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Chapter 12

ORGANIC DIET DEVELOPMENT FOR BLACK TIGER PRAWNS

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ABSTRACT

The survival and growth of black tiger prawn (*Penaeus monodon*) juveniles (~3.3 g) were compared after feeding in tanks over one month with several prepared diets based on organically certified ingredients. The extrusion process in the manufacture of pelletised experimental diets was similar to processes used in commercial plants and was closely documented. The daily feeding rate (6% of starting mean body weight) was split equally into two feeds, one in the morning and one in the afternoon. All diets tested produced high survival (97-100%).

A widely-used commercial Australian prawn feed was used as a control diet. It contained 41.2% protein with 29.5 g kg^{-1} lysine, and produced the highest ($P<0.05$) growth (117% weight gain). Three of the experimental organic diets tested (namely, 1. wheat + soy, 2. pig weaner diet + soy, and 3. pig weaner diet + dried fish waste) produced moderate growth (73–77% weight gain). These contained 33%, 36% or 31% protein, respectively, and produced better ($P<0.05$) growth than diets utilising a range of other prospective ingredients (eg: wheat + dried scallop gut, wheat + fish waste, wheat + chickpea, or wheat + macadamia meal, containing 23%, 25%, 29% or 24% protein, respectively). An unfed control-treatment produced the lowest ($P<0.05$) growth (4% weight gain).

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The water stability of the experimental diets that produced the best growth was poorer than the commercial diet, suggesting that improvements in this aspect of these organic feed's manufacture could result in additional performance benefits and possibly reduced feed wastage. Analyses revealed a linear relationship between diet performance (in terms of weight gains) and the protein and lysine contents of diets. About 70% of diet performance was explained by these factors. The superior performance of the commercial diet could be attributed primarily to its formulation using mainly marine proteins, as well as a range of other unknown factors (commercial in confidence). These other factors range from use of feed attractants, better knowledge of ingredient nutrient availability, different extrusion conditions and the use of other unspecified micro-nutrients not present in the experimental diets.

The organic diets studied still require a degree of fine-tuning before structured commercial uptake. This would sensibly include further detailed investigations of the composition and nutrient availabilities of these and other organic dietary ingredients, and refinement of the extrusion process for formulated diets.

INTRODUCTION

The black tiger prawn, *Penaeus monodon*, has traditionally been the most commonly farmed marine prawn species in Australia and the world. It is a hardy species that can grow to large sizes at high densities and withstand low salinities in outdoor ponds. This later attribute makes it suitable for culture in inland areas, as has been demonstrated in Thailand over the last decade. There, disease outbreaks in coastal areas have encouraged farmers to move operations inland, into freshwater environments which have a lower incidence of such pathogens. It is prudent for Australia and other countries to also investigate this strategy, given the potential future effects of market globalisations and inadvertent disease translocations.

Several other countries including Brazil, China, Ecuador, India, Israel, Mexico, Peru, and the United States have fostered the development of inland prawn farms (Boyd 2002). In the face of failing coastal operations, and indeed through new opportunities created, these inland practices have provided great economic benefits despite some environmental concerns for adverse impacts on agricultural soils and freshwater qualities (Flaherty *et al.*, 2000). In Queensland (Qld), Australia, the focus of inland prawn farming opportunities is more on the expansive marginal areas of land with pre-existing soil salination problems (Collins *et al.*, 2005). Whilst offering a productive use of these existing salt basins, this land use would not compromise soil qualities and would provide better opportunities for containing environmental impacts.

During proof of concept work by DPI&F at a demonstration farm in Queensland, these environmentally and socially responsible aspects of farming prawns in salt degraded tracts of land were recognised to align well with the pursuits of certified organic food production systems. Their geographical isolation would avail high biosecurity and low physical and chemical contamination risks, and the large tracts of otherwise unproductive land that could be available for such developments could permit low intensity production systems to operate according to the basic organic principles of natural systems with low power inputs.

Accordingly, our previous investigations into organic prawn production focussed on extensive production systems in inland areas (see previous article in this publication).

It was also expected that low culture densities would be necessary to adhere to organic certification standards; we had previously assumed that larger ponds (eg: 2–10 ha) on low-cost salt affected parcels of land, low stocking densities of prawns (up to 10 m^{-2}), and management regimes which minimised the most expensive input components (labour, electricity, fuel and feed), would be necessary for organic prawn farming to be economically viable in Australia. We had anticipated that the infrastructures and overheads of existing coastal prawn farms, which were geared to semi-intensive production (30-40 prawns m^{-2}), could not accommodate the lower production capacities of an extensive approach, even with the price premiums offered by certified organic product. This caused us to initially focus our work on the more herbivorous banana prawn, *Penaeus (Fenneropenaeus) merguiensis*, and simple feed inputs which provided pond fertilisation for increased natural feeds as well as some direct feeding.

Whilst these assertions may still be true to varying degrees, interest in certified organic prawn production from existing coastal farmers wishing to improve the competitiveness and marketing opportunities for their products prompted us to review several of these guiding preconceptions. Firstly, since prawns are a schooling species in nature, a strong argument can be made for higher culture densities in organic standards. Perhaps much higher than the densities that can be sustained by natural pond productivities alone. To accommodate higher culture densities, a more balanced diet that is consumed directly would be necessary.

Secondly, the banana prawn was more problematic than black tiger prawns in our previous low-salinity farm trials. Several physical and biological factors are thought to be responsible for these difficulties, including:

a) the tolerances of these species for very low salinities and various ionic imbalances that can exist in the source ground waters
b) the tolerances of these species for high temperatures which prevail during the growing season in such areas, and
c) changes to these tolerances in aging prawns.

A greater focus on the more tolerant black tiger prawn was therefore recommended. These directional changes also increased the relevance of the present work to the existing Australian industry, namely coastal *P. monodon* farmers interested in differentiating their products with organic certification.

Consequently the present study:

1) focused on developing an improved organic diet for *P. monodon* cultured at moderate densities (~20 m^{-2})
2) examined a number of new potential organic feed ingredients which could potentially be supplied in sufficient quantities for commercial use
3) used a similar feed testing environment to that previously applied to banana prawns, and
4) manufactured the experimental feeds using a documented extrusion process not unlike those used by commercial feed mills.

MATERIALS AND METHODS

Experimental Tank System

The experimental tank system used for the trial was the same as that described in previous growth and survival research with banana prawns at the Bribie island Aquaculture Research Centre (BIARC) (Palmer *et al.*, 2005). This experimental system was managed in a similar way to that described by Palmer and Slattery in a preceding article contained in this collection.

Experimental Design

Nine experimental treatments (T) included:

T1 - an unfed control used to demonstrate the minimum growth likely from the low level of natural food sources present in supply water and tanks.

T2 - a commercial reference diet (Ridley Aquafeed Prawn Starter #2) used to demonstrate the maximum growth possible in the testing system.

T3-9 - seven different feed formulations (diets 3 – 9 as displayed in Table 1) based on preliminary calculations made for ingredient moisture and protein contents. Dietary markers were also added for future analyses not reported here.

Table 1. Experimental diet composition – ingredient amounts (g) estimated to yield > 1 kg dry matter (DM) of each diet

Treatment	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Name	Wheat fish	Wheat scallop	Wheat soy	Wheat chickpea	Wheat macadamia	Weaner fish	Weaner soy
Ingredients							
Wheat flour	950	950	550	550	550	0	0
Pig weaner diet	0	0	0	0	0	950	550
Fish frames*	190	10	190	190	190	190	190
Scallop gut*	10	190	10	10	10	10	10
Soy flour	0	0	400	0	0	0	400
Chick pea flour	0	0	0	400	0	0	0
Macadamia meal	0	0	0	0	400	0	0
Molasses	50	50	50	50	50	50	50
Casein	50	50	50	50	50	50	50
Kelp meal	10	10	10	10	10	10	10
Markers**	8.5	8.5	8.5	8.5	8.5	8.5	8.5
TOTAL	1268.5	1268.5	1268.5	1268.5	1268.5	1268.5	1268.5
~DM yield	1152	1152	1175	1171	1152	1152	1175
~Protein %	32.2	32.2	41.1	34.8	31.6	38.8	44.9

* Oven dried at <60°C;

** Titanium dioxide 3.3 g, barium hydroxide 3.3 g and celite 10.0 g.

Table 2. Experimental feed ingredients, actual costs for the project in Australian dollars, and expected costs with an industrial bulk supply (estimates only)

