Chapter 11

USING WHEAT FLOUR IN THE PRODUCTION OF ORGANIC BANANA PRAWNS

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ABSTRACT

Banana prawn (*Fenneropenaeus merguiensis*) juveniles (1-2 g) were compared for survival, growth and condition after feeding in tanks over one month with several simple diets based on organically certified whole wheat flour. All feeds were applied once per day at 6% of the starting body weight, and produced high survival (>94%). A commercial Australian prawn feed used as the control diet produced the highest (P<0.05) growth (101% weight gain) and condition measured as the length of antennae (13.2 cm). The unfed control had significantly (P<0.05) lower survival (56%), and resulted in a weight loss (3.1%) and the shortest antennae (9.4 cm). Adding free flour to tanks produced lower (P<0.05) growth (6.9%) and shorter (P<0.05) antennae (10.3 cm) than adding pelletised flour with low levels (dry weight) of additional nutritional substances and feed attractants (chicken's whole egg: 1.5%, polychaete slurry: 1.1% and 6.8%, molasses: 4.2%). Rolling the flour into a dough ball also appeared to marginally improve its direct utilisation by the prawns. These results are considered within the context of appropriate nutrition for Penaeids and successfully producing certified organic prawns in Australia.

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INTRODUCTION

The banana prawn *Penaeus* (*Fenneropenaeus*) *merguiensis* is one of the most popular prawn species cultured with extensive farming practices in Southeast Asia (Boonyaratpalin, 1998; Thongrod and Boonyaratpalin, 1998). It has also been the subject of several recent studies in Australia focussing on its particularly amenable culture attributes (Hoang, 2001; Palmer *et al.*, 2005ab). To investigate the production of organically certified banana prawns in inland saline systems, a replicated tank-based experiment was undertaken at the Bribie Island Aquaculture Research Centre (BIARC) in Southeast Queensland (Qld), Australia. This trial was intended to provide information about the use of simple feeds in extensive prawn production systems, and if possible, to provide immediate feedback to managers of related farm trials, to maximise the on-farm growth of prawns using certified-organic feeds.

Since a certified-organic fish-meal-based prawn diet which had previously been planned for use in the research project was unexpectedly not available, the feed trial focussed on the use of supplemental organic feeds that were nevertheless in use at the farm. Certified-organic whole wheat flour had been in regular use in the organic farm trials since stocking prawn seed. Its regular addition to ponds was intended to lift natural pond productivity whilst also being consumed directly by the prawns. Farm operators had observed the prawns eating the flour directly when added as a powder or thick paste (dough balls placed on feed trays), and gross inspection of these prawns' digestive tracts indicated that they were feeding directly on this organic feed supplement. Certified-organic molasses from sugar cane was also being applied directly to ponds to help control algal blooms. Chickens egg and polychaete worms were two other ingredients considered to be readily organically certifiable and potentially useful in prepared diets at low inclusion levels as feeding stimulants.

Wheat flour is generally used as a binder and nutrient source in many pelletised aquaculture feeds (Tacon, 1990ab; Cheng *et al.*, 2002) and is a common ingredient in commercial prawn feeds used in Australia (eg: Ridley Aquafeed prawn feed; Charoen Pokphand prawn feed). It is one of the least expensive sources of carbohydrate and is generally used in prawn feeds as a dietary energy source to spare the more costly protein for growth (Shiau and Peng, 1992). Alone it could be expected to lack a balanced nutritional profile (eg: some essential amino acids and fatty acids), but in combination with the natural foods that prawns utilise in eutrophic ponds including zooplankton, algae and detritus, and at low prawn stocking densities, significant growth may be possible with this as a supplemental source of nutrition.

This trial therefore sought to assess a range of ways that wholemeal wheat flour could be used as the main organic feed ingredient. Treatments were designed to identify the most efficient way to feed wheat flour and pellets consisting mainly of wheat flour, in terms of prawn survival, growth, and condition, assuming that prawns can gain nutritional benefits from its direct consumption, in addition to its indirect stimulation of natural feed sources. The trial diets were crude formulations based on available organically certifiable ingredients intended for application as supplemental feeds in an extensive pond grow-out situation.

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 BIARC (Palmer *et al.*, 2005a). It incorporated 26 round plastic tanks (diameter of 1550 mm) with netting covers (to prevent bird predation and prawns jumping out), all housed inside a greenhouse designed to minimise diurnal temperature fluctuations. Each tank was fitted with a 50 mm standpipe for screened water to overflow to waste, giving a constant water depth of 900 mm and maximum water volume of 1700 L. Each tank was also fitted with a 4 mm airline, which delivered constant moderate aeration ($\sim$ 6 L min^{-1}) to the centre of each tank in mid-water column.

All tanks were initially clean and dry. Unfiltered seawater was continuously pumped into each tank at similar rates (3 L min^{-1}) for the duration of the trial and for four days prior to stocking prawns. The unfiltered seawater supply carried a low level of silt and naturally occurring suspended matter (eg: phytoplankton and zooplankton) from the adjacent waters of Moreton Bay. Supply lines were flushed daily and designed so that each tank received a similar load of suspended matter. Debris that collected on the tank bottoms during the trial was left undisturbed for the duration of the trial.

Experimental Design

Nine experimental treatments involved a range of different feeds based on wholegrain wheat flour and control diets. They were assigned randomly to each tank (Appendix Table A1) in a completely randomised design. There were three replicates for all treatments except treatment nine which had two (due to the availability of only 26 tanks for the experiment). The treatments were as follows:

1. Commercial Australian prawn feed (Ridley Aquafeed Prawn Starter #2) – control treatment for maximum growth possible in the testing system.
2. No feed – control treatment for minimum growth likely on low level of natural food sources present in supply water.
3. Wheat flour added as powder – simplest feed additive.
4. Wheat flour rolled into 1 dough ball – second simplest feed additive.
5. Wheat flour only pellet – consisting of 100% wheat.
6. Wheat flour + chicken egg pellet – consisting of approximately 1.5% egg as attractant.
7. Wheat flour + marine worm (lower level inclusion) pellet – consisting of approximately 1.1% worm as attractant.
8. Wheat flour + molasses pellet – consisting of approximately 4.2% molasses as attractant.
9. Wheat flour + marine worm (higher level inclusion) pellet – consisting of approximately 6.8% worm as attractant.

