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Effective use of microbial biomass products to facilitate the complete replacement of fishery resources in diets for the black tiger shrimp, *Penaeus monodon*

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Abstract

A series of experiments were conducted with black tiger shrimp (*Penaeus monodon*) juveniles to firstly determine the effects of reducing fishmeal inclusion in a diet and then to evaluate the potential for a microbial bioactive to support complete replacement of both fishmeal and fish oil in feeds when fed under clear-water and green-water conditions. The isoproteic and isoenergetic replacement of fishmeal resulted in a consistent decline in growth performance indicating that at every decrease in fishmeal below an inclusion level of 45% there was a decline in performance. In a subsequent trial undertaken in a clear-water tank system diets devoid of both fishmeal and fish oil fed to shrimp were demonstrated to produce poorer performance than a fishmeal and fish oil reference diet. However the addition of a microbial bioactive to the diet resulted in not only a compensation for the replacement of these ingredients but additional growth. Replication of the clear-water trial in a green-water tank system produced similar results, but also showed that the green-water system largely compensated for the performance lost through replacement of fishmeal and fish oil. However it was also shown that the use of the microbial bioactive in the diets still resulted in improved growth performance of shrimp. This study has effectively demonstrated a viable strategy for not only a complete replacement of all fishery products in shrimp diets, but an improved performance strategy.

1. Introduction

There has been a considerable amount of research in the past few decades to improve the capacity to utilize alternative raw materials in diets for many aquaculture species, including shrimp (Gatlin et al., 2007; Glencross et al., 2007). Recent progress in the use of raw materials other than fishmeal and fish oils in diets for shrimp has resulted in significant advancements in the ability to replace fishmeal and fish oil with terrestrial grain and animal resources (Davis and Arnold, 2000; Davis et al 2002; Alvarez et al., 2007; Cruz-Suarez et al., 2001; 2007; Smith et al., 2007). However, most of these studies still have a certain amount of fishmeal or fish oil in the diet, typically rarely lower than 10% for fishmeal (Cruz-Suarez et al., 2007; Smith et al., 2007). Replacement of fish oil in many instances has proven even more difficult with few other lipid resources able to provide the necessary long-chain polyunsaturated fatty acids or provide the required short-chain polyunsaturates suitable for trophic upgrading (Deering et al., 1997; Lim et al., 1997; Glencross and Smith 1999).

However, studies with *Litopenaeus vannamei* have suggested that, when replaced with co-extruded poultry and soybean meals, fishmeal inclusion could be decreased to 0%, though shrimp performance (based on shrimp weight gain) improved with increasing replacement of fishmeal and this effect was not explained (Samocha et al., 2004). In another study it was shown that canola and soybean meals could be used effectively with as little as 6% fishmeal (Suarez et al., 2009). Pond studies by Amaya et al. (2007) indicated that all of the fishmeal in diets for *L. vannamei* could be replaced when soybean and poultry-byproduct meals were used. Indeed in that study neither growth nor feed use efficiency was compromised through the reduction in fishmeal content. This appears to indicate that under pond production systems the endogenous feed sources in the pond can help ameliorate this loss in performance seen with a reduction in fishmeal (Amaya et al., 2007), which has not been seen in laboratory tank trials (Suarez et al., 2009). None of these successes in achieving very low fishmeal inclusion in the diets of more carnivorous shrimp species, like *Penaeus monodon*, have been reported.

The recent invention of a microbial biomass based growth promoter (microbial bioactive) (Novacq™, CSIRO, Dutton Park, QLD, Australia) has resulted in the ability to stimulate shrimp growth in excess of 50% above that of a standard reference diet of the same basic nutritional specifications (Glencross et al., 2013). This product therefore also offers some potential in terms of being able to off-set poorer performance due to a range of formulation changes, including the complete replacement of any fishery derived resources in the diet of shrimp. Similar such products appear to have been reported by Burford et al. (2004) and Kuhn et al. (2008, 2009), who used another microbial biomass products to achieve significant improvements in performance in *L. vannamei*.