Ingredient	Product description	Supplier, location	Project cost (price per kg)	Anticipated industrial costs per tonne*
Wheat flour	Organic high protein white flour (HPWUB25)	Kialla Pure Foods Pty Ltd, Greenmount, Qld 4359	\$38.27 / 25 kg (\$1.53 / kg)	\$473
Fish frames	Diver whiting frozen waste - heads, frames and gut	Pinzonne and Sons, Warana, Qld 4575	Free sample	\$1200 **
Scallop gut	Saucer scallop waste – gut and gonad	Queensland Sea Scallop Ltd, Burnett Heads, Qld 4670	Free sample	\$1200 **
Soy flour	Organic soy flour (SF25)	Kialla Pure Foods Pty Ltd, Greenmount, Qld 4359	\$53.82 / 25 kg (\$2.15 / kg)	\$900
Chick pea flour	Organic besan flour (BES25)	Kialla Pure Foods Pty Ltd, Greenmount, Qld 4359	\$52.35 / 25 kg (2.09 / kg)	\$450
Macadamia meal	Dried macadamia by-product after oil extraction	Mr L. Minter, Ewingsdale, NSW 0481	Free sample	\$500
Weaner pig diet	Organic pig weaner mash (FFPWM25)	Kialla Pure Foods Pty Ltd, Greenmount, Qld 4359	\$750.19 / tonne (\$0.75 / kg)	\$750
Molasses	Stockfeed-grade organic certified molasses	Rocky Point Sugar Mill, Woongoolba, Qld 4207	\$220 / 200 L (\$1.10 / L)	\$1,100
Casein	2nd grade floor sweepings	Total Food Tech	\$5 / kg	\$10,000
Kelp meal	Imported kelp from sustainable harvest	Mr L. Minter, Ewingsdale, NSW 0481	Free sample	\$2,500

* Based on market prices of raw ingredients at 25th June 2007, and allowing a 50% price premium for organic production. Comparative figures for non-organic pig weaner were \$475 t^{-1}, with non-organic wheat \$315 t^{-1} (drought price), non-organic soy meal \$448 t^{-1}, non-organic full fat soy \$600 t^{-1} and non-organic chick pea gradings \$300 t^{-1} allowing \$35 t^{-1} diet mixing/processing costs.

** Non-organic imported fishmeal was \$1200 t^{-1}, and we assumed an equivalent cost for fish and scallop processing waste after drying.

Treatments were assigned randomly to each tank (Appendix Table A1) in a completely randomised design. There were three replicates for all treatments except treatment one which had two (due to the availability of only 26 tanks for the experiment).

Experimental Feed Ingredients and Diet Preparation

All ingredients used in experimental feeds were either organically certified, or what was assumed to be organically certifiable with a reasonable rationale. Table 2 provides a list of the feed ingredients, the respective product description, the supplier, the price (AU$) paid for the product for the trial, and an estimate of the expected cost of bulk supplies when produced in industrial quantities.

The manufacture of extruded diets based on the above formulations and raw materials was contracted to Dr Peter Torley, School of Land and Food Sciences at The University of Queensland. This was undertaken to ensure a controlled and documented extrusion process was undertaken for each of the diets. Pictures of the laboratory-scale extrusion system that was utilised are provided in Appendix Figure A1. The processing was similar in nature to that which could be used in an industrial plant. The report detailing extrusion conditions that were applied is available on request from the corresponding author. Since this extrusion process did not provide a completely dry product, the extruded material was dried overnight in an oven at <60°C before being crushed with a corn cracking machine and sieved to retain the 1–2 mm fraction. All experimental feeds were prepared one week before the trial commenced, and were stored in sealed plastic jars at 4°C. Daily aliquots for each tank were weighed to the nearest 0.01 g from these bulk packs each day.

Pellet Water Stability Assessments

All pellet diets (including the control) were qualitatively assessed for their water stability in high salinity (36 ppt.) seawater at 26°C. Two grams of each feed was immersed in still seawater (2 L) in transparent hemispherical fish bowls with regular observations and physical comparisons made thereafter. At 5, 10 and 20 min and 1, 3 and 6 h after submersion, each diet was mixed with a small spoon and allowed to settle. Comparisons focused on any apparent differences in the experimental diets with the commercial control diet, in terms of the texture and integrity of the pellet, and the clarity of water in each bowl. A series of photos of each bowl were also taken after the 3 h inspection.

Prawn Acquisition, Transport, Handling and Tank Management

Juvenile prawns for the trial were obtained from a commercial coastal prawn pond at Gold Coast Marine Aquaculture Pty Ltd, Woongoolba, Qld, following clear health and histological examinations performed one week earlier. They were collected from feed trays repeatedly baited with commercial prawn feed. To maximise survival during handling and transport, this was undertaken from early to mid morning to minimise water temperatures. Trays were lifted periodically and captured prawns were tipped into bins filled with pond water. They were gently counted into smaller buckets and then carefully released into an 800 L fibreglass transport tank supplied with oxygen. The transport tank was previously half-filled with the same pond water, and after adding the prawns was slowly topped up with clean filtered seawater before transport to BIARC.

On arrival at BIARC the prawns whilst still in the transport tank were given a slow (1 hr) 100% water exchange using unfiltered seawater from the same supply that was supplying the tanks in the experimental unit. They were then funnelled out of the transport tank's 50 mm bottom drain, directly into a 4,000 L parabolic tank filled with seawater, where they were held overnight to screen out any prawns damaged during the transfer. During this time they were fed once with commercial prawn feed at approximately 6% of body weight. Unlike the banana prawns in a previous trial, these tiger prawns had come from a commercial farm that was following normal culture routines, and thus were accustomed to the availability of artificial pellet diets.

Only healthy prawns were used to set up the experiment the following day. The trial began when 20 randomly collected prawns were stocked into each tank after weighing their collective bulk wet weights. Feeds were added to the tanks twice per day on the opposite side of the tank to the overflow point. The selected feeding rate of 6% of body weight per day followed conventional feeding charts and was derived from the overall mean prawn biomass weight across all tanks at the start of the trial. This was calculated to be 4 g daily, which was equally split into a morning and afternoon feed (2 g per tank).

The day after stocking, each tank was inspected for mortalities or unhealthy individuals during a short period without air or water supply. This yielded 1 dead prawn in tank 1 and one dead prawn in tank 9. These were weighed and replaced with two healthy weighed spare prawns that were left in the parabolic tank from allocations the day before. Starting weights for each of these tanks were adjusted accordingly. From this point no further mortalities were detected in the experimental tanks. As with previous trials, silt and detrital build up on tank bottoms remained undisturbed until just before harvest and trial termination 28 days later, when it was siphoned to waste prior to prawn collection with soft nets.

Data Collection and Statistical Analyses

Max/min water temperatures were recorded daily from tank 10 which was located in the middle of the tank complex. On several occasions during the trial, water pH, temperature and dissolved oxygen readings were taken from each tank to determine between-tank consistencies in water qualities. Qualitative measures of prawn activity, survival and condition were undertaken the day after stocking and every few days thereafter. Prawn bulk weights (total prawn biomass per tank) were measured before stocking and after harvest for each tank. In weighing procedures, the time prawns spent in a net draining before being placed into a tared bucket of seawater was standardised at 10 sec. The number of prawns in each bulk weight was also recorded. The general condition of prawns in each tank was also qualitatively assessed.

Data were analysed using GenStat® for PC/Windows XP, Eighth Edition. Analyses of variance were performed for continuous data followed by comparisons of means using least significance difference (LSD) testing with a 5% level of significance. Percentage survival data were analysed using a generalised linear model (McCullagh and Nelder, 1989) with the binomial distribution and logit link (GenStat, 2005), followed by protected t-tests to determine significant differences between the means.

RESULTS

Pellet Stability in Water

All pellets sank to the bottom of the water column in a similar fashion. The pellet form of the commercial reference diet remained stable throughout the trial period and it did not have any apparent effect on water clarity. Experimental diets 3, 4, 6 and 8 also displayed acceptable stability for the first 20 min. Of these, diets 3, 4 and 6 remained stable after 6 h, however diet 8 began partial disintegration at 1 h, and although it did not affect the clarity of water its pellet form was fully compromised after 6 h.

On the other hand, diets 5, 7 and 9 began leaching material soon after submersion causing water clouding. Diets 5 and 7 had a particularly pronounced effect on water clarity by the 5 min mark. The pellet form of all three of these later diets was markedly compromised after 1 h in seawater. Photos taken of the bowls containing each diet at the 3 h interval are provided in Appendix Figure A2.