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. The flour used as the base ingredient was Organic Stoneground Wholemeal plain wheat (*Triticum aestivum*) flour certified by Organic Food Chain TOFC 27 and manufactured by Kialla Pure Foods, Greenmount Qld 4359. The specified organic standard states that it is free from genetically modified organisms (GMO), chemicals, infestation, mould and foreign materials. The chicken egg was large fresh whole egg (without shell) marketed as Coles Organic free range (Australian Certified Organic, in conversion 101081C). The marine worms (Polychaeta: Nereididae) were small (individuals <0.5 g wet weight) cultured *Perinereis* spp. grown without any chemical additives and exclusively fed on prawn farm effluent (phytoplankton and other naturally derived organic matter). These were frozen following purging for 2 hr after harvest from constructed sand beds. Thawed worms were blended into homogenous slurry prior to feed inclusion. The molasses used was certified as organic produce from the Rocky Point Refinery at Woongoolba in Southeast Qld.

Preparation of experimental feeds involved blending ingredients to homogeneity with minimal use of reverse osmosis (RO) water to make similar consistency pastes. The amounts of egg or molasses added to the pastes (wet weights as a percentage of flour) were approximately 5%, and for the worm inclusion diets were 5% and 33%. This gave a 1.45% dry weight (DW) inclusion for the egg, a 4.15% DW inclusion for the molasses, and 1.14% and 6.81% DW inclusions for the worm diets, due to the different moisture contents of different feed additives (exact formulation given in Appendix Table A2). For pelletised feeds this paste was immediately extruded through a 4 mm die, steamed for 8 min, and dried overnight (20-24 hr) at 40°C. Dried strands were then crumbled/crushed and sieved to provide similar sized particles of >1 mm and < 2 mm. The commercial prawn feed control was also crushed and sieved in this manner. 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 27°C. Two grams of each feed was immersed in still seawater (1.5 L) in transparent hemispherical fish bowls with regular observations and physical comparisons made thereafter. Comparisons focused on any apparent differences in the experimental diets with the commercial control diet, in terms of texture and integrity.

Prawn Acquisition, Transport, Handling and Tank Management

Juvenile prawns for the trial were obtained from a saline-bore-water-supplied pond at a demonstration inland farm at Bauple in Southern Queensland, following clear health and histological examinations performed one week earlier. Only certified organic feeds had been previously used in the pond. The prawns were collected 39 days after stocking as PL18, from

feed trays repeatedly baited with commercial prawn feed. To maximise survival during handling and transport, this was undertaken from early to mid morning whilst water temperatures were low. Trays were lifted periodically and captured prawns were tipped onto wetted shade cloth to gently contain them. From there they were carefully transferred with soft nets into an 800 L fibreglass transport tank supplied with oxygen. To adjust prawn juveniles from the low salinity at the Bauple site (2 ppt.) to the high salinity at BIARC (36 ppt.), and to minimise stresses associated with pH and other water chemistry changes, the transport tank was filled with 40% BIARC water and 60% Bauple pond water, providing transport conditions of approximately 16 ppt and 27°C.

A total of 800 juvenile prawns were collected and transported by road (3 hour transit time) to BIARC. On arrival they were funnelled out of the transporter's 50 mm bottom drain, directly into a 4,000 L parabolic tank filled with seawater. They were held there overnight (without feeding) to screen out prawns damaged during the transfer. The trial began the next day when 25 randomly collected prawns were stocked into each tank after weighing their collective bulk wet weights. Feeds were added to the tanks once per day on the opposite side of the tank to the overflow point. The daily feeding rate for each tank (2.0 g) was calculated as 6% of the overall mean prawn biomass weight across all tanks. The day after stocking, each tank was inspected for mortalities or unhealthy individuals. These would have been clearly visible on tank bottoms when the air and water supplies were turned off, due to the white discoloration of dead prawns against the green tank bottoms, or against the dark coloured silt/detritus which had previously accumulated in small areas of the tank bottoms. This silt/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 occasion 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. After harvest at the end of the trial, the condition of prawns in each tank was qualitatively assessed and the length of each prawn's longest antennae was measured to the nearest mm (base of eye stalk to tip of antennae).

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 reacted similarly when added to seawater. A small portion (2-5%) of each of the experimental diets floated with surface tension, sinking with slight disturbance of the water surface. After 5 minutes submersion, all diets (including the control) had softened to a similar texture. Similarly after 20 mins submersion, similar textures prevailed with all pellets easily able to be smeared into a paste between the fingers, but the pellet shape without disturbance remained unchanged. After 1, 2, 3 and 7 hr submersion there were no apparent changes in any of the feed's condition.

Feed Ingredient Proximal Analyses

Table 1 provides some previously documented proximal analyses for feed ingredients generally used in prawn feeds (ie: whole grain wheat; chicken's egg excluding shell; and various fish meals excluding bone and offal meal), for feeds used in this study (product manufacturers specifications), and for the polychaete worms (% wet weight) used in this study after harvest from a prawn farm wastewater remediation system (DPI&F unpublished data).

The organic wheat flour used in the present work had slightly higher protein and fat levels than generally reported for whole wheat, whilst the organic eggs had a slightly lower level of total fat compared with typical eggs reported in the literature (Tacon, 1990b). The marine worms used in the present study had comparable moisture content to chicken eggs, and relatively similar protein levels to both whole wheat and eggs, but lower, and much lower, levels of fat compared with whole wheat, and eggs, respectively. On the other hand, molasses is almost entirely made up of water and soluble carbohydrates (see Table 1) such as sucrose (33.4%) and reducing sugars (15.2%), although these contents can vary by several percent yearly and between batches (pers.com. Rocky Point Mill).

Table 1 further shows how fish meal and commercial prawn feeds generally have much higher levels of protein than the ingredients used in the experimental feeds. The types of protein (amino acids) and lipids (fatty acids) are also quite different (see discussion).

Tank Trial Conditions

Careful inspections conducted the day after stocking (9-11 am) did not reveal mortalities on the tank bottoms or in the clutches of live prawns (as is often the case as they are slowly consumed). Only one live prawn in tank 17 appeared to have the white-muscle syndrome typical of some banana prawns after handling, which is often unavoidable and presumably due to muscle cramp. It was not replaced. All prawns also appeared to have full gut tracts during these initial inspections. Similar silt and detritus levels were present on tank bottoms, covering approximately 20% of bottom areas.