In the present study, a series of experiments were undertaken with black tiger shrimp (*Penaeus monodon*) in an attempt to define critical inclusion limits of fishmeal and fish oil for shrimp diets. In addition to this replacement of fishmeal and fish oil the use of a microbial bioactive was also examined for its ability stimulate growth performance and to be able to potentially aid the complete

replacement of fishmeal and fish oil. The work was undertaken in both indoor laboratory ‘clear-water’ and outdoor, zero-exchange ‘green-water’ conditions. This study aimed to test the hypotheses’ that at a critical threshold of fishmeal and fish oil inclusion that feed intake and growth would decline, but that the use of a microbial bioactive would ameliorate those declines.

2. Materials and Methods

2.1 Study design

A series of three experiments were undertaken to define; 1) the thresholds to replacement of fish meal in a clear-water tank experiment, 2) the capacity of a microbial bioactive to support the complete replacement of fishmeal and fish oil in a clear-water tank experiment, and 3) the capacity of a microbial bioactive to support the complete replacement of fishmeal and fish oil in a green-water tank experiment.

2.2 Diet manufacture

Each diet was based on using a standard reference diet of 42% protein and 7% lipid which was a mimic of the commercial feeds typically used in the Australian shrimp farming industry and which acts as our industry equivalent performance benchmark (Glencross et al., 1999a). Variants of this diet were then made by increasing inclusion of both poultry offal meal and a lupin kernel meal. Details and composition of all ingredients used in this study are presented in Table 1. Each diet was prepared by ensuring all ingredients were milled to <750 μm, prior to mixing in an upright planetary mixer (Hobart, Sydney, NSW, Australia). Water was then added during the mixing to form a dough which was subsequently screw-pressed (Dolly, La Monferrina, Castell'Alfero, Italy) through a 3mm die and cut to pellet lengths of about 6mm. The pellets were then steamed at 100ºC for 5 minutes before being oven dried at 60ºC for 24h. Diets were kept at -20ºC when not being fed.

2.3 Experiment 1

In experiment 1 a series of diets were formulated to the same specifications as the reference diet but with the fishmeal progressively diluted out of the formulations from 20% to 0% inclusion at 5% increments. In addition to the series of diets with fishmeal diluted out, two additional diets with 5% and 0% fishmeal were formulated which also included 10% of a microbial bioactive (Novacq™, CSIRO, Dutton Park, QLD, Australia) as a replacement for the wheat component of the diet. An additional two diets maintained high levels of fishmeal, but also had 5% and 10% inclusion levels of the microbial bioactive in replacement of wheat as a reference (Table 2).

2.3.1 Shrimp collection and trial management

Several hundred individuals (~8 g) of a wild-type genotype of black tiger shrimp (*Penaeus monodon*) were collected from a grow-out pond at Truloff's Prawn Farm (Woolgoolga, QLD, Australia) by cast-netting and transferred to a holding tank (10,000 L) where they were held pending allocation to trial tanks. During the holding period (~7 days) they were fed a standard commercial grower diet (Prawn Grower, Ridley Aquafeeds, Narangba, QLD, Australia).

Six shrimp were then allocated to each of 50 x 100 L tanks in an indoor laboratory system. The mean initial weight across all tanks was 8.19 ± 0.72 g. Tanks of the shrimp were maintained with

flow-through seawater at a rate of 500 mL/min. Temperatures of each tank were maintained at 29.2 ± 0.28ºC and dissolved oxygen maintained at 6.4 ± 0.14 mg/L. Light was maintained on a 12:12 light:dark cycle. Shrimp were individually weighed at day 0, 14, day 28 and again at day 42. The mean weight of each tank was determined at each assessment point to calculate the mean weight for each treatment, with tanks used as the replicate (n = 5 per treatment). During this period the shrimp were manually fed the diets twice daily to marginal excess and the amount of feed remaining the following day scored and used to adjust the next day's ration (increase or decrease) according to a feed intake score. Uneaten feed was siphoned from each tank daily after scoring. The assessment was also used to provide a quantitative measurement of uneaten feed in each tank. This method was also used to estimate as accurately as possible feed intake within each tank on each day (Smith et al., 2007b).

