and the trial was expanded into 12 research cages (i.e. 6 groups of small and large fish). At this time and because there were no significant differences in average weight of fish at the end of stage 1, the number of small and large fish in each research cage was equalised in order that a two-way ANOVA could be employed to statistically examine the results at the conclusion of the experiment. The adjustment in fish number and size represented the commencement of stage 2. Most importantly, all fish remained assigned to their original diet allocation and fish graded from primary replicate cages were split into small and large subgroups. Stage 1 was conducted between the 9th July 2013 and 18th November 2013. Stage 2 commenced on the 2nd December 2013 and concluded on the 2nd April 2014. The period between the end of November and start of December being necessary to weigh, count and reassign the remaining fish to the appropriate research cages.

Fish were hand fed to apparent satiation from pre-weighed, labeled buckets on a daily basis and according to the prevailing weather conditions. On occasions when it was difficult to observe fish feeding below the water surface or judge their feeding behavior, a restricted feeding protocol was employed based on estimated biomass. Used in conjunction, these two feeding methods assured most feed offered to fish was consumed and little was wasted due to pellets falling through the cages or being washed out at the surface. Feed input data was recorded on a daily basis.

Representative samples of initial fish and fish from the end of stage 1 and end of stage 2 were taken and frozen for determination of chemical composition. Twenty whole fish were taken from each research cage at the end of stage 2 for determination of yield data such as head on gutted weight (HOG), fillet recovery, hepatosomatic (HSI) and visceromatic (VSI) indices.

Chemical analysis of the soy-based feeds (i.e. FM18 and FM28) and the control diet (Nutrilis-C) indicated there were only minor differences in the nutrient and energy composition of different batches over time (Table 2). The highest variability was recorded in the moisture content of separate batches. Chemical analysis of FM18 and FM28 indicated that the targeted levels of protein, amino acids, fat and energy etc. were similar to predicted formulation values. In addition, the nutrient values measured in FM18 and FM28 were similar to those determined for the control diet (Nutrilis-C), with the exception of tryptophan content, which was more than double that of soy-based feeds (Table 3).

Initial pellet quality of the control and soy-based feeds was poor. This was attributed to the small batch-sizes used when making each of the aquafeeds on their research extruder for the first time. Pellet quality of all diets improved once larger batches of feed were being produced, reportedly due to the ability to refine and adjust the extruder settings over a longer period of feed production (Marc Campet, *personal comm.*; Ocialis).

Table 1. Formulation of optimized soy-based trial feeds; fishmeal 18% (FM18) and fishmeal 28% (FM28) and commercial control (Nutrilis-C)

INGREDIENT %	FM18	FM28	Nutrilis-C*
Wheat flour	12.00	12.00	15.6
HP 300 - Hamlet SPC	5.00	5.00	0*
Fish oil	5.20	5.70	5.20
SBM TTX 46.5 /1.3 - SBM	25.10	25.40	20.0
Poultry meal POM 66	25.00	15.00	7.5
Blood meal 93.6	6.00	6.00	5
Fish meal - Premium 65	2.90	8.20	13.5
Fishmeal Super Premium 68	15.10	19.80	31.5
Mon Calcium Phosphate (MCP)	1.80	1.43	0.32*
L-lysine sulfate 70	0.70	0.35	0.35*
DL Methionine (Pure)	0.53	0.46	0.1*
VIT C 35% coated	0.07	0.07	0.07
Choline chloride 60%	0.10	0.10	0.10
Globatiox	0.024	0.024	0.024
B-Glucans	0.10	0.10	0.10
Globalmold	0.10	0.10	0.10
Premix Fish	0.30	0.30	0.30
NUTRIENTS (estimated)			
Dig Energy MJ/kg	14.8	15.0	15.1
Protein %	51.0	51.0	51.1
Fat %	11.3	11.3	10.1
Ash %	10.6	10.9	9.7
Fiber %	1.0	1.0	
Starch %	8.1	8.1	10.4
Linoleic acid %	1.76	1.6	
Omega 3 %	1.98	2.37	
Omega 6 %	1.92	1.78	
C20:5 n-3 % (EPA)	0.67	0.83	
C22:6 n-3 % (DHA)	0.64	0.78	
Cholesterol %	0.05	0.08	
Calcium g/kg	22.8	23.3	
Phosphorus g/kg	18.1	18.2	
Disposable phosphorus g/kg	14.5	14.5	
Sodium %	0.3	0.3	
NDF %	2.7	2.7	
Methionine g/kg	14.5	14.4	13.4
Meth + cystine g/kg	20.8	20.4	
Lysine g/kg	35.1	35.0	41.5
Threonine g/kg	19.8	20.1	22.5
Tryptophan g/kg	5.4	5.6	
Arginine g/kg	31.7	31.4	
Glycine g/kg	33.1	30.9	30.8
Cystine disposable g/kg	4.6	4.5	
Methionine disposable g/kg	13.2	13.2	
Meth + cystine disposable g/kg	17.8	17.7	
Lysine disposable g/kg	30.6	31.0	
Threonine disposable g/kg	16.1	16.7	
Tryptophan disposable g/kg	4.6	4.8	
Arginine disposable g/kg	28.4	28.4	

Table 2. Measured composition of batches of FM18, FM28 and the commercial control (Nutrilis-C) used during the trials

Aquafeed	Feed Sample	Pellet Size (mm)	Crude Fat (%)	Crude Protein (%)	Ash (%)	Moisture (%)	Energy kcal/100g	Phosphorus (%)
Nannolis-2*	1	2	8.17	55.40	13.00	6.80	361.65	2.07
Nutrilis-C1	2	3	9.32	51.80	12.10	9.80	359.00	1.90
Nutrilis-C1	3	3	11.80	52.90	13.30	5.20	385.00	2.09
Nutrilis-C1 A	5	3	10.60	52.10	12.80	4.40	384.20	1.80
Nutrilis-C2	4	5	10.18	54.30	13.20	5.50	376.10	2.10
Nutrilis-C2 A	8	5	11.29	52.30	12.30	4.50	389.25	1.75
Nutrilis-C3 A	11	7	10.09	50.90	12.10	7.80	370.85	1.85
Nutrilis-C3 A	14	7	11.51	55.20	12.50	2.70	396.75	2.02
		mean	10.68	52.80	12.60	5.70	380.16	1.93
		stdev	0.89	1.49	0.50	2.37	12.57	0.14
FM18 B	6	3	11.19	52.00	10.90	4.90	392.15	2.10
FM18 B	9	5	10.98	52.00	10.70	5.40	390.50	1.99
FM18 B	12	7	10.48	50.30	9.90	10.20	372.00	1.99
FM18 B	15	7	11.61	52.90	10.40	5.90	392.85	2.13
		mean	11.07	51.80	10.50	6.60	386.88	2.05
		stdev	0.47	1.09	0.43	2.43	9.97	0.07
FM28 C	7	3	10.94	51.40	10.60	6.70	385.50	1.94
FM28 C	10	5	11.44	53.10	10.80	4.70	395.20	1.92
FM28 C	13	7	11.19	51.60	10.80	6.40	387.15	1.93
FM28 C	16	7	10.89	54.90	11.00	5.30	389.25	1.95
		mean	11.12	52.80	10.80	5.80	389.23	1.94
		stdev	0.25	1.62	0.16	0.94	4.24	0.01