Feed Ingredient Proximal Analyses

Table 3 provides some previously documented proximal analyses for similar feed ingredients, and information that could be supplied by the manufactures of feeds and ingredients used in the study. Table 4 provides the analysed proximate and amino acid results (DM basis) of all major ingredients used in the experimental diets, and Table 5 provides their fatty acid compositions. These analyses were performed by DPI&F laboratories at the Animal Research Institute, Yeerongpilly Qld. Table 6 provides estimates of the nutrient contents of the experimental diets as calculated from ingredients.

The commercial prawn feed bag label indicated the diet as having an energy level of 18.66 MJ kg^{-1}, and was made from ingredients that include steam dried fish meal, squid meal, squid liver meal, antioxidant–treated marine and vegetable oils, oilseed meal, wheat flour, phospholipids, vitamins and minerals. The organic pig weaner diet that was used as the base in two of the experimental diets was certified by the Organic Food Chain 0451. It had a list of ingredients that included wheat flour, corn, wheat, fishmeal, meat meal, soybean meal, sunflower or safflower meal, and a mineral mix. Since it is normally produced in a coarse mash for piglets, prior to inclusion in our diets it was ground to a powder in a hammermill.

Tank Trial Conditions

Careful inspections conducted the day after juveniles were stocked into the experimental tanks revealed 1 dead prawn in each of tanks 1 and 9. These were weighed and replaced as discussed previously. All remaining prawns in the tanks appeared active and healthy at that time and faeces were apparent on the bottoms of all tanks. Over the next few days, there were

Table 3. Feed ingredient manufacturer's information and other sources (% air dry basis)

Feed type	Source data	Moist -ure	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Ash	Calcium	Phos-phorus	Energy
Wheat feed flour	Tacon, 1990	12.0	11.7	1.2	1.3	73.3	0.5	0.03	0.18	-
High protein white plain wheat flour	Manufacturer spec	13.0	13.5	1.2	3.8*	70.6**	-	-	-	15.04
Dried catfish processing waste	Tacon, 1990	-	42.0	35.0	-	-	16.0	5.40	2.80	-
Dried whiting processing waste ^	DPI – see Table 4									
Dried scallop processing waste^^	DPI – see Table 4									
Mill feed soy flour	Tacon, 1990	10.3	12.9	1.7	32.5	37.9	4.7	0.41	0.18	-
Soya flour	Manufacturer spec	10.0	43.0	20.7	12.7*	22.4**	-	-	-	18.51
Brown chick pea flour	Manufacturer spec	13.0	22.4	6.7	10.8 *	48.0**	-	-	-	15.31
Macadamia meal	DPI									
Pig weaner diet	Manufacturer spec	-	21.9	-	3.29	-	-	-	-	14.2
Molasses	Nutr.Data.com (2006)	21.9	0.0	0.1	0.0	74.7**	3.3	0.21	0.03	-
Molasses	Manufacturer spec	31.4	4.8#	-	-	60.8**	12.2	0.71#	5.06	-
Casein	DPI – see Table 4									
Kelp meal	DPI – see Table 4									
Commercial prawn feed	Manufacturer spec	-	43.0	6.0	3.0	-	13.0		-	-

* dietary fibre.

** total carbohydrate; #data from different batch of molasses produced at same mill in 2002.

^ Original material DM 21.5%.

^^ Original material DM 13.4%.

Table 4. Proximate chemistry and amino acid composition of organic prawn diet ingredients on a dry matter (DM) basis

Parameter	Unit	Wheat flour	Fish frames oven-dried	Scallop gut oven-dried	Soy flour	Chick pea flour	Macadamia meal	Pig weaner diet	Molasses	Casein	Kelp meal	Fish frames freeze-dried*	Scallop gut freeze-dried*
Total DM	%	87.1	19.7	12.4	90.5	89.9	90.1	92.1	75.6	89.0	82.3	19.7	12.4
DM	%	87.1	97.4	97.2	90.5	89.9	90.1	92.1	75.6	89.0	82.3	94.4	93.9
Ash	%	0.7	29.6	35.6	5.0	2.3	1.7	8.8	-	3.9	28.6	32.7	34.7
N	%	2.68	10.21	8.32	6.79	4.57	2.11	3.78	-	14.96	1.06	9.95	8.74
Protein	%	16.75	63.81	52.0	42.44	28.56	13.19	23.63	0.0	93.5	6.63	62.19	54.63
Fat	%	1.6	5.1	2.9	22.2	5.7	13.9	6.5	-	0.1	2.9	5.0	4.2
Crude fibre	%	7.0	0.2	8.3	4.3	0.6	37.1	3.7	-	1.0	-	-	-
NDF	%	1.7	-	-	6.6	3.1	47.0	13.8	-	1.3	-	-	-
ADF	%	0.3	-	-	5.4	1.1	39.2	5.6	-	1.6	-	-	-
GE	MJ	18.55	16.58	13.97	23.62	19.87	22.53	18.76	-	23.71	-	-	-
Ca	%	0.03	10.36	3.95	0.21	<0.1	<0.1	1.79	-	<0.1	-	-	-
P	%	0.16	3.79	0.61	0.63	0.25	0.24	1.05	-	0.34	0.12	4.01	0.64
Amino acids													
Aspartic Acid	g/kg	6.57	72.09	58.63	62.28	32.69	14.32	14.53	-	73.69	7.83	73.74	63.29
Threonine	g/kg	4.00	22.70	15.99	14.30	8.60	3.96	7.48	-	35.39	2.17	21.34	17.37
Serine	g/kg	6.91	21.23	16.95	18.17	12.91	4.78	9.12	-	48.32	2.07	20.84	18.00
Glutamic acid	g/kg	59.46	106.43	77.01	96.14	46.89	28.96	40.08	-	233.48	12.60	105.31	85.63
Proline	g/kg	15.34	26.71	16.56	18.42	9.97	4.42	14.66	-	90.88	2.30	27.30	18.94
Glycine	g/kg	5.15	39.95	46.02	13.56	8.99	4.80	13.29	-	14.19	2.30	39.75	49.26
Alanine	g/kg	4.32	43.49	23.09	18.20	9.90	5.19	10.57	-	27.17	3.31	43.49	26.49
Valine	g/kg	5.32	23.16	15.33	15.97	9.75	4.34	8.28	-	51.13	2.42	23.09	17.09
Isoleucine	g/kg	4.99	20.62	14.72	16.85	10.52	3.57	7.48	-	42.71	2.04	20.43	16.36
Leucine	g/kg	9.55	36.26	24.12	27.85	17.80	6.62	13.93	-	78.84	3.41	36.53	27.06
Tyrosine	g/kg	4.30	12.72	9.72	9.94	6.65	3.12	5.42	-	43.56	0.92	12.43	10.52

Table 4. (Continued)

Parameter	Unit	Wheat flour	Fish frames oven-dried	Scallop gut oven-dried	Soy flour	Chick pea flour	Macadamia meal	Pig weaner diet	Molasses	Casein	Kelp meal	Fish frames freeze-dried*	Scallop gut freeze-dried*
Phenylalanine	g/kg	7.03	16.52	11.65	15.37	15.47	3.18	8.37	-	41.77	1.96	16.60	12.73
Lysine	g/kg	2.95	55.25	30.54	30.33	15.29	5.49	11.23	-	73.30	2.85	51.36	33.46
Histidine	g/kg	3.08	9.43	6.69	7.97	6.62	2.11	4.94	-	22.39	0.59	9.94	8.39
Arginine	g/kg	5.81	24.33	24.54	24.97	28.50	11.96	13.87	-	31.30	1.99	31.84	28.16
Tryptophan	g/kg	2.00	5.00	4.47	5.73	2.41	1.27	1.96	-	12.51	0.49	5.49	4.78
Cystine	g/kg	4.16	6.46	7.09	8.94	4.42	4.33	4.53	-	3.79	1.32	6.47	7.45
Methionine	g/kg	3.02	20.22	11.76	8.59	4.64	2.46	5.43	-	36.20	1.54	18.18	11.90

* Provided for general comparison with the oven-dried product used in experimental diets.