2.4 Experiment 2

In Experiment 2, a series of diets were formulated to the same specifications as the reference diet but with the fishmeal reduced in the formulations to either 10% or 0% inclusion. In addition to the series of diets with the reduced fishmeal, a corresponding series of diets (with 10% and 0% fishmeal) were formulated with linseed oil replacing all fish oil. A further additional corresponding series of diets (with 10% and 0% fishmeal) were formulated with the microbial bioactive in replacement of the wheat component of the diet. A final additional corresponding series of diets was formulated with linseed oil replacing all fish oil and also including the microbial bioactive (Table 3).

2.4.1 Shrimp collection and trial management

Several hundred individuals (~4 g) of a wild-type genotype of shrimp were collected from a grow-out pond at the Bribie Island Research Centre (DEEDI, Woorim, QLD, Australia) by cast-netting and transferred to a holding tank (10,000 L) where they were held pending allocation to trial tanks. During the holding period (~7 days) they were fed a standard commercial grower diet (Prawn Grower, Ridley Aquafeeds, Narangba, QLD, Australia).

Six shrimp were then allocated to each of 40 x 100 L tanks in an indoor laboratory system. The mean initial weight across all tanks was 4.35 ± 0.04 g. Tanks of the shrimp were maintained with flow-through water at a flow rate of 500 mL/min. Temperatures of each tank were maintained at 28.9 ± 0.15ºC and dissolved oxygen maintained at 5.3 ± 0.11 mg/L. Light was maintained on a 12:12 light:dark cycle. Shrimp were individually weighed at day 0, 14, day 28 and again at day 42. The mean weight of each tank was determined at each assessment point to calculate the mean weight for each treatment, with tanks used as the replicate (n = 4 per treatment). During this experiment feeding of the shrimp was managed the same as detailed for Experiment 1.

2.5 Experiment 3

In Experiment 3 the same series of diets as used in Experiment 2 were fed to shrimp kept in green-water conditions. Green-water conditions were established in each of 30 x 2400 L tanks about 7 days before being stocked with shrimp and maintained as a zero-water-exchange system for the duration of the experiment. Freshwater was added to tanks as required to maintain water level and salinity change from evaporation. To generate a green-water culture each tank was filled with seawater filtered through a 100μm screen and then fertilised with 70g of Aquasol™ (Yates, Padstow, NSW, Australia). Each of the 30 tanks was then recirculated through a common sump to ensure homogeneity in the tank conditions. After seven days each tank had established a consistent microalgal bloom within and this was consistent across all 30 tanks.

2.5.1 Shrimp collection and trial management

Several hundred individuals (~3 g) of a wild-type genotype of shrimp were collected from a grow-out pond at the Pacific Reef Farms Pty Ltd (Ayr, QLD, Australia) by cast-netting and transferred by air-freight to Brisbane where they were placed in several holding tanks (10,000 L) and held pending allocation to trial tanks. During the holding period (~7 days) they were fed a standard commercial grower diet (Prawn Grower, Ridley Aquafeeds, Narangba, QLD, Australia).

Thirty shrimp were then allocated to each of 30 x 2400 L tanks in an outdoor tank system kept in a glasshouse. The mean initial weight across all tanks was 3.89 ± 0.07 g (mean ± S.D.). Tanks were maintained on a static water basis with regular aeration and a top up with fresh seawater to maintain volumes after evaporation. Temperatures of each tank were maintained at 28.1 ± 1.8ºC and dissolved oxygen averaged 7.1 ± 0.25 mg/L. Light was ambient over the period of the study (8th November 2012 to 10th January 2013). Water salinity 38.1 ± 1.01 ppt and pH 8.1 ± 0.11ºC. Secchi .depth was also measured weekly and over the 63 days averaged 75 ± 10.6 cm. Shrimp were individually weighed at day 21, day 42 and again at day 63. The mean weight of each tank was determined at each assessment point to calculate the mean weight for each treatment, with tanks used as the replicate (n = 3 per treatment). During this period the shrimp were manually fed the diets twice daily on either of two feed trays within each tank to marginal excess and the amount of feed remaining the following day scored and used to adjust the next day's ration (increase or decrease) according to feed intake score. This method was also used to estimate as accurately as possible feed intake within each tank on each day. Uneaten feed was emptied from the feed tray after scoring each day. This feeding method was adapted from Smith et al (2002).