*Hatchery feed used prior to switching Asian seabass to Nutrilis-C

Results

Mortality

There was a significant increase in the mortality of juveniles after the stocking event, despite using vaccinated fish. The rapid spike in mortality was attributed to post-handling effects rather than dietary treatment or disease and decreased after progressive weight checks were increased from 2 to 4 weekly intervals. There did not appear to be any relationship between running mortality and water temperature at the research cage site. Water temperature was reasonably stable between July and October 2013 varying between 28-30°C but declined thereafter, reaching a low of 22°C at the end of December 2013. The fall in water temperature roughly coincided with the commencement of stage 2. Not unexpectedly, feed intake of Asian seabass declined under the cooler water temperatures that prevailed over the winter period. According to factorial models, optimum growth and feed intake in Asian seabass is achieved when water temperature is between 28-32°C. Our initial stocking density was around 20 fish m^{-3} (i.e. 2500 fish /125m^{-3} cage) or 2.0kg m^{-3}. At harvest the highest biomass in any cage was less than 5.0kg m^{-3} which is extremely low, especially compared to stocking densities of Asian seabass raised in some recirculating aquaculture systems operated in Australia (75kg m^{-3}) (Nick Arena, *personal comm*; Tailor Made Fish Farm).

Table 3. Measured amino acid composition of FM18, FM28 and the commercial control (Nutrilis-C)

	Aquafeed Treatment (7mm pellets stage 2)		
Amino acid (%)	**Nutrilis-C**	**FM 18**	**FM 28**
Alanine	2.83	2.74	2.66
Arginine	3.58	3.36	3.25
Aspartic acid	4.83	4.86	4.84
Cystine	0.51	0.56	0.45
Glutamic acid	7.66	7.53	7.42
Glycine	3.46	3.24	3.05
Histidine	1.45	1.54	1.45
Isoleucine	1.84	1.92	1.86
Leucine	3.61	3.88	3.67
Lysine	3.63	3.49	3.28
Methionine	1.39	1.46	1.34
Phenylalanine	2.13	2.32	2.20
Proline	2.42	2.47	2.33
Serine	2.23	2.15	2.08
Threonine	1.89	1.87	1.82
Tryptophan	1.43	0.67	0.63
Tyrosine	1.51	1.52	1.46
Valine	2.66	2.76	2.63
Sum of Amino Acids (%)	49.06	48.34	46.42
Hydrolysed fat %	11.12	11.21	11.19
Crude protein %	52.51	53.35	51.59
Ash %	12.85	10.82	10.72
Dry matter %	96.00	95.48	93.06
Moisture %	4.00	4.52	6.94
Gross energy (kcal/100g)	20.09	20.09	19.67
Phosphorus %	1.88	1.78	1.78

Ongoing mortality of Asian seabass during stage 2 was somewhat higher than industry expectations for this species (Jorge Alarcon *personal comm.*; General Manager MFV). The General Manager MFV was of the opinion that the small square nets were not ideal for their more exposed sites (i.e. compared to where local farmers would place such a system), and that the fish usually look for cover and protection by congregating around the net folds and corners. This behavior coupled with stronger wave action likely resulted in enhanced skin abrasion and onset of secondary infections which caused the increased and constant background mortality. MFV grow-out sites were specifically selected for depth, current and good water quality. Ironically, while these conditions are suitable for large Polarcirkel cages which handle site conditions much better, they may have been less suitable for the research platform (Jorge Alarcon, *personal comm.*; General Manager MFV).

Growth and Feed Outcomes End of Stage 1

By the end of stage 1 the average weight of surviving fish was around 450g. Due to the significant number of mortalities recorded in the first weeks after stocking and the ongoing rate of background mortality, the estimated loss in biomass from most cages by the end of stage 1 was around 200kg, or 30%. The highest loss was recorded in cage FM28-R2 at just over 300kg or 42%. Despite the high mortality in this cage, the average harvest weight of fish was approximately 20g higher than other replicates. This is probably due to the increased mortality of smaller fish in this cage at the beginning of the trial compared to the other cages, the result being that the sample weights of fish drawn from this cage at the end of stage 1 are based on a population of fish skewed towards the larger surviving animals.

One-way ANOVA found no significant difference in the average weight, biomass of mortalities, live biomass or total biomass of Asian Seabass fed different diets at the end of stage 1. In addition, there was no significant difference between the total amount of feed consumed by each group or the resultant eFCR or bFCR (Table 4). By completion of stage 1 approximately 6 tonnes of trial feed had been consumed across all cages. Average specific growth rate (SGR) was close to 1.03%d^{-1}. Whole of stage 1 data indicated that there was an overall mortality rate of 31% equating to 1292kg in lost biomass. Overall feed utilization, calculated as eFCR and bFCR at the end of stage 1 was 1.90 and 1.35, respectively.

Protein retention of Asian seabass fed Nutrilis-C, FM18 or FM28 was estimated to be 28.8±1.13%, 26.8±1.15% and 29.4±0.9%, respectively. Fat retention in the same order was estimated to be 52.1±10.7%, 54.1±1.71% and 42.5±0.34%, respectively. Percent phosphorus retention was highest in fish fed Nutrilis-C (40.5±6.7%) followed by fish fed FM18 (33.3±2.70%) and those fed FM28 (25.3±3.26%). Gross energy retention was close to 100% in all groups. According to one-way ANOVA there were no statistical differences between the retention of nutrients or energy among diets.