Table 1. Feed ingredient proximal analyses (%)

Feed type	Wat	CP	EE	CF	NFE	Ash	Cal	Phos
Wheat*	12.1	12.0	1.7	2.5	70.0	1.7	0.05	0.36
Wheat**	12.0	13.1	2.1	11.2 df	52.4 tc	-	-	-
Egg*	74.4	12.4	11.0	0.0	1.3	0.9	0.06	0.18
Egg**	-	12.8	10.1	-	<1.0 tc	-	-	-
Worms***	80.7	13.3	1.2	-	1.0 tc	3.8	-	-
Molasses****	21.9	0.0	0.1	0.0	74.7 tc	3.3	0.21	0.03
Molasses**	31.4	4.8#	-	-	60.8 tc!	12.2	0.71#	5.06
Fish meal*	7.0-9.1	57.0-72.7	4.2-9.3	0.8-1.0	0.8-4.4	10.1-26.0	2.04-7.86	1.42-4.21
Commercial prawn feed**	-	43.0	6.0	3.0	-	13.0	-	-

Wat = water, CP = crude protein, EE = lipid or ether extract, CF = crude fibre, NFE = nitrogen-free extractives, Ash, Cal = calcium and Phos = phosphorus.
* given by Tacon (1990b); **Manufacturers specifications for product used in this study; ***DPI&F unpublished data; ****NutritionData.com (2006); df = dietary fibre; tc = total carbohydrate; #data from different batch of molasses produced at same mill in 2002.

The salinity of supply and tank water remained high (35 ppt.) throughout the trial. Water temperatures ranged from 26.5°C to 31°C (see Appendix Table A3), and between-tank variations of <0.2°C prevailed throughout the experimental system. Similarly, pH remained stable at 8.2 (<0.04 difference between tanks), and dissolved oxygen levels remained high (6.3 – 6.6 mg L^{-1}). At the end of the trial, a small portion of sediments on the bottom of all tanks was in a reduced state, as evidenced by the anaerobic odour when tank bottoms were siphoned to waste prior to harvest. Despite this the harvested prawns were vigorous and healthy, free from shell fouling and with full gut tracts.

Prawn Survival and Growth

Similar (P>0.05) numbers of prawns were retrieved from all treatments except the unfed control (Table 2). Unfed prawns had lower (P<0.05) survival (54%) than fed prawns, and all fed treatments had a similarly (P>0.05) high survival (>94%) (Figure 1). The average prawn weight at the start of the trial was 1.3 (± 0.02) g as calculated from the mean (± se; n = 26) prawn bulk weight across all tanks of 33.6 (± 0.46) g (Appendix 1). This initial biomass estimate provided the feeding rate of 2.0 g per day (6% body weight) applied to each tank for the duration of the trial. The mean (± se; n = 26) prawn bulk weight across all tanks at the end of the trial was 41.5 (± 2.41) g.

There were no differences (P>0.05) between the mean bulk weights or mean prawn weights in different treatments at the start of the feeding trial (Table 3). However on completion, significant effects (P<0.05) were detected for bulk weights and prawn weights. The commercial pellet by far produced the fastest growth with an average of 101.6% weight gain (Figure 2). Prawn growth was negative in the unfed control (weight loss of 3.1%), and low in the free flour treatment (weight gain of 6.9%). Most pellets produced a higher weight

gain than the free flour treatment, particularly when a feeding attractant was included (weight gains from 29.9% to 46.1%). There were no significant differences (P>0.05) in the growth of prawns fed pellets with different feed attractants (ie: egg, low or high worm, or molasses). Rolling the flour into a dough ball produced larger (P<0.05) mean bulk weights and prawn weights than adding it as a powder (Table 3), but when compared as a percentage weight gain this difference was not significant (P>0.05) (Figure 2).

Table 2. Numbers of banana prawns surviving in different tanks after supply with different feeds over one month. Means with similar superscripts are not significantly different (P>0.05)

Feed type	Number surviving in each replicate	Mean ± se. number surviving
Commercial pellet	23, 25, 28*	$25.3^b \pm 1.45$
No feed	13, 13, 16	$14.0^a \pm 1.00$
Free flour	24, 24, 23	$23.7^b \pm 0.33$
Dough ball	24, 24, 25	$24.3^b \pm 0.33$
Flour only pellet	23, 24, 25	$24.0^b \pm 0.58$
Flour + egg pellet	25, 22, 25	$24.0^b \pm 1.00$
Flour + low worm pellet	23, 23, 25	$23.7^b \pm 0.67$
Flour + molasses pellet	24, 25, 25	$24.7^b \pm 0.33$
Flour + high worm pellet **	23, 25	$24.0^b \pm 1.00$

* Unexplained error in initial number stocked with 100% survival assumed in later calculations; ** n = 2.
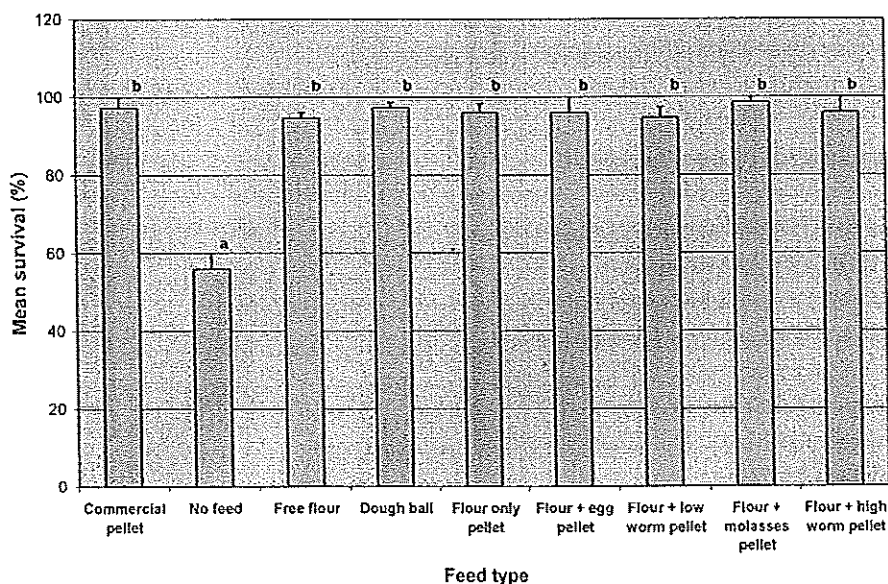
Figure 1. Mean (± se; n = 3) banana prawn survival expressed as a percentage of the number stocked (25) into each tank after supply with different feeds over one month. Means with similar letters are not significantly different (P>0.05).