2.6 Chemical analysis

Ingredient samples were analysed for dry matter, ash, nitrogen, amino acids, total lipid, carbohydrate and gross energy content. Diets and whole shrimp samples were also analysed for fatty acid composition. Dry matter of the samples was calculated by gravimetric analysis of a milled sample following oven drying at 105ºC for 24 h. Protein levels were calculated from the

191 determination of total nitrogen by CHNOS auto-analyser, based on N x 6.25. Amino acid analysis
192 involved the samples being hydrolyzed at 110ºC for 24 h in 6M HCl with 0.05% Phenol. Cystine was
193 derivatized during hydrolysis by the addition of 0.05% 3-3-dithiodipropoinic acid. The acid
194 hydrolysis destroyed tryptophan making it unable to be determined. Separation was by HPLC on a
195 Hypersil AA-ODS 5μm column using an 1100 series Hewlett Packard HPLC system. Gross ash
196 content was determined gravimetrically following loss of mass after combustion of a sample in a
197 muffle furnace at 550°C for 12 h. Total lipid content of the diets was determined gravimetrically
198 following extraction of the lipids using chloroform:methanol (2:1). Fatty acids were analysed as
199 methyl ester derivatives. Lipids were esterified by the method of O'Fallon et al. (2007) and analysed
200 by gas chromatography (GC) using flame ionization detection. Peaks were identified by comparing
201 relative retention times to standards. Carbohydrates were estimated based on dry matter content of the
202 feed minus the lipid, ash and protein contents. Gross energy was determined by adiabatic bomb
203 calorimetry. All methods were consistent with those recommended by AOAC (2005).
204
205 *2.7 Statistical analysis*
206 All values are means ± standard deviations unless otherwise specified. Significant differences
207 were determined using an ANOVA followed by a Tukey's HSD test with critical ranges were set at $P < 0.05$. These tests were undertaken using the Statistica™ v6.0 software (Statsoft®, Tulsa, OA, USA).
209 Linear regression analysis and line fitting of those relationships was undertaken using the data
210 analysis tools and graphics elements of Microsoft Excel.

3. Results

3.1 Experiment 1

There were significant effects on shrimp growth due to the reduction in fishmeal inclusion (Table 4). Growth (as defined by final weight, weight gain, gain rate) of those shrimp fed the reference diet was typical for that of this species in a clear-water tank system over a six week period (0.91 ± 0.11 g/wk/shrimp) (Smith et al., 2007b). The progressive replacement of fishmeal with a balance of poultry offal meal and lupin kernel meal resulted in a significant decline in growth ($P=0.026$ as regression; Fig. 1) to a lowest performance at 10% inclusion of fishmeal, though this was not significantly different from the growth of shrimp fed diets with either 5% or 0% fishmeal. Based on an ANOVA analysis the critical inclusion level where growth was poorer than the reference diet (F45:M0) was the diet with 10% fishmeal (F10:M0). The addition of the microbial bioactive resulted in a numerical, but not a significant ($P=0.063$) improvement in growth. The addition of the microbial bioactive to diets with either low or no fishmeal resulted in those shrimp subsequently fed those diets performing equal to the reference diet (F45:M0). Feed conversion ranged from 3.92 to 6.42 and but was not significantly affected ($P=0.116$) by fishmeal inclusion level.

3.2 Experiment 2

In clear-water indoor laboratory conditions the complete replacement of both fish meal and fish oil resulted in a progressive decline in growth (as defined by final weight, weight gain and gain rate) of those shrimp in experiment 2 (Table 5). Growth of shrimp fed the diet completely free of fish meal and fish oil (F0:O0:M0) was significantly ($P<0.001$) poorer than that of those shrimp fed the reference diet (F50:O2:M0). This effect was despite that the reduction in fish meal and fish oil resulted in increased feed intake by the shrimp (Table 5). The addition of the microbial bioactive (F50:O2:M10) resulted in a significant increase in growth ($P<0.001$) and feed intake ($P=0.034$) by the shrimp. When added to diets with low or no fish meal and/or fish oil (diets F10:O0:M10, F0:O0:M10, F10:O2:M10 and F0:O2:M10) this increase in growth induced by the microbial bioactive exceeded the losses in growth observed with the complete replacement of fish meal and fish oil (Table 5). Feed conversion was not significantly affected by any treatment, largely due to the inherent variability associated with this parameter.