Growth and Feed Outcomes End of Stage 2 (I.E. Harvest Data)

Data for stage 2 is summarized in Table 5. After grading, the average weight of small and larger fish was about 340 and 550g, respectively. However, because of the higher level of mortality in the FM28 cages during stage 1, there were ultimately less small-graded fish to move into separate cages for on-growing. Fish continued to grow through the cooler winter months, but the overall specific growth rate (SGR) slowed to 0.3%d^{-1}. Mortality continued during the second stage with most cages losing between 50-70 fish per month. This represented a mortality rate across all cages of about 27%, reaching a high of 33% in one of the smaller grades assigned to Nutrilis-C. Ongoing mortality appeared to be lower in cages that were graded with the large cohort.

Statistical analysis (factorial ANOVA) indicated there was no effect of diet type on the harvest weight of barramundi, regardless of size at stocking. The calculated bFCR for individual cages, which takes account of all biomass gained and lost during the period varied between 2.26 and 4.02. Nonetheless, there was no effect of diet type, fish size or interaction between diet type and fish size on bFCR of Asian seabass at harvest.

Percent survival of Asian seabass was not affected by diet type or the interaction between diet type and fish size. However, the percent survival of larger fish (76.3%, n=6) was significantly better than percent survival of smaller fish (69.9%, n=6). This indicated that irrespective of the diet being fed, smaller fish were more prone to morbidity than larger individuals under the conditions experienced in stage 2.

Table 4. Mean (stdev) production data (by feed group) at the end of stage 1

Index	Nutrilis-C treatment	FM18 treatment	FM28 treatment	Anova	P value
Number fish stocked	2528.5±4.9	2517.5±10.6	2532.0±9.9	NS	*P=0.36*
Ave. stock weight ($gfish^{-1}$)	100.6±0.1	99.8±8.1	98.6±2.1	NS	*P=0.91*
Est. stock biomass (kg)	254.2±0.3	251.3±21.6	249.5±4.1	NS	*P=0.93*
Ave. harvest weight ($gfish^{-1}$)	443.8±11.6	438.9±17.3	459.7±17.3	NS	*P=0.47*
Est. biomass mortalities (kg)	188.9±0.2	200.6±11.2	256.7±66.5	NS	*P=0.32*
Est. live biomass at harvest (kg)	814.2±26.0	777.9±13.2	740.9±71.5	NS	*P=0.38*
Total biomass harvest (kg)	1003.1±25.8	978.5±24.4	997.6±4.9	NS	*P=0.53*
Total feed used (kg)	1000.7±0.7	1000.9±8.3	995.6±9.5	NS	*P=0.74*
eFCR	1.79±0.08	1.91±0.14	2.05±0.29	NS	*P=0.50*
bFCR	1.34±0.05	1.38±0.09	1.33±0.00	NS	*P=0.72*
%mortality	27.4±0.6	29.6±1.3	36.2±8.3	NS	*P=0.31*

Condition factor *K* was not affected by diet type, fish size or the interaction between diet type and fish size. The average ± stdev condition factor *K* for fish fed Nutrilis-C, FM18 or FM28 was 1.36±0.04, 1.39±0.07 and 1.39±0.04, respectively (n=4 pooling across fish size).

When considered holistically, the percent survival for stage 2 was 73.3% with a bFCR of 2.90 and an estimated production output of 5019kg. The latter figure represents an estimate of harvest biomass based on the number of surviving fish multiplied by the average weight of 200 fish sampled from each cage at the conclusion of the trial. The calculated eFCR for different aquafeeds at the end stage 2 was high and proved to be nonsensical due to the significant loss of biomass from many of the replicate cages. Mortality of fish across all treatments in stage 2 accounted for 1493kg in lost production.

Temperature dependent models of growth have been published for Asian seabass and it was of interest to benchmark the growth of fish in the trial against predicted weight gain. As can be seen in Figure 1, there was significant divergence in the average growth of fish over the course of the trial compared to published models, both before and after grading. However, when the average weight gain of the top 10% of Asian seabass was estimated from monthly assessment data, the model and trial data for these fish converge to a greater extent. This would appear to indicate that 1) despite sourcing "high quality" fingerlings from Singapore there was wide variation in the growth potential (i.e. genetics) of the stock used in our trial and 2) the growth rate of the top 10% of fast growing fish was not restricted by the nutritional quality of the trial feeds.

Effects of Diets on Carcass Composition

Several minor changes in carcass composition were noted in fish at the end of stage 1 and at in composition of fish at harvest time. For example ANOVA found no significant difference in the *as received* content of crude fat, ash, moisture energy or phosphorus of

Asian seabass fed different dietary treatments at the end of stage 1, but fish fed FM18 had a minor, but significantly lower protein content than fish fed the other diets.

Table 5. Mean (n=2 cages) production data (by size and feed group) at the end of stage 2 (i.e. harvest)