Table 3. Mean (± se; n = 3) starting and finishing bulk and individual banana prawn weights in tanks fed different feeds over one month. Within columns, means with similar superscripts are not significantly different (P>0.05)

| Feed type	Mean ± se start bulk weight (g)	Mean ± se start prawn weight (g)	Mean ± se finish bulk weight (g)	Mean ± se finish prawn weight (g)
Commercial pellet	33.2[a] ± 1.82	1.3[a] ± 0.07	64.6[e] ± 2.31	2.6[e] ± 0.09
No feed	33.4[a] ± 2.13	1.3[a] ± 0.09	18.2[a] ± 2.07	1.3[a] ± 0.15
Free flour	33.0[a] ± 1.89	1.3[a] ± 0.08	33.0[b] ± 1.50	1.4[a] ± 0.06
Dough ball	33.4[a] ± 0.75	1.3[a] ± 0.03	40.0[ct] ± 1.04	1.6[bc] ± 0.04
Flour only pellet	33.2[a] ± 0.58	1.3[a] ± 0.02	38.9[c] ± 2.00	1.6[b] ± 0.08
Flour + egg pellet	34.1[a] ± 2.18	1.4[a] ± 0.09	45.8[d] ± 1.64	1.9[d] ± 0.07
Flour + low worm pellet	34.2[a] ± 1.50	1.4[a] ± 0.06	43.8[cd] ± 1.36	1.9[d] ± 0.06
Flour + molasses pellet	35.5[a] ± 0.61	1.4[a] ± 0.02	45.4[d] ± 0.71	1.8[cd] ± 0.03
Flour + high worm pellet *	32.3[a] ± 0.44	1.3[a] ± 0.02	45.2[d] ± 1.49	1.9[d] ± 0.06

* n = 2.
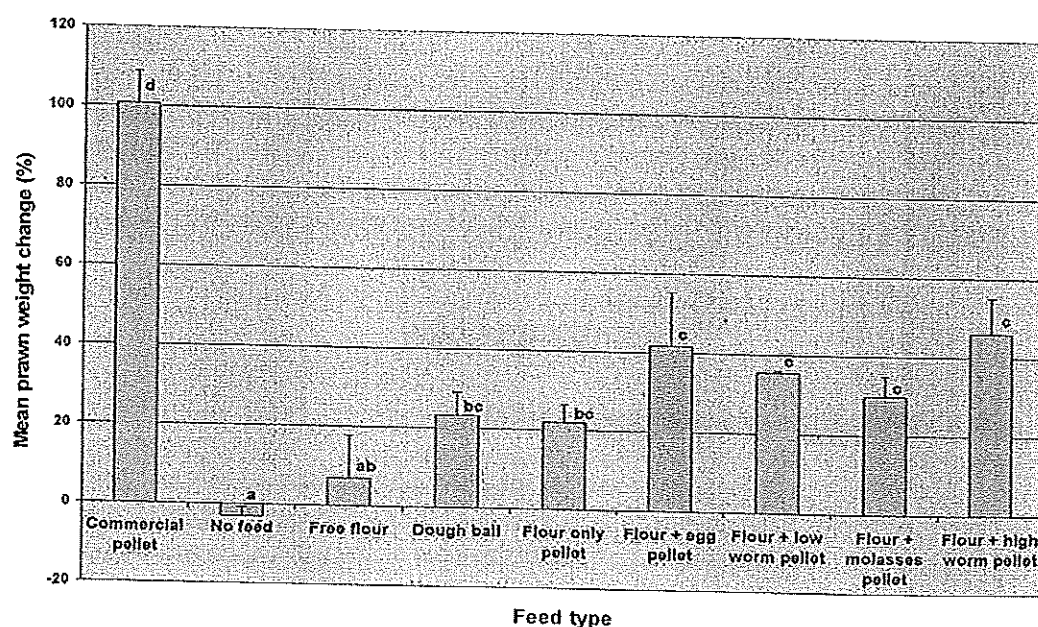
Figure 2. Mean (± se; n = 3) banana prawn weight changes expressed as a percentage of mean starting weights after supply with different feeds over one month. Means with similar letters are not significantly different (P>0.05).
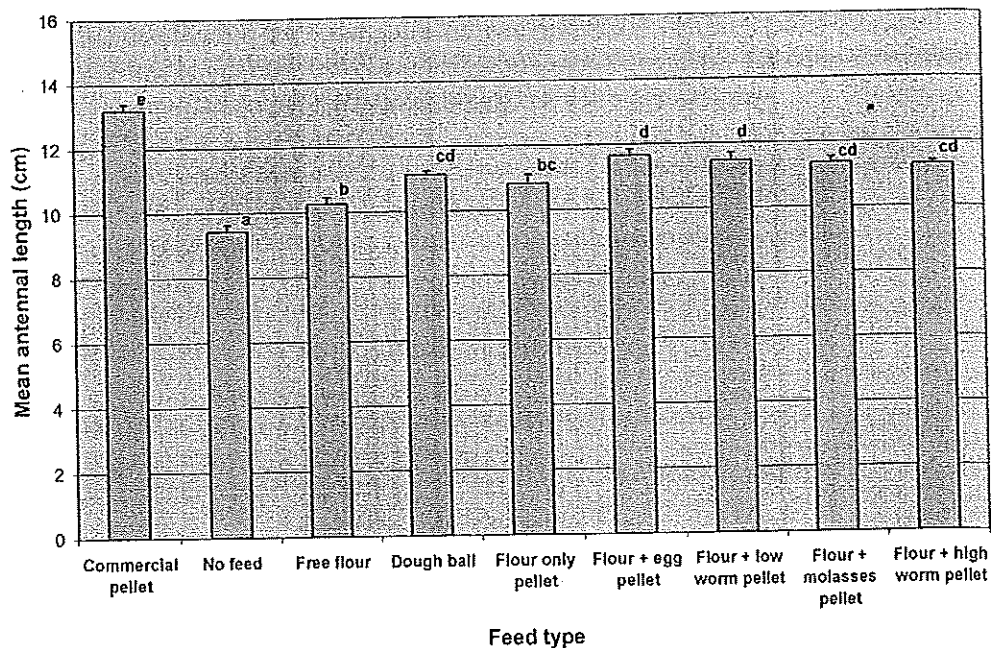
Figure 3. Mean (± se; $n = 3$) banana prawn antennal lengths after supply with different feeds over one month. Means with similar letters are not significantly different ($P>0.05$).

Prawn Condition

Prawn condition as measured by antennal length was highest in prawns fed the commercial pellet (13.2 cm) and lowest in the unfed treatment (9.4 cm). Adding free flour to tanks produced shorter ($P<0.05$) antennae (10.3 cm) than adding pelletised flour with the feed attractants included (ie: egg, worms, molasses: 11.3-11.7 cm). Rolling the flour into a dough ball produced antennal lengths (11.1 cm) that were not significantly different ($P>0.05$) to that produced with pelletised feeds, but longer ($P<0.05$) than when it was added as a powder.