Table 5. Fatty acid composition (mg g^{-1} of dry sample) of organic prawn diet ingredients

Fatty acid	Wheat flour	Fish frames oven-dried	Scallop gut oven-dried	Soy flour	Chick pea flour	Macadamia meal	Pig weaner diet	Molasses	Casein	Kelp meal	Fish frames freeze-dried*	Scallop gut freeze-dried*
14	-	1.2	0.5	20.9	-	0.8	0.5	-	1.1	1.4	1.0	0.5
14:1n-5	-	-	-	-	-	-	-	-	0.1	-	-	-
15	-	0.4	0.1	-	-	-	0.1	-	0.1	0.1	0.3	0.1
16	2.5	6.1	3.4	-	5.9	10.9	10.0	0.3	3.3	2.2	6.2	3.1
16:1n-7	-	1.4	0.5	-	-	21.5	0.4	-	0.1	0.2	1.4	0.5
17	-	0.6	0.4	-	-	-	0.3	-	0.1	0.0	0.5	0.3
17:1n-8	-	-	-	-	-	-	-	-	-	-	-	-
18	0.2	4.1	1.9	8.4	0.7	4.6	4.8	0.0	1.4	0.2	3.8	1.8
18:1n-9	2.1	3.4	0.7	52.6	12.0	69.0	20.8	0.2	2.5	8.3	3.6	0.6
18:1n-7	0.1	1.5	0.5	2.8	0.6	4.8	0.8	-	0.1	0.1	1.5	0.4
18:2n-6	8.3	0.2	0.4	106.8	30.2	2.7	24.3	0.5	0.3	1.5	0.4	0.2
19	-	-	-	-	-	-	-	-	-	-	-	-
18:3n-3	0.4	-	0.2	13.0	1.5	0.2	2.3	0.1	0.1	0.4	0.1	0.1

Table 5. (Continued)

Fatty acid	Wheat flour	Fish frames oven-dried	Scallop gut oven-dried	Soy flour	Chick pea flour	Macadamia meal	Pig weaner diet	Molasses	Casein	Kelp meal	Fish frames freeze-dried*	Scallop gut freeze-dried*
18:4n-3	-	-	0.4	-	-	-	-	-	-	0.2	0.1	0.3
20	-	0.3	0.3	0.7	0.3	3.6	-	-	-	0.1	0.2	-
20:1n-11	-	0.8	0.3	-	-	-	-	-	-	0.0	0.7	0.3
20:1n-9	0.1	0.2	0.2	0.4	0.2	3.1	0.2	-	-	0.0	0.2	0.2
20:1n-7	-	0.1	-	-	-	0.2	-	-	-	-	0.2	-
20:2n-6	-	-	-	-	-	-	-	-	-	0.2	0.2	-
20:3n-6	-	-	-	-	-	-	-	-	-	-	-	-
20:4n-6	-	0.8	1.1	-	-	-	-	-	-	0.9	2.7	0.5
20:3n-3	-	-	-	-	-	-	-	-	-	0.0	-	-
20:4n-3	-	-	-	-	-	-	-	-	-	-	-	-
20:5n-3	-	0.5	1.3	-	-	-	-	-	-	0.4	2.8	0.9
22	-	0.3	-	0.9	0.2	1.1	0.3	-	-	0.0	0.2	-
22:1n-11	-	-	-	-	-	-	-	-	-	-	-	-
22:1n-9	-	-	-	-	-	0.3	-	-	-	-	-	-
22:1n-7	-	-	-	-	-	-	-	-	-	-	-	-
22:4n-6	-	0.2	0.2	-	-	-	-	-	-	-	0.6	-
22:3n-3	-	-	-	-	-	-	-	-	-	-	-	-
22:5n-6	-	-	0.5	-	-	-	-	-	-	-	0.5	0.2
24	-	0.3	-	0.3	-	0.6	-	-	-	0.0	0.4	-
22:5n-3	-	0.2	0.1	-	-	-	-	-	-	-	1.0	-
22:6n-3	-	1.0	4.5	-	-	-	0.3	-	-	-	5.8	2.4
24:1n-9	-	0.4	-	-	-	-	-	-	-	-	0.3	-

* Provided for comparison with oven-dried product used in experimental diets.

Table 6. Calculated organic prawn diet nutrient contents

Diet Total DM content	Unit	2 Commercial (control) diet	3 Wheat fish	4 Wheat scallop	5 Wheat soy	6 Wheat chick pea	7 Wheat macadamia	8 Weaner fish	9 Weaner soy
Ash	%	7.33	5.63	6.49	6.92	6.11	5.93	11.51	10.33
N	%	6.6	4.02	3.74	5.28	4.6	3.88	4.92	5.8
Protein	%	41.23	25.12	23.39	32.99	28.78	24.22	30.74	36.25
Fat	%	5.88	1.92	1.6	8.1	3.15	5.6	5.53	10.19
GE	MJ	18.29	16.3	15.9	18.0	16.9	17.7	19.7	22.4
Ca	%	1.23	1.65	0.72	1.71	1.66	1.66	3.54	2.96
P	%	0.99	0.71	0.25	0.85	0.74	0.73	1.36	1.23
Amino acid									
Aspartic acid	g/kg	49.16	18.75	16.78	35.49	26.58	21.12	24.77	38.98
Threonine	g/kg	13.42	7.65	6.67	10.78	9.06	7.68	10.32	12.33
Serine	g/kg	14.56	9.9	9.27	13.35	11.75	9.33	11.77	14.43
Glutamic acid	g/kg	97.14	66.23	61.94	77.87	63.04	57.74	54.55	71.11
Proline	g/kg	19.99	18.07	16.59	19.16	16.61	14.96	18.18	19.22
Glycine	g/kg	16.05	10.54	11.4	13.11	11.73	10.49	16.63	16.64
Alanine	g/kg	22.35	10.81	7.85	15.01	12.51	11.12	15.5	17.73
Valine	g/kg	16.01	9.2	8.06	12.45	10.57	8.96	11.55	13.81
Isoleucine	g/kg	15.09	8.27	7.41	11.87	9.96	7.9	10.26	13.03
Leucine	g/kg	25.82	15.19	13.43	20.77	17.73	14.41	18.73	22.82
Tyrosine	g/kg	8.79	6.57	6.13	8.31	7.31	6.26	7.55	8.88
Phenylalanine	g/kg	12.91	8.98	8.27	11.55	11.55	7.9	10.22	12.27
Lysine	g/kg	29.49	13.43	9.84	21.65	17.12	14.22	19.53	25.19
Histidine	g/kg	8.59	4.43	4.03	5.93	5.51	4.17	5.9	6.77
Arginine	g/kg	19.74	9.05	9.08	14.85	15.85	10.95	15.11	18.36
Tryptophan	g/kg	4.06	2.63	2.55	3.77	2.77	2.43	2.68	3.8
Cystine	g/kg	6.18	4.03	4.12	5.51	4.15	4.13	4.47	5.76
Methionine	g/kg	10.38	6.59	5.36	8.29	7.1	6.46	8.45	9.37

variable amounts of uneaten feeds on the tank bottoms, but due to the pellet stability issues, depth of tank and a variable degree of water turbidity it was not possible to assess this in a meaningful way for feeding comparisons.

As in the previous trial with banana prawns, similar silt and detritus levels remained on all tank bottoms, covering 10 - 20% of bottom areas. Towards the end of the trial, small portions of this became reduced (anoxic) and this was siphoned to waste immediately prior to harvest.

The salinity of supply and tank water remained high (36 ppt.) throughout the trial. Water temperatures ranged from 22.5°C at the beginning of the trial up to 28.0°C towards the end (see Appendix Table A2). This pattern of slow temperature increase during the trial and daily variations of only 3 - 4°C provided ideal conditions that should have been conducive to high survival, and which mirrored conditions that would likely occur in outdoor ponds. Between-tank variations of <0.3°C were observed within the experimental system. Similarly, pH values in tanks were stable (8.1 - 8.2) and dissolved oxygen levels remained high (6.3 – 7.1 mg L^{-1}).

Prawn Survival, Growth and Condition

Survival was very high in the trial and no significant differences were detected between treatments (Table 7). The average prawn weight at the start of the trial was 3.3 ± 0.06 g as calculated from the mean (± se; n = 26) prawn bulk weight across all tanks of 65.6 ± 1.22 g (see Appendix Table A1). This initial biomass estimate provided the feeding rate of 4 g per day (6% of tank biomass average = 3.94 g) which was applied to each tank for the duration of the trial (split into 2 x 2.0 g feeds daily).