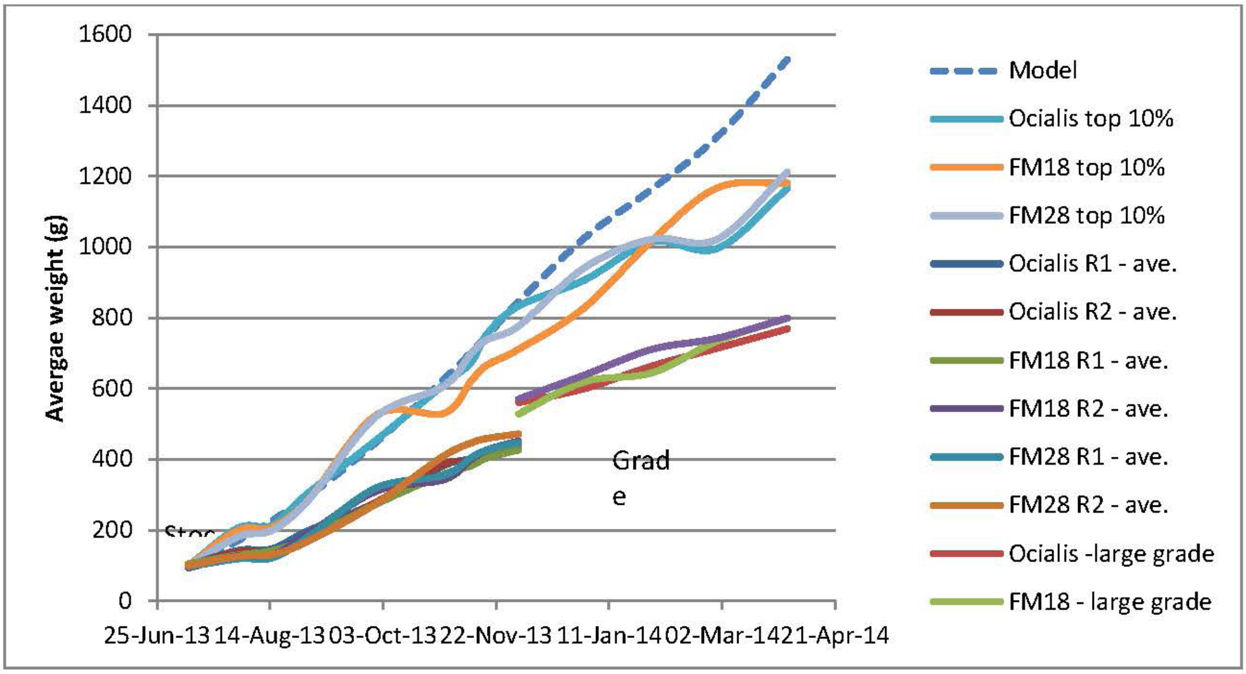
	Large Graded Fish Stage 2			Small Graded Fish Stage 2		
Treatment	**Nutrilis-C**	**FM18**	**FM28**	**Nutrilis-C**	**FM18**	**FM28**
Number fish stocked	937.0	913.5	932.5	837.0	871.0	750.0
Ave. stock weight ($gfish^{-1}$)	560.8	528.1	570.1	326.8	349.7	349.2
Est. stock biomass (kg)	525.9	481.7	531.1	273.5	303.8	261.8
Number mortalities	212.0	242.0	206.0	258.0	246.5	234.0
Number fish left	725.0	671.5	726.5	579.0	624.5	516.0
Est. biomass mortalities (kg)	141.1	160.0	141.0	104.7	103.0	96.8
Est. live biomass at harvest (kg)	557.8	535.3	581.8	280.2	308.0	246.6
Total biomass harvest (kg)	698.9	695.3	722.8	384.9	411.0	343.4
Total feed used (kg)	533.8	541.5	538.5	318.3	310.0	310.0
bFCR	3.1	2.5	3.0	2.9	2.9	3.8
%mortality	22.6	26.4	22.1	30.8	28.1	31.2
K condition factor	1.37	1.44	1.40	1.36	1.35	1.39
$SGR\%day^{-1}$	0.27	0.35	0.28	0.33	0.29	0.26

At harvest, the moisture content of Asian seabass reared on FM18 (while not significant) tended to be lower than fish fed FM28 and the commercial diet. However, we found no difference in the protein content of fish due to diet. Both ash and phosphorus content was affected by diet, with fish fed diet FM18 having a significantly lower ash content (5.38%; n=4) than those fed FM28 (6.16%; n=4). The ash content of fish fed Nutrilis-C was intermediate (5.78%; n=4) and did not differ from the ash content of FM18 or FM28. Phosphorus content of seabass fed FM18 was significantly lower than the phosphorus content (0.95%; n=4) than those fed FM28 (1.07%; n=4). The phosphorus content of fish fed Nutrilis-C was intermediate (1.03%; n=4) and did not differ from the phosphorus content of FM18 or FM28. The low body stores of phosphorus in fish fed FM18 might be indicative of low phosphorus availability from this diet, however the fact that the phosphorus content of fish fed Nutrilis-C was centered between the other two diets likely discounts this hypothesis (i.e. Nutrilis-C is high in fishmeal which is a generally a good source of available phosphorus). Availability of phytate bound phosphorus from plant proteins such as soybean meal is low compared to animal meals. For this reason care was taken during formulation to ensure that available phosphorus in the form of MCP was increased in FM18 and FM28. Fat content was significantly affected by diet type. In general, large graded fish were fatter than smaller graded fish and larger fish fed FM18 had a much higher fat content than larger fish fed the other two diets.

The fatty acid composition of whole fish (both small and large grades at harvest) was also examined to determine if longer term feeding of soy based diets, especially those with lower fishmeal and higher poultry meal content affected the level of beneficial fatty acids present in the flesh (especially omega n-3 such as EPA C20:5n-3 and DHA C22:6n-3). Results from ANOVA on the total sum of n-3 fatty acids (as mg $100g^{-1}$ sample) indicated a significant effect of diet type but not fish size.

Pooled across fish size, the total n-3 content of whole fish was significantly higher in fish fed Nutrilis-C (691.6 mg $100g^{-1}$; n=4) than fish fed FM28 (566.3 mg $100g^{-1}$; n=4). The n-3 content of fish fed FM18 (593.3 mg $100g^{-1}$; n=4) was not statistically different to fish fed FM28. Levels of EPA and DHA were not affected by fish size or the interaction term but were affected by diet type. Both EPA (118.8mg $100g^{-1}$; n=4) and DHA (261.1mg $100g^{-1}$; n=4) were significantly higher in fish fed Nutrilis-C; the aquafeed with the highest level of fish meal.

Figure 1. Plot of average weight gain of Asian seabass vs top 10% vs published model