DISCUSSION

Nutrient Sources and their Suitabilities

The organic certification process for products derived from cereals and grains is well developed within Australia due to their direct and indirect consumption by humans. Cereals and grains are also some of the least expensive food sources that are available for intensive livestock, and they are used widely as energy sources and binders in manufactured feeds for fish and shrimp (Tacon, 1990b). Generally, they are low in protein (8-12% of dry matter), but high in carbohydrates (60-80%) comprised mainly of starch in the grains' endosperm (amylase 25%, amylopectin 75%). Of the protein that does exist in grains, the amino acids lysine and threonine are generally low, and the fatty acids present (eg: mainly linoleic and oleic acid) are normally unsaturated. These and other constituents (eg: fibre, vitamins,

minerals) vary with different grain species, but notably, cereal grains are also considered to be a good source of phosphorus (as phytates) (Tacon, 1990b).

Fish meals are generally used as the main source of protein and fat in aquaculture diets. They have what is considered to be a better balance of amino acids for aquaculture feeds and a much broader range of fatty acids. Table 4 provides some previously documented amino acid contents for wheat (% of grain), for typical chicken's egg (excluding shell), for various fish meals (excluding bone and offal meal), and for whole dried *P. monodon* juveniles (average wet weight of 1.5 g). Here it can be seen that for most amino acids, wheat and eggs have comparatively similar levels whilst fish meal is several fold higher. Compared with fish meals, wheat is particularly low in methionine, arginine, threonine and tyrosine (<20% of the minimum values given in Table 4), as well as in isoleucine, lysine, valine, and histidine (<15% of the maximum values given in Table 4). Although prawns generally require lower amounts of protein in their diets as they grow larger, suboptimal growth is often due to amino acid imbalances (Akiyama and Dominy, 1989). Compared with levels in *P. monodon* juveniles, wheat also has a several fold lower content for all major amino acids, and is particularly low in methionine, arginine, threonine, isoleucine, valine and phenylalanine (all <20% of the values given in Table 4).

Fish and prawns generally have a high dietary protein requirement (24-57%) and preferentially derive energy from protein over carbohydrate (Akiyama and Dominy, 1989; Tacon, 1990b). Despite this there has been considerable research over the last decade directed at the dietary substitution of carbohydrate for protein in aquaculture feeds, and particularly with regard to replacing fish meal with terrestrial based products. Starch (from cereal grains) is one of those most studied, even though it is not generally found in marine food sources. Starch from wheat has been shown to have good digestibility in several Penaeids, and particularly after it has been gelatinised through cooking (Cousin *et al.*, 1996). However, the digestibility of carbohydrates differs between Penaeid species (Catacutan, 1991) and there is still a paucity of relevant information and some general scepticism about carbohydrates as suitabl nutrients for prawns.

Table 4. Documented amino acid contents* (%) for two feed ingredients used in this trial, for a range of different fish meals*, and for *P. monodon***

Feed type	Arg	Cys	Met	Thr	Iso	Leu	Lys	Val	Tyr	Try	Phe	His
Wheat	0.60	0.13	0.15	0.38	0.50	1.35	0.95	0.57	0.31	0.26	0.58	0.28
Egg	0.76	0.29	0.40	0.61	0.76	1.07	0.83	0.86	0.50	0.19	0.70	0.30
Fish meals	3.25-4.62	0.4-0.76	1.46-2.14	2.31-3.25	2.41-3.5	3.81-5.3	4.04-6.6	2.8-4.37	1.86-2.29	0.56-0.8	2.16-2.92	1.3-1.88
P.monodon	4.99	-	1.50	2.69	2.65	5.96	4.40	3.04	0.80	0.66	2.97	1.18

Arg = arginine, Cys = cystine, Met = methionine, Thr = threonine, Iso = isoleucine, Leu = leucine, Lys = lysine, Val = valine, Tyr = tyrosine, Try = tryptophan, Phe = phenylalanine, and His = histidine.
*given by Tacon (1990b).
** given by Sarac (1994).

Shiau (1998) provides a useful summary of carbohydrate research for various Penaeid species. Generally, simple or reducing sugars are thought to be less effective 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. Earlier work by Andrews *et al.* (1972) had also shown that for *P. setiferus* juveniles (4 g), 30% starch provided higher growth rates and reduced the effect on growth of variable protein levels. Pascual *et al.* (1983) also 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.

In contrast, the present study demonstrates that for *P. merguiensis*, high survival can be achieved with very high levels of complex carbohydrates (wheat flour) over short periods (one month), but suggests that without supplemental protein growth will be suboptimal. This result may have been due to low levels of natural feeds present in the intake water supplying essential nutrients for health maintenance, but insufficient protein levels for substantial growth.

Natural zooplankton diets are reportedly good sources of protein, lipids, minerals, vitamins and carotenoid pigments for fish and shrimp, although a range of factors influence their value to particular species of different ages (eg: size, stain, source) (Tacon, 1990b). Suitable natural foods in the culture system can therefore permit a reduction in the protein levels of artificial feeds (Akiyama and Dominy, 1989). For example, Hari *et al.* (2004) showed that in extensive systems, the addition of carbohydrate to the water column increased the carbon to nitrogen ratio which stimulated heterotrophic bacterial growth that was then utilised as a supplemental protein source. Whilst this style pond management may alleviate the crop's protein requirements somewhat, it still appears that well balanced feeds are necessary to increase production levels. Various forms of wheat or bread flour may be a suitable base for this organic dietary development, perhaps up to a maximum inclusion level of 35% (Catacutan, 1991; Shiau, 1998).

Feeding wheat flour clearly improved survival in the present study, and in some forms, also the growth of prawns. Rolling the flour into a dough ball also appeared to marginally improve its direct utilisation by the prawns, probably by making it more accessible at higher concentration and with lower expenditure of foraging energy. Akiyama and Dominy (1989) recommend the use of a 6:1 protein:lipid ratio for energy levels in diets for shrimp. Sedgwick (1979) found that for *P. merguiensis* juveniles (0.3 - 4.9 g), the energy content of the diet affected food consumption, protein conversion efficiency and growth, and that with sufficient energy supplied in the range 2.9 - 4.4 kcal g^{-1}, maximum growth was obtained with protein and lipid levels of 34 - 42% and 7.4%, respectively. This energy level is similar to that used in contemporary commercial prawn feeds such as the control diet in the present study which contained a gross energy level of 18.66 MJ kg^{-1} (4.5 kcal g^{-1}), with 43% protein and 6% lipid. In comparison, the wheat flour used in the present study had an energy content (12.59 kJ g^{-1} or 3.0 kcal g^{-1}) in the lower end of this optimal range suggested by Sedgwick (1979), as well as lower levels of protein (13.1%) and lipid (2.1%). For an energetic prawn like *P. merguiensis*, which spends a large proportion of its time swimming in the water column, the generally lower energy content of carbohydrate combined with lower inclusions of energy

rich food groups (ie: lipid and protein) may also compound to cause minor energy deficiencies with the 100% wheat diet.