Table 7. Numbers and percentages of black tiger prawns surviving from 20 stocked into different tanks and fed different feeds over a one month period

Feed type	Number surviving in each replicate	Mean ± se. % survival
No feeding*	15, 20	87.5a ± 12.50
Commercial diet 2	20, 20, 21**	100.0a ± 0.00
Wheat fish diet 3	20, 20, 20	100.0a ± 0.00
Wheat scallop diet 4	20, 20, 19	98.3a ± 1.67
Wheat soy diet 5	20, 20, 21**	100.0a ± 0.00
Wheat chick pea diet 6	20, 20, 20	100.0a ± 0.00
Wheat macadamia diet 7	20, 20, 20	100.0a ± 0.00
Weaner fish diet 8	20, 20, 20	100.0a ± 0.00
Weaner soy diet 9	20, 20, 18	96.7a ± 3.33

* n = 2.

**Unexplained error in initial number stocked with 100% survival assumed in later calculations.

Means with similar superscripts are not significantly different (P>0.05).

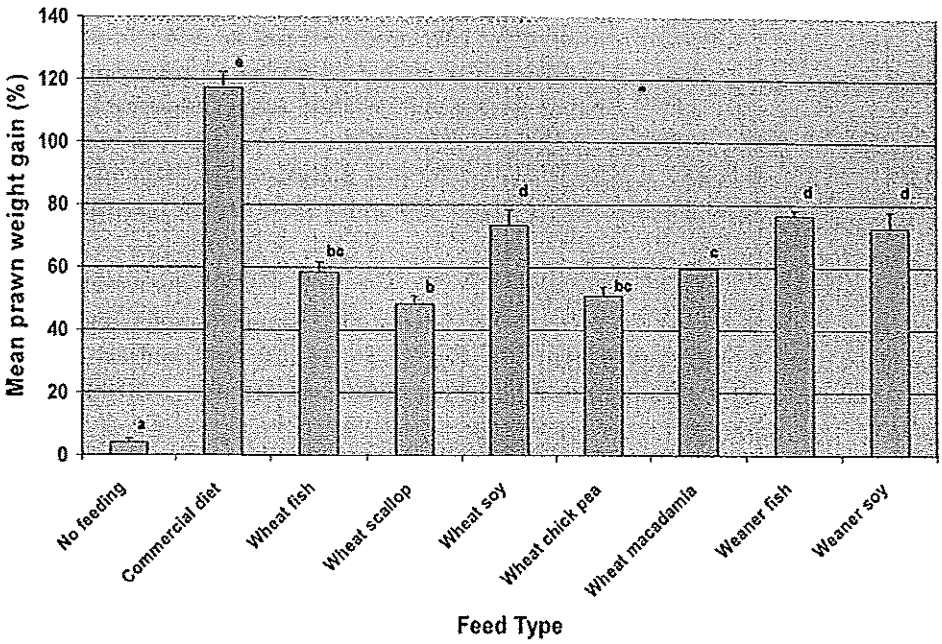

Figure 1. Mean (± se; $n = 3$) percentage of black tiger prawn weight gain for different feeds supplied to 20 prawns over a one month period. Means with similar letters are not significantly different (P>0.05).

The mean (± se; $n = 26$) prawn bulk weight across all tanks at the end of the trial was 106.4 ± 4.26 g. Figure 1 provides an assessment of the mean percentage weight increases for different feeds over the trial period. The commercial reference diet produced the highest (P<0.05) mean weight gain (117.2%). This was followed by the wheat soy (73.5%), weaner fish (76.6%) and the weaner soy diets (72.6%). All other diets produced significantly (P<0.05) lower weight gains. The unfed prawns produced only a slight weight gain (4.1%) and were fouled with epibiotic algae and other organisms, indicating that they had probably not moulted during the trial period. All prawns from the other fed treatments were free from fouling, had long antennae, were without obvious tail bites, and were vigorous and healthy when harvested.

Observed Prawn Performance Response and Relationship to Diets

Further examination of the composition of diets and the total prawn weight gains for each diet are provided in Figures 2 and 3. Plots revealed a positive linear trend in weight gains for increasing total lysine content (Figure 2) and increasing protein content (Figure 3) for all diets. This growth response to the commercial diet was much greater that that for the experimental organic diets for both the protein and lysine contents. This indicates that other factors associated with the commercial diet (of which we have little information these being confidential) result in better growth performance.

Such factors are likely to include:

1. The better long term water stability of the commercial diet pellets (refer to results above and Appendix Figure A2). This could be due to differences in extrusion parameters or due to post-extrusion coating with marine or vegetable oils. The net result of such pellet stability is less nutrient loss before consumption and lower overall feed wastage due to pellet disintegration.
2. The use of highly digestible ingredients that include steam dried fish meal, squid meal, squid liver meal, oilseed meals and wheat flour. These ingredients were not certified-organic, are generally expensive, and some of the fisheries-sourced products could be considered unsustainable in the long term, but the marine-based products are likely to be more nutrient rich and digestible for prawns. Additionally, although our organic diet ingredients have been analysed for total nutrient contents, we presently have insufficient information about the relative availability of these nutrients to prawns.
3. A greater overall intake of the commercial diet, which could have been a result of the squid meal generally used in commercial prawn feeds as an attractant. Squid meal is used in commercial prawn feeds because of its reported growth promoting and feed attractant properties at levels as low as 1.5%. This is reportedly a result of it containing low molecular weight peptides, free amino acids and betaine (Meyers, 1989; Takaoka *et al.*, 1995). Unfortunately the level of feed consumption could not be ascertained under the tank conditions of this trial. Ideally in a performance study, any variation in the resultant weight gain of an experimental animal can usually be attributed correctly by knowledge of both the actual feed consumption of the animal and determination of the nutritive value of that feed.
4. The addition of antioxidant–treated marine and vegetable oils, phospholipids, vitamins and minerals (non-organic micronutrients) which could be deficient in our experimental organic diets and hence may have affected the growth response.

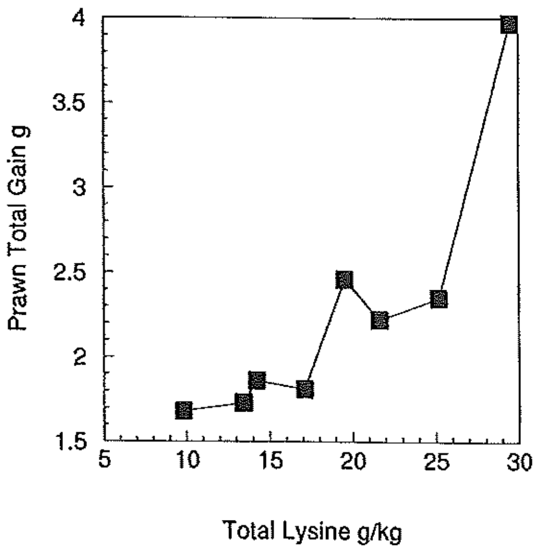

Figure 2. Plot of prawn total weight gain against total lysine content of diets.

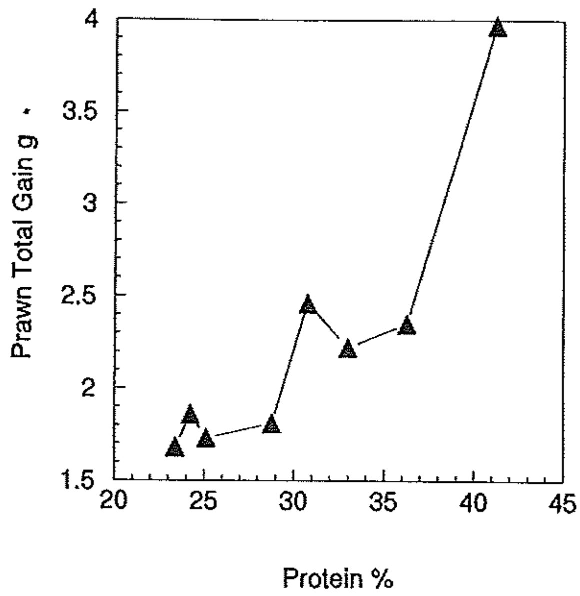

Figure 3. Plot of prawn total weight gain against protein content of diets.

DISCUSSION

Organic Market Analysis

Organic agriculture is an increasingly important food production sector. At the beginning of 2005 it involved over 550,000 certified farms covering more than 26 million hectares in 108 countries; the Australian organic market is currently valued at $400 million (BFA, 2006). Demand for organic products greatly exceeds supply in Australia, as in most other developed economies, and it is the fastest growing food and beverage category worldwide (McKinna, 2006). In 2005 the global organic seafood market was estimated to be worth US$600 million (Holmyard, 2006). However there are very few aquaculture ventures in Australia producing and/or processing organic seafood.