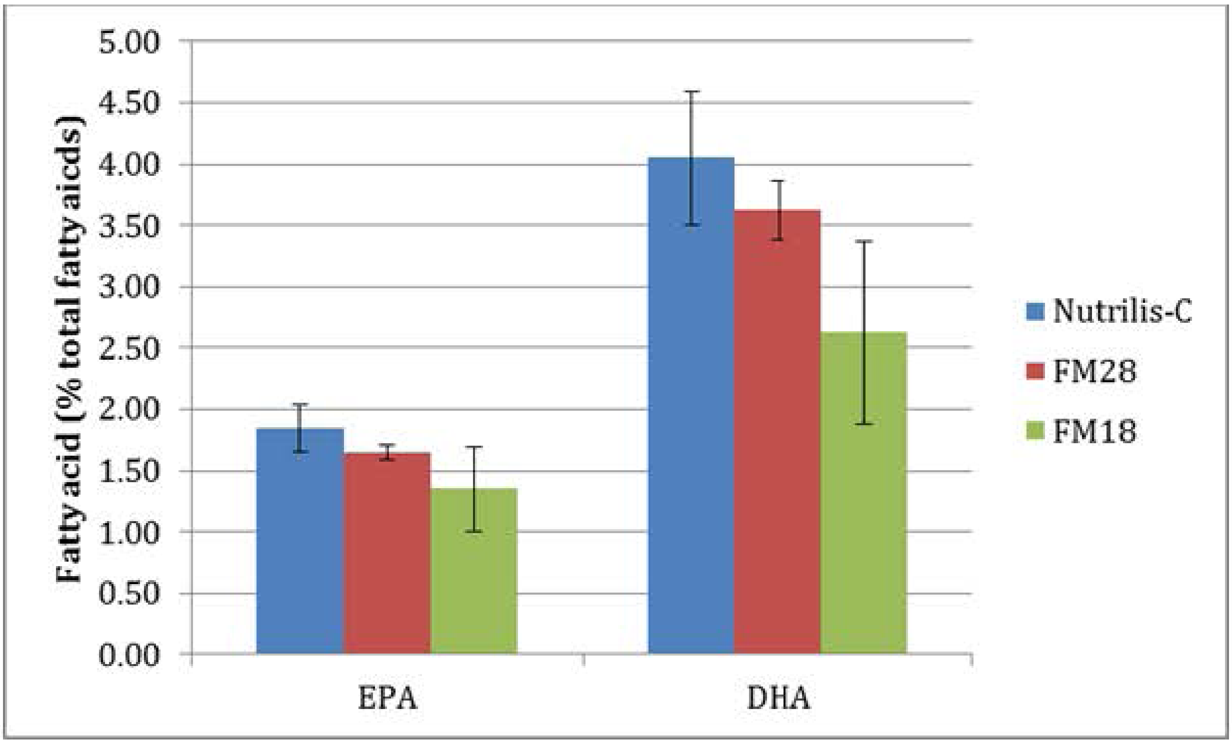


The sum of n-6 fatty acids was also significantly affected by diet type, but not by fish size. Fish fed FM18 had a significantly higher n-6 content (1291.6mg $100g^{-1}$; n=4) than fish fed FM28 (987.7mg $100g^{-1}$; n=4) or Nutrilis-C (1050.8mg $100g^{-1}$; n=4). There was a significant difference between the total PUFA content of fish fed FM18 (2067.9mg $100g^{-1}$; n=4) and fish fed FM28 (1689.3mg $100g^{-1}$; n=4). The PUFA content of fish fed Nutrilis-C was intermediate (1899.3mg $100g^{-1}$; n=4) and not different to fish fed the other diets. The content of saturated fatty acids (SFA) was affected by diet type and fish size, but interaction was absent. Smaller fish had lower total SFA content (2091.2 $100g^{-1}$; n=6) than larger fish (2438.6mg $100g^{-1}$; n=6) and fish fed FM18 had significantly higher SFA content (2577.4mg $100g^{-1}$; n=4) than fish fed FM28 (1985.6mg $100g^{-1}$; n=4). The SFA content of fish fed Nutrilis-C was intermediate (2231.8mg $100g^{-1}$; n=4) and not different from Asian seabass fed the other diets.

Most evidence suggests that the composition of body lipids tends to closely reflect that of the diet, especially over prolonged feeding. As all diets contained the same amount of fish oil (5%), variation in the content of the major protein meals and their constituent fatty acids would be the primary reason fatty acid composition of fish varied in this study. The fillet fatty acid composition of Asian seabass has been shown to vary when fishmeal or fish oil has been replaced with non-marine proteins such as meat meal and poultry meal. For example Asian seabass can be reared effectively on diets containing about 50% meat meal and 5.0 to 6.0% fish oil, however the content of SFA and some short chain MUFA increased and total PUFA of tissues decreased compared to Asian seabass reared on a fishmeal control. Our trial has indicated that feeding Asian seabass on diets high in poultry meal and soybean while reducing fishmeal content elevated MUFA content to some degree, but more striking was the systematic reduction in both EPA and the DHA content (see Figure 2). This is not unexpected as both poultry meal and soybean meal contain negligible or no EPA and DHA.

Effectively, these essential fatty acids have been "washed out" over the course of the experiment. Similar findings were found in Asian seabass fed diets that replaced fish oil with palm or poultry oil. Recent feeding experiments with juvenile Asian seabass where fishmeal and fish oil were replaced with vegetable oils rich in alpha-linolenic acid and defatted poultry meal showed that levels of linolenic acid increased in muscle tissues and liver in response to intake, but there was no corresponding increase in the n-3 lcPUFA content of tissues. Thus fish were not able to readily convert the precursor C18 alpha-linolenic acid to EPA or DHA.

Figure 2. Variation in EPA and DHA content of whole fish samples at the end of the trial



Although low in residual fat, the high inclusion level of SBM and SPC in FM18 and FM28 would be expected to leave some fatty acid signature for plant oils such as 18:2n-6 (linoleic acid). This was evident in fish fed FM18 more so than those fed FM28. Plant oils are deficient in the typical marine lcPUFA arachidonic acid (ARA, 20:4 n-6), EPA (20:5 n-3) and DHA (22:6n-3). One of the most widely used plant oils in aquafeeds, soybean oil is rich in 18:2 n-6 (52 %), 18:1 (24 %) and 16:0 (11 %) but due to its deficiency in lcHUFA, SBO and other plant oils are typically blended with fish oil when incorporated into fish diets.

Economic Evaluation

The economic appraisal of this study is based on the formulation cost of the 3 trial feeds as well as data collected on FCR. Ex-farm gate prices for whole round fish have also been used to estimate the net feed cost of production (i.e. the difference between the feed cost to produce 1kg of fish and the sale price for 1 kg of fish). Expenditure on other operating costs such as boats, travel, labour costs etc. has been ignored as these costs were shared across the whole MFV operation and pro-rata costs cannot easily be determined. Unfortunately, the broader economic outcomes are heavily influenced by the high level of mortality sustained during the experiment, although it is likely this fact would have little impact on the wholesale price offered in Vietnam for good quality fish reaching marketable size.

A broad overview of the whole trial, disregarding dietary allocations revealed the following. At start there were 15,156 fish averaging 100g stocked into the trial cages which equated to a starting biomass of 1,516kg. At the end of the trial there were 7,685 fish remaining with a total estimated biomass of 5,019kg. Feed inputs totalled 11,098kg returning an overall eFCR of 3.16 (i.e. eFCR = total feed input/biomass gain). Based on the average commercial feed cost of $US1.61/kg, the cost of producing 1kg whole fish was approximately $US5.08 (i.e. eFCR x feed cost in $US/kg). This estimate excludes all other production inputs such as purchase of fingerlings, feeding of fish prior to use in the trial, labour, overheads, fuel, maintenance etc.

The high overall eFCR is a direct result of poor survival rather than the effect of diet. This premise is supported by the reasonable bFCR recorded during stage 1. Regular monthly weight assessment of Asian seabass may have contributed to the running mortality experienced in small research cages and this strategy should be reviewed in future experiments; i.e. longer periods between weight assessment may reduce handling stress and associated mortality or use of larger circular cages (e.g. Polarcirkel) should be considered.