Molasses was added to one of the wheat pellet diets in the present work to provide an additional energy source. Levels of inclusion (4.2% DW) were well below the critically detrimental levels reported for *P. monodon* by Pascual *et al.* (1983) (discussed above), and similarly had no apparent affect on survival of *P. merguiensis*. The marginal increase in growth of *P. merguinesis* through addition of this simpler carbohydrate to the pelletised wheat flour was also not significant. Molasses used in the present work contained 15.2% reducing sugar (most likely the monosaccharides glucose and fructose), and 33.4% sucrose, a non-reducing disaccharide that has been shown to be a beneficial dietary inclusion (at 10%) for growth in at least two Penaeid species (ie: *P. japonicus* and *P. monodon*: see review by Shiau, 1998). On the other hand, Penaeids are thought to utilise glucose to a far lesser degree due to problems with digestive system absorption and metabolism (Shiau, 1998).

Nevertheless, molasses is also known to be a good nutritional source of potassium, and trace elements like magnesium and manganese (eg: 1.5% K, 0.2% Mg and 0.002% Mn: NutritionData.com, 2006). Since the low salinity ground waters that exist in Queensland have typically unbalanced ionic concentrations compared with seawater, and particularly with regards to low potassium levels (Collins *et al.*, 2005), molasses in feeds or used to help control pond water qualities may provide an additional source of this element in inland prawn farming systems. Whole grain wheat flour is also a good source of potassium (0.4%: NutritionData.com, 2006), so it's inclusion in the diets or in pond fertilisation regimes may further alleviate this potential ionic imbalance.

This study also sought to test for potential advantages of including feeding stimulants such as chicken's egg and marine polychaetes with the wheat flour. Diet palatability has recently been shown to be a factor that influenced growth of *Litopenaeus vannamei* when replacing marine animal protein with fermented grains and wheat gluten (Molina-Poveda and Morales, 2004). Free amino acids and small peptides that leach from the feed may provide attractants for shrimp (Akiyama and Dominy, 1989), and since palatable ingredients like fish meal and other seafood products were lacking in our simple diets, it was possible that such ingredients could improve palatability, feed utilisation and conversion. The inclusion levels used in the present study (1.1–6.8%) were slightly higher than those recommended by Akiyama and Dominy (1989) for fish solubles (1–5%), however, the marginal increases in weight gain apparent in our results were not statistically significant. Interestingly, even free wheat flour added to the tanks appeared to stimulate a strong foraging response in the experimental stock, so it is likely that wheat in itself may hold significant feeding stimulants for this Penaeid.

Marine polychaetes are widely recognised as feeding stimulants, and chickens egg has traditionally been used as a control diet in much nutrition research (Tacon (1990b). But regardless of their feeding stimuli, if protein levels in the basal diet are too low, their inclusion may not significantly improve growth even if it is improving its consumption. Additionally, any effect of these feed additives on growth could also have been due to slightly improved fatty acid and/or amino acid profiles of the composite diet. More detailed behavioural observations of prawns exposed to feeds containing various attractants may be necessary to better evaluate their usefulness in the future. A recent example of such work is that by Sanchez *et al.* (2005), who tested krill and squid meal added to a wheat flour base. This could be combined with digestibility and enzyme inhibition studies of the like recently

used by Lemos *et al.* (2004) to study *Farfantepenaeus paulensis*. That work used *in vitro* shrimp enzymes to screen and compare potential ingredients. They found similarly high digestibility for Brazilian fish meal, meat meal and wheat flour, intermediate digestibility for super prime Chilean and Argentinean fish meals, and least digestibility for soybean meal and blood meal, the later being impeded by inhibition of proteinases.

The commercial prawn feed used as the control diet in this study produced by far the highest prawn growth for all feeds tested. But it is more costly and a more highly processed feed, with fish meal and other ingredients that collectively may have difficulty passing organic certification standards. As discussed earlier, this better growth result can generally be attributed to it having a much higher level of protein (43%), and probably a more suitable range of amino and fatty acids, having been specifically formulated as a complete diet for Penaeid species. Its ingredients include steam dried fish meal, squid meal, squid liver meal, antioxidant–treated marine and vegetable oils, oilseed meal, wheat flour, phospholipids, vitamins and minerals. These ingredients provide many of the essential nutrients that are known to be required by Penaeids (for recent reviews see Chen, 1998; Shiau, 1998). Some of these essential nutrients, like phospholipids, are not found in wheat flour (NutritionData.com, 2006) and are not able to be synthesized or are synthesised too slowly by crustaeceans (Akiyama and Dominy, 1989), so must be included in their diets. For *P. merguiensis*, between 1 and 2% lecithin provides sufficient phospholipid for good growth and survival (Thongrod and Boonyaratpalin, 1998). In earlier work, Aquacop (1978) suggested that a diet containing 50-55% protein, 7% fish oil, carbohydrate in the form of starch, and vitamen and mineral mixes rich in vitamen C, choline, inositol, and magnesium (3%) is optimal for *P. merguiensis* juveniles.

Species, Feeds and Processes for Organic Certification

Carnivorous fish like rainbow trout are poor digesters of crude starch due to reduced intestinal amylase activity (Spannhof and Plantikow, 1983), so their ability to digest complex carbohydrates from grains is reduced. Fish meal replacement with grain products in aquaculture diets is therefore thought to be more likely to be successful for omnivorous or herbivorous fish species like tilapia and carp (Tacon, 1990a). This may also be due to their ability to extract more nutrients from natural sources in ponds, rather than due to large differences in their overall protein requirements. Banana prawns are generally considered to have a lower protein requirement and be more herbivorous than other species of cultured prawns like *P. monodon* (Hoang, 2001). This is one of the reasons for choosing banana prawns in this work. They have also been shown to grow to market size in prawn farm settlement ponds without the direct application of artificial feeds (Palmer *et al.*, 2005b), and thus have proven ability to adequately utilise a pond ecosystem for this extensive rearing approach. Like *P. vannamei*, which appears to digest gelatinised and natural wheat starch almost as well as many terrestrial animals (Cousin *et al.*, 1996), *P. merguiensis* may hold dietary advantages for production systems based largely on grain feeds.