The Queensland prawn aquaculture industry (including black tiger, banana and kuruma prawns) is under considerable competitive pressure from cheaper imports. Its value has decreased from $45.9 million in 2004 - 05 to $42.5 million in 2006 – 07, despite a small (80 tonne) increase in production over the same period. The number of producing farms has also seen a steady decline from 37 in 2003 - 04 to 26 in 2006 - 07 (Lobegeiger and Wingfield, 2006; 2008). There are a number of farms that have ceased production and are for sale or are looking at producing other products (eg: marine fish). These government surveys show that the average market price received by farms for their product in 2006 - 07 ($13.79 kg^{-1}) was similar to that received in 2003 - 04, despite the continuous escalation in production costs in recent years.

These point-of-sale and cost of production pressures have created interest within the industry in alternative products and markets such as organic prawns. More efficient production methods that utilise lower stocking densities and reduced power and feed inputs may be a viable way for local farmers to compete effectively with cheaper imports. These are the basic principles of organic production, along with avoidance of chemicals and sustainability issues which are highly favoured by discerning consumers. However, the price premiums for organic produce bring an added attraction. For example, in October 2006, cooked and peeled organic prawns in modified atmosphere packaging from Waitrose in the UK quoted an on-line price equivalent to A$73 per kg. Even with a much tempered wholesale value of A$36.50 for this product (ie: nominally 50% of retail value), this shows plenty of potential for attaining about double the current Queensland farm gate price.

Further a field, the Ecuadorian Organic Support and Advisory Department has identified the US as a major target for expanding organic shrimp sales and recently recommended a 10 fold increase in production of organic shrimp. China is also moving towards this style of aquaculture production, with a 100,000 tons per annum organic extrusion mill for aquatic feeds currently under construction in Hainan (Anon, 2007). Dragon Feeds of Aberavon are also looking towards production of organic feeds for aquaculture by incorporating farmed polychaete worms in the diets. This company was looking towards completion of a 500,000 tonnes per annum feed mill in Wales by the end of 2007 (Anon, 2006a; 2006b). These examples show a world wide trend towards organic aquaculture products, which Australia and other countries must address if they are to successfully compete in future markets.

Nutrient Requirements of P. Monodon and the Ability of Organic Feeds to Meet Needs

As prawn farming operations move from low density extensive systems to more intensive systems with higher stocking densities, the need to provide more balanced nutrition increases, along with the need for more detailed information on the specific energy and nutrient requirements of target species (Shiau, 1998; Chen, 1998). Although the sources of protein and lipids used in prawn diets have traditionally been from marine sources, these are becoming increasingly more expensive and will be limited in supply in the future. In general terms, there is a need to explore alternative terrestrial-based sources of ingredients for use in aquaculture diets in the future. Organic standards recognise the unsustainable use of wild fish meal by requiring that only waste fish is used for certified stock feeds.

Despite this clear global need to move away from marine-based feed ingredients such as fish meal in the future, terrestrial plants generally offer lower protein and higher starch contents. Fish and prawns preferentially derive energy from protein over carbohydrate (Tacon, 1990). Akiyama and Dominy (1989) recommend the use of a 6:1 protein:lipid ratio for energy levels in diets for shrimp, but several authors suggest that long chain carbohydrates can spare the use of amino acids by prawns for energy. Simple or reducing sugars are thought to be less effective in meeting prawn nutritive needs than more complex polysaccharides such as starch (Chen, 1998). For example, Shiau and Peng (1992) showed that corn starch (20-30%) provides better growth and is a better carbohydrate for protein-sparing in *P. monodon* juveniles (0.5 g) than simpler carbohydrates like dextrin and glucose.

Proteins can therefore be regarded as indispensable nutrients for prawns, with levels required varying from 30 to 57%. This requirement is influenced by the protein quality, physiological state of the prawn, its age and the environment in which prawns are grown. For example, Shiau (1998) suggests that the optimum dietary protein level is lower (40%) in juvenile *P. monodon* when reared in seawater than when reared in brackish water (in 16 ppt. they need 44% protein). That work further suggests that salinity affects protein digestibility for sources such as soy, but not for fish meal or casein. Thus, there may be benefits in developing specific diets based on protein levels and sources for different salinity environments, and in fact this may be a necessity in the future for inland culture systems. However, the apparent incapability of juvenile or adult prawns to utilise dietary crystalline amino acids has made it difficult to efficiently quantify specific essential amino acid requirements. It has been reported that prawns have a requirement for at least 10 amino acids; work in *P. monodon* has been undertaken for methionine (Millamena *et al.*, 1996), threonine (Millamena *et al.*, 1997), lysine and arginine (Millamena *et al.*, 1998), histidine, isoleucine, leucine, phenylalanine and tryptophan (Millamena *et al.*, 1999).

Wheat Flour

We used wheat flour as the basal ingredient in the present organic formulations for black tiger prawns, because it is a commonly used ingredient in commercial prawn feeds as an energy and nutrient source, and as a binder. Wheat as an ingredient in prawn feeds is reviewed more thoroughly in our previous work with banana prawns (see Palmer and Slattery in a preceding article contained in this collection)

Soy Flour

Soy products are also often used in aquatic feeds because they are a rich source of protein. However, soy flour is also high in fat (22% - see Table 4), and its high inclusion levels in feeds could contribute to excessive levels for prawns. When compared with the whiting waste, the soy flour used in this study was very high in linoleic acid (18:2n-6 – 500 fold higher) and oleic acid (18:1n-9 – 15 fold higher) but completely lacking in eicosapentaenoic acid (EPA - 20:5n-3), docosahexaenoic acid (DHA - 22:6n-3) and arachidonic acid (AA - 20:4n-6) which are considered essential fatty acids for marine fish and prawns. When compared to fish, the soy flour was also comparatively low in the amino acids threonine, glycine, alanine and lysine, and low in ash and phosphorus.

Anti-nutritional factors, such as trypsin-inhibitors, are also present in some soy products, especially if they are inadequately heat processed. This has implications in protein digestion and utilisation whereby the actions of pancreatic enzymes like trypsin and chymotrypsin are impeded. Over-heating can also pose a problem with soy products through reduced amino acid availability.

Although it was uncertain in this trial whether such anti-nutrition factors affected the results, both experimental diets that had high soy inclusion levels (wheat soy and weaner soy) were among the best performing experimental diets in terms of prawn growth. An apparent leaching of ingredients from these soy-based diets suggested that other forms of soy that are less soluble in water, or further pellet stabilisation fine-tuning procedures could provide additional benefits for the use of this ingredient.

Macadamia Meal

This ingredient was considered because it can be supplied in large quantities in Australia as an organic by-product of oil extraction activities. The product that we sourced had a relatively high level of fat (14%) compared with the fish waste (5%), and was found to be particularly high in the oleic (18:1n-9 - 69 mg g^{-1}) and palmitoleic (16:1n-7 - 22 mg g^{-1}) fatty acid classes, which are normally found at much lower levels in marine based products (eg: 1-3 mg g^{-1} in the whiting waste; see Table 5). It also had much lower levels of protein than was hoped for (13% compared with 64% in the fish waste), and consequently was much lower in most amino acids except cystine.

Balogun and Fagbenro (1995) previously reported the use of this new feed ingredient in tilapia diets. That study reported a substantially higher crude protein content of 39.5%, and a crude fibre content of only 3.0%. By comparison, the macadamia meal used in the present study had a crude fibre value of 37.1%, which indicated that it was, in fact, not a de-hulled macadamia or that the meal had a much higher than anticipated hull content.

Chick Pea Flour

Chick peas are used as feed for many terrestrial animals (eg. pigs and poultry) and can currently be sourced readily in Australia as a certified organic flour product called besan. Compared with the fish waste, it had less than half the protein level, and was lower in ash and phosphorus. Whilst it had similar total fat levels to the fish waste, this was made up with a limited range of fatty acids, though like soy with heavy representation of the linoleic (18:2n-6) and oleic (18:1n-9) acid classes. The limited availability of polyunsaturated fatty acids that are essential for marine fishes and crustaceans (eg: EPA, DHA and AA) in this feed ingredient may limit its usefulness in aqua-feeds.

Again like soy, most varieties of chick pea also contain anti-nutritional factors. Some reported levels present for trypsin inhibitor activity (TIA) and chymotrypsin inhibitor activity (CIA) in chick pea are 4.79 (range 1.4 - 7.03) and 7.72 mg g^{-1} (range 3.71-13.19), respectively (Petterson and Mackintosh, 1994). Both can impair the utilisation of nutrients by terrestrial animals, but there is a paucity of information on their effects on prawn nutrition. Studies by Mustafa *et al.* (2000) showed that heat treatment reduced the rumen escapes values of chick peas by inactivating the TIA and CIA, while Batterham *et al.* (1993) found that older pigs could tolerate dietary levels of 4.7 mg g^{-1} TIA and 4.5 mg g^{-1} CIA. In the present study, it was unclear whether there were any significant impacts on prawn growth from anti-nutrients present in the chick pea, and also whether the heated extrusion process could have reduced such factors.