The culture of marine fish across Asia is still dominated by the use of trash or low value fish sourced from commercial fishing or artisanal fisheries. Many farmers perceive the performance of their fish to be superior when fed on trash fish compared to pelleted feeds and the price of trash fish is generally cheaper than commercial feeds. A recent multi-farm trial (see table below) compared the growth and FCR of Asian seabass fed a commercial feed (Thai Union Feedmill; $US1.50/kg; CP=45%, fat=10%, GE=18MJ/kg) versus low value fish. Fish were reared in small 3m x 3m sea cages similar to those used in our trial, stocked at about 30g body weight in April 2009 and reached an average harvest weight of about 600g in October – November the same year.

Overall survival was reported to be between 83 to 100% and SGR between 1.55 and 2.07%/day on either feed type. Food conversion ratio on pelleted feed ranged between 2.59 to 2.74 at different farms, meaning the feed cost of production on pelleted feed was approximately $US3.90 to $US4.11/kg fish produced. The average FCR of Asian seabass reared on low value fish over the same period was approximately 5.51, meaning feed cost of production on this resource was about $US1.92/kg fish produced (i.e. trash fish @ $US0.35/kg). Thus, although growth performance was similar on either feed type, cost of feed production was much lower for Asian seabass reared on low value fish.

At the time our study commenced in 2013 the delivered price of feed from Invivo-Ocialis, including taxes of 5% ranged between $US1.58 to $US1.72/kg. The price range of feed fluctuated predominantly due to minor changes in the exchange rate of $US and $VND dollars rather than changes in ingredient costs. Averaging over the life of the trial feed was purchased for $US 1.61/kg. The price of specialised hatchery feeds would be more expensive.

Summary of Similar Asian Seabass Study (Bunlipatanon et al., 2014)	Feed Type	
	Pellet feed	**Low value fish feed**
FCR	2.55	5.51
Total feed fed (kg)	144.7	302.8
Fish biomass increase (kg)	62.3	65.4
Feed cost $US	1.50	0.35
Feed cost production $US/kg	3.80	1.92

The formula cost of each feed was calculated using the cost of ingredients and premixes used at the time of manufacture (ca. July-August 2013). Ingredient and additive costs were obtained from Hammersmith Marketing Reports(http://hammersmithltd.blogspot.com.au/) and bulk commodity sellers such as Alibaba (http://www.alibaba.com) and contacts in the industry. As the precise formulation of the commercial feed was not disclosed the cost was based on the known ingredient levels provided by the Invivo-Ocialis nutritionist (Frederic Baron) and by keeping the inclusion of the additives the same as that used in FM18 and FM28. Commodity prices, formulations and estimated ingredient cost of trial feeds are presented in Table 6 and are based on output from a least-cost formulation software package (Winfeed 2.8 Release 3; Cambridge University, UK).

Based on formulation costs alone, FM28 and FM18 (i.e. the diets containing high levels of SBM and moderate levels of fishmeal) would be approximately 10.5% and 18.1% cheaper, respectively than the commercial feed containing 45% fishmeal. This equates to a dollar saving of approximately $US121 and $US209 per tonne of feed. These input cost savings would be significant if they were passed onto the farmer, however the base price does not account for additional costs such as manufacturing, profit margin, freight and relevant taxes. As stated above, trial feeds were delivered to Nha Trang for an average price of $US1610 per tonne including taxes. From the retail figures we might assume that manufacturing costs equate to about $US185 per tonne and a company profit margin of about $US350 per tonne is levied.

At the time Asian seabass were harvested (ca. April - July 2014), the highest ex-farm gate price achieved for whole fish > 700g was $US 3.10/kg ($VND 65,000 dong/kg). The lowest price offered for smaller batches of whole fish < 700g was about $US1.43/kg. Local ex-farm prices for Asian seabass are generally around $US3.00/kg while Pompano and Cobia achieve about $US4.00 and $US5.00/kg, respectively (Jorge Alarcon, *personal comm.;* General Manager MFV). The average yield of Trim B from whole fish was 56.4%, meaning the value of Trim B fillets was approximately $US5.50/kg (based on current ex-farm / market prices). If fish were destined for export sale as fillets, additional value chain expenses such as transport costs from farm to plant would add $US0.15/kg (dependent on volume transported), the processing fee would add $US1.25/kg, vacuum packaging another $US0.25/kg while transport to a major city such as HCMC from Nha Trang would add a further $US0.20/kg. This scenario would equate to a free on board (FOB) price in HCMC of approximately $US7.35 (Jorge Alarcon, *personal comm.*; General Manager MFV). By way of comparison, locally grown Asian seabass sold in Australian retail outlets can command as much as $US18.00/kg whole round and up to $US30.00-35.00/kg when presented as skinned fillets ($US1.00 = $AUD0.91). Imported Asian seabass fillets from Thailand retailed for approximately $US14.50/kg in 2009 and similar imports from Taiwan recently retailed for about $US18.00/kg (see associated graphics).

Results from our trial indicate the average feed cost of production (i.e. $US5.08) would exceed the highest wholesale price we obtained for whole fish (i.e. $US3.10) by almost $US2.00/kg!. According to the 2013/14 NSW DPI Fisheries provisional aquaculture report the farm gate price of farmed Asian seabass (Barramundi) produced in recirculating production systems in NSW averaged $AUD 15.94/kg whole round (i.e. ≈$US14.30/kg).

Table 6. Estimation of formulation costs for FM18, FM28 and Nutrilis-C based on commodity pricing circa July 2013

	Formulation (%)			Ingredient Cost	Pro-data Cost $US		
	FM18	FM28	Nutrilis-C	$US/Metric Tonne	FFM18	FFM28	Nutrilis-C
Wheat Flour	12	12	15.6	275	33	33	42.9
HP 300 – Hamlet SPC	5	5	0	900	45	45	0
Fish Oil	5.2	5.7	5.2	2150	111.8	122.55	111.8
SBM TTX 46.5/1.3-SBM	25.1	25.4	20	500	125.5	127	100
Poultry Meal POM 66	25	15	7.5	750	187.5	112.5	56.25
Blood Meal 93.6	6	6	5	10.53	63.18	63.18	52.65
Fish Meal – Premium 65	2.9	8.2	13.5	1550	44.95	127.1	209.25
Fish Meal Super Premium 68	15.1	19.8	31.5	1700	256.7	336.6	535.5
Mon Calcium Phosphate (MCP)	1.8	1.43	0.32	500	9	7.15	1.6
L-Lysine Sulfate 70	0.7	0.35	0.35	2000	14	7	7
DL Methionine (Pure)	0.53	0.46	0.1	4000	21.2	18.4	4
Vit C 35% Coated	0.07	0.07	0.07	3000	2.1	2.1	2.1
Choline Chloride 60%	0.1	0.1	0.1	900	0.9	0.9	0.9
Globatiox	0.024	0.024	0.024	4500	1.08	1.08	1.08
B-Glucans	0.1	0.1	0.1	3000	3	3	3
Globalmold	0.1	0.1	0.1	5000	5	5	5
Premix Fish	0.3	0.3	0.3	7000	21	21	21
				Formula Cost $US/Tonne	**$ 944.91**	**$ 1032.56**	**$ 1154.03**