However other factors may also drive the selection of species for the organic systems under investigation. For example, their general salinity tolerance and changing osmoregulatory abilities and temperature tolerances as they get older may be overriding factors in extensive low salinity inland systems. In related farm trials, *P. merguiensis*

postlarvae have been shown to successfully acclimatise and grow to small sizes at low salinities, but they are yet to yield adequate production levels for economic assessment. Seed stocks have also sometimes proved more problematic than *P. monodon* during low salinity acclimation, and they have generally shown lower survival as they grow. Despite its more carnivorous nature, *P. monodon* may therefore be a better candidate for this research direction in the future, particularly if the economic viability of extensive rearing systems can only be realised in inland regions using low salinity bore water, and in areas where high temperatures can also often prevail. This of course would be more contingent on identifying suitable and organically certifiable protein sources.

The use of low protein feeds may also be important in the future to help avoid excessive eutrophication in low-water-exchange culture ponds. In this regard the levels of digestibility and waste produced with different feeds are of high importance. Water stable feeds which do not leach excessive ingredients before ingestion, and which hold their form for several hours before breaking down, are instrumental in avoiding pond bottom fouling and wasted nutrients. A high inclusion of cooked wheat flour provides this water stability, along with greater than 34% moisture content of the extrusion paste (Cheng et al., 2002). In the present study, the steaming of extruded paste provided this stabilising gelatinisation, and the moisture contents of paste ranged from 33.4% (egg pellet) through to 39.3% (high worm pellet), which appeared to provide similar and acceptable physical stability after several hours in seawater.

The economics associated with low density or extensive rearing systems requires that it is performed on a large scale. This is why the current project's focus was on the use of saline ground waters that exist at considerable scale in inland areas of Queensland (Collins *et al.*, 2005). Low feed costs and labour would also be vital to minimising the costs of production, so that acceptable profit margins are achieved. The feeding rate and frequency used in this trial were therefore kept to a minimum (6% once per day), assuming that in an extensive pond, natural feeds would likely make up many nutritive shortfalls. The need for these practical considerations in nutritional studies with crustaceans is highlighted by Tacon (1996), who has criticised laboratory based trials in artificial environments due to their potential to skew experimental results and provide misleading findings. Whilst the present study can be similarly criticised for differing salinities, ionic concentrations and natural feeds in the culture water compared with inland extensive pond environments for which it was intended, these factors can vary significantly between potential sites making it difficult to practically address all possible scenarios that could affect such nutrition research.

Other Operational Considerations for Conversion

The United Kingdom Register of Organic Food Standards (UKROFS) require that organic production systems are designed to avoid the use of agricultural chemical inputs and minimise any related damage to the environment and wildlife. They seek to encourage the integration of enhanced biological cycles involving micro-organisms, plants and animals in sustainable farming systems. As far as possible, renewable resources and closed farming systems are employed with recyclable or biodegradable materials used or produced. Pollution needs to be minimised, human rights, workers satisfaction and safety aspects of the operation are maximised, and the wider social and ecological impacts are also considered. Product traceability is built into the certification and marketing of products and producers are bound

to report any actual or suspected contaminations. Where organic products are being produced along-side non-organic products, separation of products must be ensured with efficient operational and record keeping activities. Genetically modified organisms and their derivatives are prohibited at all stages of production. Veterinary drugs, in-feed medications/antibiotics and pest control materials (including pyrethrum) are prohibited or severely restricted (except for approved rodent baits), and emphasis is placed on good hygiene, house keeping and exclusion. Some pesticide ingredients are allowed with prior approval from the certifying body and the use of stacked or composted manures is closely regulated. Whilst referring more to land based agricultural systems, organic seed is preferable but parent crops for seed production can be non-organic.

Cavilli *et al.* (2005) provides a description of proposed practices in Brazil where the first recognised organic aquaculture farm in the world (Primar Aquaculture) was established in December 2003. Polyculture is practiced on this farm, producing shrimp (*Litopenaeus vannamei*), oysters (*Crassostrea rizophorae*), crabs (*Callinectes* sp.), mullet (*Mugil* sp.) and macroalgae (*Gracilaria*). In addition to many of the UKROFS principals stated above, the animals natural behaviour patterns guide culture practices, and local resources are used wherever possible. For example, lined ponds without bottom substrate are not considered to provide adequate burrowing habitats for prawns. Uncontaminated water supplies are a prerequisite, on-farm water recirculation schemes are recommended, and artificial aeration devices are not considered appropriate. Standards comprehensively address all operational conditions, such as site selection avoiding destruction of natural habitats, ethical slaughter of animals (immediate dipping in cold water), and processing and storage for maximum product quality. They suggest that only local species should be farmed to avoid ecological impacts of escapements. It is also proposed that fish meals used in diets should only be from the waste products of fish caught for human consumption (eg: offal and fillet trimmings), and that organic ingredients suitable for human consumption should not be used in the diets. Rather, ponds should be managed with organic inputs to increase natural productivity. Interestingly, these authors suggest that underground waters should not be used, but they fail to provide justification for this. They also suggest that antibiotic usage be restricted to post-larval production or curative measures where no homeopathic method is available.

In Australia, many of these considerations are already built into best practice requirements of the overarching industry body (Australian Prawn Farmers Association) and the licensing controls of governing bodies (Departments of Primary Industries and Fisheries, Environmental Protection Agencies, Departments of Health). For example, permits to construct farms are considered in the context of acceptable environmental impacts and appropriate site selection. Safeguards such as implementing systems to prevent stock from escaping into adjacent environments are part of licence conditions. Water discharge limits are tailored to the capacities of the receiving environments to assimilate nutrient loads, and disease reporting requirements are supported by the ready availability of high quality diagnostic services. Strict chemical usage is routinely regulated through the Australian Pesticides and Veterinary Medicines Authority (APVMA) (eg: prophylactic antibiotic use is illegal).

The two organic certifying bodies in Australia are the Biological Farmers Association (BFA) and the National Association for Sustainable Agriculture Australia (NASAA). Neither body requires that prawn postlarvae for production systems are organically certified. NASAA do not allow use of fishery by-catch in feeds but the utilisation of waste streams (eg: offal and

fish frames) is in line with the organic principles of recycling, reusing and composting. No chemicals for use with prawns are currently approved by these bodies. Those used in production of other organic foods (sulphites, ascorbic acid) would need to be assessed within the context of specific applications. Regarding growth densities, the BFA accept that fish have a tendency to school in nature. Prawns do exist at high densities in natural environments and their behavioural needs may provide a reasonable argument for more intensive production levels, as long as environmental aspects and stressors that can cause disease are minimised. Regarding water qualities, the main focus is on contaminants infiltrating the culture systems. Audits take into account what industries and other land uses are upstream, and if the water source quality is poor it is likely that closed systems with water recirculation would be necessary.