Whiting Fish Frames (Processing Waste)

The whiting processing waste was chosen because there is current production of this from wild harvested whiting (stout whiting *Sillago robusta*). Unlike traditional fish meals, which are primarily produced from fish harvested for fishmeal production, these fish are harvested primarily for human use and the by-product or residue could be used for prawn feeding. This adheres well to organic standards. It had a high protein content (64%), reasonable (5%) fat levels made up of a wide range of fatty acids, but relatively high ash content (30%). Of particular note was the lower levels of EPA, DHA and AA in oven-dried compared with freeze-dried product (only one replicate tested - see Table 5), and this may indicate that better drying methods could be employed for this ingredient in the future.

Whiting were also chosen because they are a small species which have relatively soft bones, allowing them to be more easily pulverised into meals suitable for feed plants. The tolerances for hard particles which could jam in mechanical bores and other moving parts of these plants are often quite small, so it is important to ensure ingredients are well screened or soft enough not to cause costly extrusion plant failures.

Additionally, there may also be a significant potential in the future to farm whiting (sand whiting *Sillago ciliata*) for the butterfly fillet market. Research into this pursuit is currently underway at BIARC. If such whiting were also being cultured according to organic principles, it would create a substantial organic by-product feed resource (approx. 50% of biomass produced would be head, skin and bone frames) that could be utilised as a feed ingredient in the formulation of organic diets for prawns. And in keeping with an organic whole-of-farm approach which limits food-transport-miles to minimise carbon-dioxide emission factors, and maximise farm gate profits, prawns processed on farms would yield waste heads and shells which would be of particular use in diets for organic whiting, thereby creating the potential for maximum nutrient use efficiencies and almost complete processing by-product utilisation.

Scallop Gut Waste

The scallop gut ingredient was also included in the study because there is potential for the availability of this material to increase substantially in the future due to scallop restocking efforts in the Hervey Bay region of Qld for the saucer scallop *Amusium balloti*. The company that supplied this ingredient for our trial is directly involved in these restocking endeavours.

The primary role of this ingredient's inclusion across all our experimental diets was as a prawn feeding attractant. Its value as a protein source (52%) was also examined in one dietary treatment group (diet 4), but this did not appear to provide significant prawn growth advantages. Compared with the fish waste ingredient, it was the only other experimental ingredient which also contained EPA, DHA and AA, and several other fatty acids.

Although unspecified studies have found that scallop gut waste can contain levels of cadmium, this would be unlikely to cause concerns neither for the health of the prawns nor for the organic certification process when used at low dietary inclusion levels. Another potential problem in the use of this waste stream is the potential degenerative action of the proteolytic enzymes that are present in this product, which would necessitate rapid processing (freezing and/or drying) to limit or inhibit this activity and help maintain ingredient stability.

Kelp Meal

Kelp meal was added to all experimental diets to add further feeding stimulants, provide additional trace elements and potentially assist in the binding of the diets. Seaweed meals from *Kappaphyycud* sp. and *Gracilaria* sp. have been included as useful binders in diets for juvenile *P. monodon* (Dy Penaflorida and Golez, 1996). Since kelp is of marine origin, it was also expected to provide some factors lacking in the diets based more on terrestrial based inputs. Analyses revealed that it contained several fatty acids that were not present in the terrestrial products, but lacked DHA and was high in ash (29%).

There may be other species of seaweed that could provide similar advantages to those sought in the kelp meal for formulated aquatic feeds, and which could be more conveniently produced within aquaculture premises. These include species in the *Enteromorpha / Ulva* complex which can be produced in specialised facilities on waste nutrients made available from pond discharge waters (DPI&F unpublished data). In some of our preliminary analyses these other seaweeds have been shown to contain higher protein contents (22.8 - 25.9%) than we found in kelp; in *Ulva* sp. we have also found higher lysine (8.54 g kg^{-1}) than in kelp (2.85

g kg^{-1}), 0.6 - 0.85% calcium, 0.3 - 0.4% phosphorus and 11 MJ kg^{-1} gross energy. But unfortunately, the level of ash in air dry *Ulva* sp. appears to be higher at 34 - 42%.

Molasses

Molasses was added to the formulations at the time of experimental diet extrusion to potentially provide an additional energy source. As discussed earlier it may afford a degree of protein sparing effects, and may also assist in pellet binding (McDonald *et al.*, 1998).

It consists almost entirely of water and soluble carbohydrates (see Table 3) such as sucrose (33.4%) and reducing sugars (15.2%). Pascual *et al.* (1983) found that for *P. monodon* juveniles (1.8 g), sucrose (10%) provided the highest survival (56%) for a range of simple and complex carbohydrates tested, but that at 40% inclusion all carbohydrates (particularly maltose and molasses) provided very low survival. The final levels of inclusion in the present study were much less than those detrimental levels.

Water Stability of Feeds

Water stability is well known to be an important aspect for aquatic feeds, both in terms of optimising feed consumption and waste minimisation. Future work in this area will be necessary for our organic diets because some which provided the best weight gains also demonstrated less than desirable water stability. Since prawns are fed every four to six hours, the pellets need to hold their integrity for at least this length of time, and preferably longer to avoid water and pond bottom fouling.

Gelatinised starch is a very useful ingredient for achieving increased pellet stability. However, at the time of these trials we could not identify any organically certified sources of starch which could be supplied in commercial quantities. Alternatively, aquatic feed pellets are also often sprayed with oil to provide a water stabile coating. But again, the fish oils that are normally used in commercial feeds would not likely adhere to some of the basic organic principles, and it is uncertain how palatable organic vegetable oils are for prawns, or what effects they may have on nutrient digestion. Sourcing and testing approved starches for increased protein and pellet stability, and testing alternative organic-oil coatings are likely to be the next steps in this progression of organic feeds development for prawns.

CONCLUSION

This work sought to identify and test a range of new ingredients for organic prawn diets and explore further the potential for production of this new seafood in Australia for market differentiation purposes. An increase in the protein level of supplemental diets for grow-out ponds had been recommended by our earlier trials, despite the relatively low rearing densities that were previously targeted. However, higher culture densities may arguably be allowable in organic standards, and this would place even greater emphasis on the provision of balanced and more complete diets which can promote healthy growth. Despite the inclusion of some organically certifiable protein-rich ingredients that could potentially be sourced in commercially relevant quantities, the test diets again (as in our previous trials) produced weight gains that were inferior compared with an uncertified commercial Penaeid feed.

There are several considerations necessary and pathways which can be recommended for fine-tuning and developing more complete organic diets for prawns. For example:

1. Better pellet stability may increase the feed utilisation to improve the performance of our current diets. This will have the advantage of reducing nutrient and feed loss through leaching and pellet breakdown. From a production viewpoint this means that the unit prawn production is maximised for each unit of feed. Additionally, it reduces the fouling of pond water by uneaten feed which is particularly nutrient rich. This better pellet stability could be achieved by a number of techniques, such as by:
   a. increasing or manipulating the starch content of diets
   b. adding other ingredients with binding properties (eg: seaweed with alginates). Of course these additional products would need to comply with organic standards
   c. varying the extrusion parameters, and/or
   d. coating pellets with a water resistant barrier such as a certifiable proxy for the fish oil used in current commercial diets.

2. The suitability of future terrestrial protein sources for prawns in general, and particularly for use in organic diets for prawns is of particular concern in this initiative. Two of the main concerns are their availability in commercial quantities and their nutrient suitability and digestibility values.

3. Diets based on terrestrial protein sources will need optimisation:
   a. either through adding better attractants ensuring that the majority of food is being consumed, or
   b. by investigating protein treatment methods that may improve their digestibility by aquatic species.

One method that is being increasingly used to treat and stabilise seafood waste for future use as aquaculture feed is hydrolysis using endogenous proteases (Lian *et al.*, 2005). This process increases digestibility by increasing the availability of peptides and free amino acids for uptake, and since it uses the enzymes that naturally occur in the waste products it is likely to be organically certifiable. However, for this approach to be a viable pursuit, seafood processing plants would need to implement new systems, equipment and procedures to treat wastes in a more controlled manner. They would need to be willing to foster a new product line rather than dispose of the waste in the most convenient way. A ready organic feed market for such improved products could drive the implementation of this and other waste utilisation strategies through economics, and create significant new fisheries business opportunities based on organic aquaculture products.