Data on the estimated ingredient price of formulations, bFCR and sale price of whole round fish was used to compare the basic feed production costs of different diets during stage 1 (Table 7). Harvest weight and bFCR were constrained to 450g and 1.35, respectively to standardise the analysis because the average harvest weight and other biometric indices were not different among treatments at the end of stage 1. Savings due to formulation cost have a significant impact on the net value of whole fish at the end of stage 1. Formulations FM28 and FM18 increase the net value of whole fish by 23 and 39%, respectively above the net value of fish fed the high-fishmeal commercial formulation. Based on the constraints applied the net value of fish at the end of stage 1 on a per kilogram basis (whole round) was positive and ranged between $US0.71 and $US0.99.

Table 7. Basic economic evaluation of FM18, FM28 and Nutrilis-C during stage 1

		Nutrilis-C	FM18	FM28
A	Feed (est. formulation cost - WinFeed) $US/tonne feed	1,154.03	944.91	1,032.56
B	Feed (est. manufacturing costs) $US/tonne	185.00	185.00	185.00
C	Feed (est. margin) $US/tonne	350.00	350.00	350.00
D	Feed (est. tax & freight) $US/tonne	80.00	80.00	80.00
E	Est. feed price delivered Nha Trang $US/tonne (A+B+C+D)	1,769.53	1,560.41	1,648.06
F	Actual averaged feed price delivered Nha Trang $US/tonne	1,610.00	1,610.00	1,610.00
G	Formula saving $US/tonne vs Nutrilis-C	0.00	209.12	121.47
H	bFCR – constrained	1.35	1.35	1.35
I	Sale price whole round $US/kg	3.10	3.10	3.10
J	Harvest wt (kg) - constrained	0.450	0.450	0.450
K	Feed cost to produce 1kg fish whole round (H x E) $US	2.39	2.11	2.22
L	Net value of fish end of stage 1 $US/kg whole round (I-K)	0.71	0.99	0.88

A similar strategy was applied to assess the basic economic performance of stage 2 (Table 8), however in this case the average bFCR and harvest weight were constrained to 3.0 and 800g, respectively. Only fish from the largest grade was evaluated. Based on the sale price for whole fish of $US3.10/kg, the net value of fish during stage 2 was negative and ranged between $US-1.58 to $US-2.21. The fundamental reason for the negative return is high FCR. The overall appraisal of each diet (Table 9) was evaluated by calculating the total amount of feed given to each dietary group across stage 1 and stage 2 and calculating the total biomass gain as estimated at the end of the trial (i.e. eFCR). An estimate of lost biomass due to mortality was also tabulated. On a per kilogram basis this equated to 42, 44 and 46% of production, respectively for Nutrilis-C, FM18 and FM28 Economic FCR on an as fed basis ranged between 3.07 and 3.20 and bFCR ranged between 1.72 and 1.78. Based on estimates of bFCR (i.e. if they were achieved) the feeding cost of production would be highest for fish fed Nutrilis–C while modest savings could be made using FM18 and FM28. Under this scenario the net value of fish fed FM18 and FM28 would be positive rather than negative.

The General Manager MFV (Jorge Alarcon) predicts the cost of feeds for Asian seabass could be discounted by as much as 10-15% for large volume orders (subject to feedback from feed companies). This would potentially reduce the cost of Asian seabass feeds to $US1.35 - 1.45/kg delivered to Nha Trang. As discussed the production costs and losses estimated in this report are based solely on the feed cost. As a general rule, feeding cost of production represents about 60-80% of total production costs in a sea-cage farming system depending on how efficiently other major expenses such as fuel and labour are utilised. In Vietnam there is a slight advantage because of the low cost of labour, but larger farms, such as the one contracted for this study, are often a considerable distance from their shore base (i.e. MFV ≈ 25 km), so fuel costs tend to be higher. As a guide, the additional cost of production for marine fish in Vietnam is probably 25-30% above the feeding cost of production (Jorge Alarcon, *personal comm.;* General Manger MFV). Based on their experience in Vietnam and assuming ideal farming conditions (e.g. larger circular cages) coupled with improved biological results (e.g. eFCR of 2.3 or better) and discounted aquafeeds (i.e. $US1.45/kg), MFV estimates the baseline cost of production for cage-farmed Asian seabass would be about $US 4.45/kg. With additional marketing / promotion or future trends in popularity the farm gate price for whole round fish might conservatively reach about $US3.75. This would still result in a production loss of around $US0.70/kg, but probably represents a realistic appraisal of the local Vietnamese market in today's terms.

Table 8. Basic economic evaluation of FM18, FM28 and Nutrilis-C during stage 2

	Stage 2 Basic Economic Review	Nutrilis-C	FM18	FM28
E	Est. feed price delivered Nha Trang $US/tonne (A+B+C+D)	1,769.53	1,560.41	1,648.06
G	Formula saving $US/tonne vs Nutrilis-C	0.00	209.12	121.47
H	bFCR – constrained	3.0	3.0	3.0
I	Sale price whole round $US/kg	3.10	3.10	3.10
J	Harvest wt (kg) - constrained	0.800	0.800	0.800
K	Feed cost to produce 1kg fish whole round (H x E) $US	5.31	4.68	4.94
L	Net value of fish end of stage 1 $US/kg whole round (I-K)	-2.21	-1.58	-1.84

Table 9. Basic economic evaluation of FM18, FM28 and Nutrilis-C start to finish of trial