Several other factors associated with the quality assurance and shelf life of harvested product are also important for the organic certification of seafoods. Modified atmosphere packaging (MAP) is an approved alternative to the chemical preservatives that are commonly used in some foods, as has been shown for prawns in research associated with the present study. Marketing initiatives will benefit from the extended shelf life that this packaging system can provide, particularly for chilled (unfrozen) product, because of critical delays in transport and handling before going into retail environments.

CONCLUSION

This was the first work conducted in Australia towards organic prawn production. It encompassed some preliminary trials of simple feed formulations that may be applicable to extensive production systems, and took into consideration a range of factors that may impinge on a commercial operation for this new food sector. Although the experimental diets that were tested in a tank-based system fell well short of the growth achieved with an uncertified commercial feed, their protein levels were also much lower as the result of no fish meal inclusion, and they were presumably also not as well balanced with nutrients known to be essential for Penaeids. Feed ingredients that can fulfil these needs and be supplied at large scale will need to be identified before commercial operations can proceed. Culture at low densities in extensive production systems with a high prevalence of natural feeds would likely alleviate the nutritional deficits of simple supplemental feeds like those demonstrated in this study, but it is possible that the resulting low production levels would not provide enough incentive for businesses to develop along these lines; particularly in coastal regions where land values are high requiring high returns on investments and at existing facilities that have capital-intensive infrastructures.

To support this initiative, future research in Australia could therefore productively focus on developing organic diets for more intensive production systems, and on investigating other economic drivers that can assist this environmentally and socially responsible approach to seafood production. It is still uncertain whether industry in Australia will embrace this highly regimented approach to food quality assurance, particularly since best practice in Australia already has much in common with organic standards. Whilst this suggests relative ease for conversion, there are still a range of site-specific factors which would need to be addressed on a case by case basis, which adds uncertainty and complexity to business structures. Whilst the

economic assessments that have been made thus far for organic prawn farms are encouraging (eg: Xie *et al.* 2005; Wainberg, 2005), they have been undertaken in other countries (China and Brazil) where different cost structures and regulatory considerations will greatly affect operating environments and the competitive advantages this may provide. Economic assessments in Australia will require application under localised conditions so that the full production and marketing cycle can be comprehensively evaluated.

ACKNOWLEDGMENTS

APPENDIX

Table A1. Treatment allocation for tanks and starting tank bulk weights

Treatment-replicate	Tank	Prawn bulk weight
1-1	9	32.94
1-2	13	30.22
1-3	26	36.52
2-1	21	31.54
2-2	7	30.98
2-3	6	37.63
3-1	20	34.87
3-2	2	29.21
3-3	18	34.89
4-1	4	31.88
4-2	12	34.35
4-3	1	33.85
5-1	3	33.67
5-2	10	33.94
5-3	19	32.09
6-1	8	33.65
6-2	22	30.58
6-3	15	38.10

Table A1. (Continued)

Treatment-replicate	Tank	Prawn bulk weight
7-1	23	37.14
7-2	5	33.23
7-3	16	32.20
8-1	24	35.21
8-2	14	36.64
8-3	25	34.56
9-1	11	32.75
9-2	17	31.88

Mean biomass per tank = 33.635 g

Mean prawn weight = 1.345 g

Table A2. Experimental feed formulation

Experimental diet	Feed formulation
Flour dough ball	Certified organic stoneground wholemeal plain flour (2 g) freshly mixed with 22 drops (1 mL) of tap water and immediately kneaded to a homogenous dough and rolled into a sphere
Flour only pellet	Flour (as above) (900 g) mixed with 300 mL reverse osmosis (RO) water to make extrusion paste. Dried pellet consisting of 100% wheat
Flour + egg pellet	Flour (as above) (950 g) mixed with one whole chicken's egg without the shell (48 g) and 350 mL RO water to make extrusion paste. Assuming a moisture content for flour of 12% (Kialla Pure Foods), and 74.4% for the egg (Tacon, 1990b), this final dried pellet consisted of approximately 1.45% dry weight of egg
Flour + low worm pellet	Flour (as above) (950 g) mixed with worm (50 g) slurry and 350 mL RO water to make extrusion paste. Assuming moisture content of flour as above (12%) and 80.7% for blended worm slurry (DPI&F unpublished data), this final dried pellet consisted of approximately 1.14% dry weight of worm
Flour + molasses pellet	Flour (as above) (900 g) mixed with molasses (50 g) and 300 mL RO water to make extrusion paste. Assuming moisture content of flour as above (12%) and 31.4% for the molasses (pers. com. Rocky Point Refinery, 2006), this final dried pellet consisted of approximately 4.15% dry weight of molasses
Flour + high worm pellet	Flour (as above) (900 g) mixed with worm slurry (300 g) and 200 mL RO water to make extrusion paste. Assuming moisture content of flour as above (12%) and 80.7% for the worm slurry (DPI&F unpublished data), this final dried pellet consisted of approximately 6.81% dry weight of worm

**Table A3. Minimum and maximum water temperatures (from tank 10)
and feeding times**

Day	Min – max temp (°C)	Time feed added
Tues	29.0	5.30 pm
Wed	27.0 – 29.0	3.30 pm
Thurs	–	3.30 pm
Fri	28.5 – 30.0	1.00 pm
Sat	28.9 – 29.0	10.20 am
Sun	28.0 – 30.0	10.20 am
Mon	27.0 – 29.0	1.30 pm
Tues	26.5 – 29.0	4.00 pm
Wed	27.0 – 29.5	3.00 pm
Thurs	27.5 – 29.5	3.00 pm
Fri	27.5 – 29.5	1.30 pm
Sat	27.5 – 29.5	10.20 am
Sun	28.0 – 29.5	10.30 am
Mon	28.0 – 29.0	9.45 am
Tues	27.5 – 30.0	1.30 pm
Wed	28.5 – 30.0	1.00 pm
Thurs	28.0 – 30.5	2.30 pm
Fri	28.0 – 30.5	1.45 pm
Sat	29.5 – 30.5	10.30 am
Sun	28.5 – 29.5	10.30 am
Mon	28.0 – 30.0	12.15 pm
Tues	28.0 – 30.0	12.30 pm
Wed	28.0 – 30.0	2.30 pm
Thurs	28.0 – 30.0	2.00 pm
Fri	28.5 – 30.5	12.30 pm
Sat	28.5 – 30.5	10.45 am
Sun	27.5 – 31.0	10.45 am
Mon	27.0 – 28.5	3.30 pm
Tues	Harvest	

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