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APPENDIX

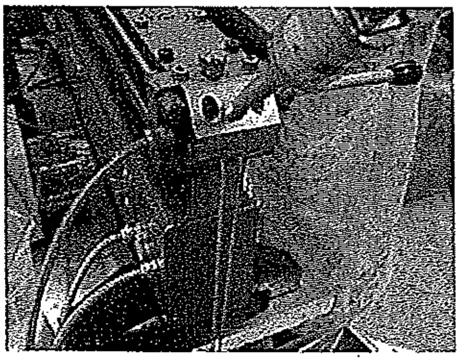
Table A1. Treatment allocation for tanks, primary prawn starting bulk weights, and adjusted prawn starting bulk weights after replacement of morbid individuals

Treatment-replicate	Tank	Primary prawn starting bulk weight	Adjusted prawn starting bulk weights
1-1	8	68.33	68.33
1-2	14	68.57	68.57
2-1	4	69.92	69.92
2-2	11	74.43	74.43*
2-3	26	62.91	62.91
3-1	3	56.14	56.14
3-2	19	63.90	63.90
3-3	23	56.25	56.25
4-1	1	61.99	60.59
4-2	9	75.88	75.52
4-3	22	72.70	72.70
5-1	7	53.94	53.94*
5-2	16	61.46	61.46
5-3	20	70.83	70.83
6-1	2	75.73	75.73
6-2	13	76.26	76.26
6-3	25	61.80	61.80
7-1	5	66.09	66.09
7-2	21	60.14	60.14
7-3	24	61.64	61.64
8-1	12	63.62	63.62
8-2	15	66.01	66.01
8-3	18	63.38	63.38
9-1	6	67.43	67.43
9-2	10	67.22	67.22
9-3	17	60.31	60.31

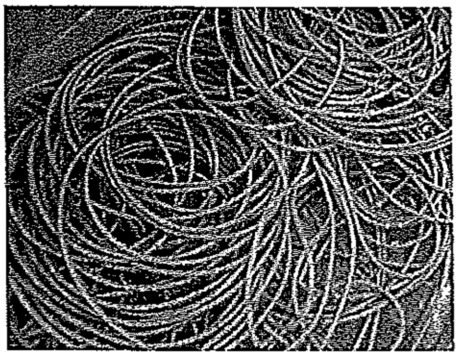
* 21 rather than 20 prawns stocked.
Mean biomass per tank after adjustment = 65.58 g. Mean prawn weight after adjustment = 3.27 g.

Table A2. Minimum and maximum water temperatures (from tank 10) and feeding times

Day	Min – max temp (°C)	Times feed added
Sat	24.5 when stocked	Not fed 4.00 pm
Sun	22.5 – 25.0	9.00 am 5.25 pm
Mon	22.5 – 26.0	9.30 am 4.00 pm
Tues	23.0 – 26.0	Not fed 4.30 pm
Wed	23.5 – 26.5	9.00 am 4.40 pm
Thurs	23.5 – 26.5	9.00 am 5.30 pm
Fri	24.0 – 26.5	9.00 am 5.00 pm
Sat	24.5 – 27.0	9.00 am 2.30 pm
Sun	24.5 – 27.0	9.00 am 5.30 pm
Mon	25.0 – 27.5	9.15 am 4.40 pm
Tues	25.5 – 28.0	9.00 am 5.00 pm
Wed	25.5 – 28.0	8.30 am 3.45 pm
Thurs	25.5 – 28.0	8.30 am 5.30 pm
Fri	24.5 – 26.0	9.30 am 3.00 pm
Sat	25.0 – 27.5	9.40 am 3.00 pm
Sun	26.0 – 28.0	10.30 am 4.50 pm
Mon	25.0 – 27.5	9.00 am 4.00 pm
Tues	24.0 – 26.5	9.00 am 4.00 pm
Wed	24.0 – 27.0	8.40 am 5.00 pm
Thurs	24.0 – 26.5	9.00 am 4.00 pm
Fri	24.5 – 26.5	9.00 am 4.00 pm
Sat	24.5 – 26.5	8.45 am 5.00 pm
Sun	24.5 – 27.0	8.30 am 4.00 pm
Mon	24.5 – 27.0	9.30 am 3.30 pm
Tues	24.0 – 27.0	9.00 am 3.30 pm
Wed	24.5 – 27.0	9.00 am 5.00 pm
Thurs	25.0 – 27.5	9.30 am 5.00 pm
Fri	25.0 – 28.0	Harvest 9 am – 1 pm



Prawn diet exiting extruder die



Extruded organic wheat fish diet

Figure A1. (Continued)


Lab scale Prism extruder

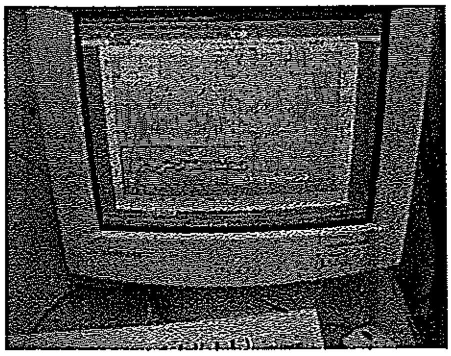
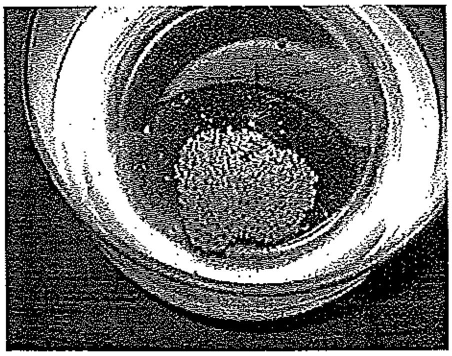
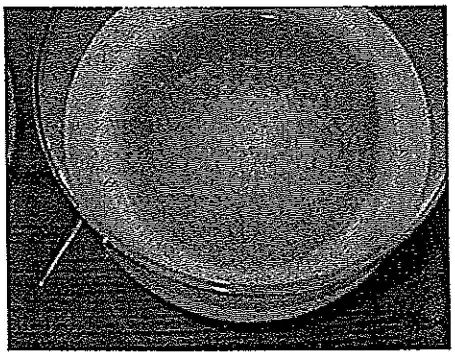

Monitoring process parameters

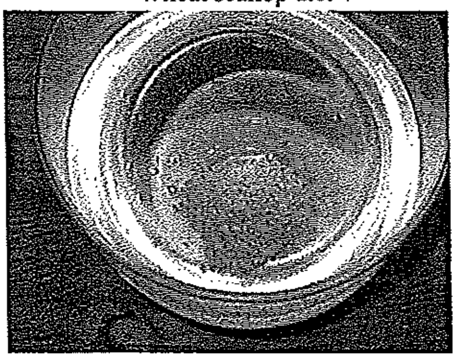
Figure A1. Pictures of the laboratory-based extrusion of organic prawn diets.

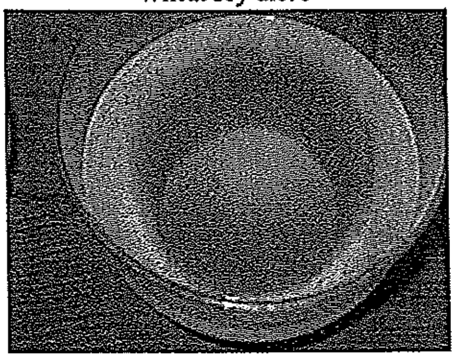

Commercial diet 2


Wheat fish diet 3

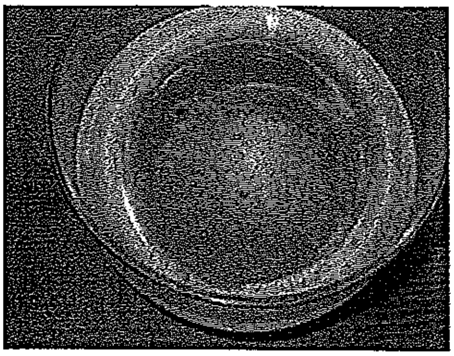

Wheat scallop diet 4


Wheat soy diet 5


Wheat chick pea diet 6


Wheat macadamia diet 7

Weaner fish diet 8



Weaner soy diet 9

Figure A2. Pictures of the different diets investigated after 3 hours submersion in seawater.

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