	Overall Trial Economic Review	Nutrilis-C	FM18	FM28
E	Est. feed price delivered Nha Trang $US/tonne (A+B+C+D)	1,769.53	1,560.41	1,648.06
G	Formula saving $US/tonne vs Nutrilis-C	0.00	209.12	121.47
I	Sale price whole round $US/kg	3.10	3.10	3.10
M	Start biomass (kg)	508.7	502.5	499.3
N	End biomass (live wt) (kg)	1715.8	1673.9	1657.8
O	Biomass loss (Stage 1+ Stage 2) (kg)	869.3	927.2	988.9
P	eFCR	3.07	3.20	3.18
Q	bFCR	1.78	1.77	1.72
K	Feed cost to produce 1kg fish whole round (Q x E) $US	3.14	2.76	2.83
L	Net value of fish end of stage 1 $US/kg whole round (I-K)	-0.04	0.34	0.27

Recommendations

- Despite the ongoing mortality experienced in this field trial, Asian seabass fed extruded aquafeeds containing optimized levels of soy product performed as well as Asian seabass fed a commercial diet high in fishmeal. For this reason we recommend repeating the trial or conducting similar trials in larger sea cages or at more protected sites. These strategies may reduce cage related mortality.
- The high level of background mortality experienced over the life of the trial precludes definitive recommendations on the efficacy of the feed formulations with respect to growth potential. However, the n-3 fatty acid content of Asian seabass fed FM18 and FM28 decreased compared to fish fed the commercial diet, illustrating that carcass composition was affected by our ingredient choice; that is increased use of poultry meal and decreased use of

fishmeal probably elicited these changes. As such future diets high in soybean meal and low in fishmeal may need to consider additional use of fish oil or use of finishing diets that are rich in n-3 to ensure n-3 content is kept at acceptable levels for consumers of these fish.

Future nutrition research on the use of SBM and SPC in feeds for Asian seabass should ensure that fingerlings are of the highest quality. This is critical for field experiments where control of many other research variables is lost. If possible, it would be prudent to conduct lab-based feeding trials at the same time as larger field based trials to confirm the validity of the results and the performance of Asian seabass fed soy optimized feeds.

Conclusions

This field trial was a joint collaboration between NSW DPI, MFV and Invivo-Ocialis. Considerable effort and in-kind support has been provided by the General Manager (Jorge Alarcon) and many staff employed by Marine Farms Vietnam. The purpose of the 10 month trial was to evaluate two aquafeeds containing high levels of soybean meal (30%) and reduced levels of fishmeal against a commercial Asian seabass feed. The trial was conducted in small replicated sea-cages north of Nha Trang under practical, real world conditions. The trial was initially stocked with 15,000 vaccinated advanced Asian seabass fingerlings in July 2013 at which time each of the feeds were randomly allocated to n=2 research cages. Fish were later graded into smaller and larger cohorts as per normal management practices, but original dietary allocations were retained such that 12 research cages were deployed. Fish were then on-grown aiming for an average market size of about 1000g. The trial was ended in April 2014 at which time fish were offered to the local market so performance and economic outcomes could be assessed. The major outcomes of the experiment were;

- There was no statistical difference between the harvest weight of fish fed Nutrilis-C (high fishmeal control diet) and fish fed FM18 or FM28. The soy-based trial feeds contained optimized levels of SBM and SPC and low levels of fishmeal and were formulated to the same digestible protein (DP) and digestible energy (DE) content but used varying levels of similar feed ingredients, premixes and additives.
- There was no major difference in the proximate carcass composition or nutrient retention of Asian seabass fed different feeds at the end of stage 1 or stage 2. However, fish fed FM18 had slightly lower ash and phosphorus content than fish fed FM28 or Nutrilis-C.
- At the end of the trial, larger graded fish were higher in fat and gross energy content than smaller graded fish.
- Diet type had an impact on fatty acid composition of fish (FAME). Fish fed soy-optimized diets containing lower levels of fishmeal had decreased n-3 fatty acid content (i.e. EPA and DHA) at the end of the trial compared to fish fed Nutrilis-C. In addition, n-6 fatty acid concentration was higher in fish fed FM18 and FM28 compared to Nutrilis-C.
- Fish growth slowed dramatically over the winter period. Specific growth rate declined from about 1.0%d^{-1} at 28°C to around 0.3%d^{-1} at 24°C.
- Collective mortality of Asian seabass during the experiment was high. Morbidity was higher in smaller graded fish then larger graded fish. The quality of the fingerlings imported from Singapore and the efficacy of the vaccination procedures applied to juveniles prior to stocking is questionable. Future nutrition trials with Asians seabass should consider use of larger circular cages (e.g. Polarcirkel) which may reduce mortality.
- The formulation cost of diets FM18 and FM28 were 10.5% and 18.1% cheaper, respectively than Nutrilis-C.
- Based on bFCR, the combined feeding cost of production (stage 1 + stage 2) was

calculated to be $US3.14, $US2.76 and $US2.83 per kg of whole fish for Nutrilis-C, FM18 and FM28, respectively.

Whole Asian seabass offered for sale at the end of the trial sold for between $US1.43 and $US3.10/kg. Fillet recovery (Trim B) was approximately 57%.

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Dr Booth is a Senior Research Scientist with 25 years' experience in aquaculture nutrition research at the Port Stephens Fisheries Institute (PSFI). He has worked on a variety of major national and international programs researching the nutritional requirements and development of aquafeeds for species such as silver perch, mulloway, yellowtail kingfish, barramundi, Atlantic salmon and prawns.

Dr Booth is currently focused on refining the nutritional requirements of yellowtail kingfish and developing sustainable aquafeeds which will underpin the expansion of this industry in NSW and other parts of Australia. He conducts feed development research and provides advice to a variety of commercial aquafeed companies in Australia.

Soy In Aquaculture Program

This technical paper was created through the USSEC Soy In Aquaculture (SIA) program and the USSEC Southeast Asian Regional Program. USSEC works with target audiences in Southeast Asia and globally to show the utility and benefits of using United States soybean products in aquaculture diets.

The SIA program replaces the Managed Aquaculture Marketing and Research Program (the AquaSoy Initiative, funded and supported by the United Soybean Board and American Soybean Association) which was designed to remove the barrier to soybean meal use in diets fed to aquaculture species.

The objective of the SIA is to optimize soy product use in aquaculture diets and to create a preference for U.S. soy products in particular, including but not limited to U.S. soybean meal, soybean oil, soybean lecithin, and "advanced soy proteins" such as fermented soy and soybean protein concentrate.

This paper follows the tradition of USSEC to provide useful technical materials to target audiences in the aquaculture industry.

For more information on soybean use in aquaculture and to view additional technical papers, please visit the Soy-In-Aquaculture website at www.soyaqua.org.

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