There were a range of significant effects of the dietary treatments on the composition of the shrimp (Table 7). Notable changes included a decrease in the LC-PUFA with a decrease of either (or both) fishmeal and fish oil in the diet. This was caused principally by a decline in the percentage of C20:5n-3, C22:5n-3 and C22:6n-3. This decline in the LC-PUFA and notably the Total n-3 also resulted in a significant change to the n-3 : n-6 ratio in the fatty acid composition of the shrimp, which was further exacerbated by an increase in the Total n-6 fatty acids.

3.3 Experiment 3

In green-water zero-exchange outdoor tank conditions the complete replacement of both fish meal and fish oil had no significant (P=0.994) effect on growth (as defined by final weight, weight gain and gain rate) of those shrimp in experiment 3 (Table 6). However, the addition of the microbial bioactive to the diet resulted in a significant (P=0.005) increase in growth and feed intake by the shrimp. When added to diets with low or no fish meal and/or fish oil this increase in growth induced by the microbial bioactive was greater than that observed when the diets had high levels of fish meal and fish oil (F50:O2:M10) (Table 6).

As with experiment 2 there were also a range of significant effects on the composition of the shrimp due to the dietary treatments in experiment 3 (Table 8). In contrast to experiment 2, there were no significant differences among treatments in the percentages of Total LC-PUFA in experiment 3. Although there was some variability in the percentages of C20:5n-3 and C22:6n-3 with changes in dietary treatments in this experiment, it was the substantial increase in the levels of C20:4n-6 that caused the absence of a significant difference in the Total LC-PUFA.

4. Discussion

The use of fishmeal in shrimp diets has long been identified as one of the critical issues affecting sustainability of feed use in this aquaculture sector (Tacon and Metian, 2008). However, despite this apparent reliance on the use of fishmeal there was little data in the literature on the consequences of diluting the use of fishmeal through the inclusion of other protein sources. In addition to attempts to define critical inclusion limits of fishmeal for shrimp diets, the development of a microbial bioactive (Novacq™; CSIRO, Brisbane, QLD, Australia) also holds promise for being able to potentially aid the complete replacement of both fishmeal and fish oil (Ju et al., 2008; Kuhn et al., 2009). This study therefore aimed to test a series of hypotheses. Firstly, that at a critical threshold of fishmeal inclusion feed intake and growth would decline. Secondly, that the use of a microbial bioactive would ameliorate those declines and those also attributable to complete replacement of fish oil as well. It was also hypothesised that these effects would be seen in both clear-water and green-water conditions.

4.1 Thresholds to fishmeal inclusion

From experiment 1 it was clear that reducing the fishmeal inclusion in diets for *P. monodon* had a negative effect on performance. Based on the regression analysis a decline in growth was observed with every level of reduction in fishmeal. Though, based on an ANOVA analysis though this difference was only significant at the 10% inclusion level of fishmeal and below. This observation supports the fact that although regression analysis presents a case for decline in performance with any replacement of fishmeal, it tangibly only becomes an issue once fishmeal is as low as 10%. However, the results from experiment 1 provide clear support to the claims of importance of fishmeal use in diets for shrimp (Tacon and Metian, 2008).

Other researchers who have examined the replacement of fishmeal with other alternative protein sources have also noted a similar response. Lim and Dominy (1990) replaced fishmeal (mixed with squid and shrimp meal) in series of diets for *L. vannamei* using soybean meal and achieve diets with 0 g/kg fishmeal. However, these authors observed that below 213 g/kg inclusion of the fishmeal mix that growth began to significantly deteriorate. Other studies using co-extruded poultry and soybean meals as replacements found that they could achieve equal performance with 0g/kg fishmeal inclusion, and interestingly shrimp performance appeared to improve with increasing replacement of fishmeal, though this observation wasn't rationalised. Notably, though the addition of 10 g/kg of krill meal made a notable improvement to a diet with 0 g/kg of fishmeal (Samocha et al., 2004). In other studies it has been demonstrated that use of both rapeseed and soybean meals can be effective with as little as 6% fishmeal inclusion in the diet (Suarez et al., 2009). Though notably, most of these studies have relied on the addition of a krill or squid meal supplement to achieve equal or slightly poorer performance of shrimp fed fishmeal free diets.

4.2 Growth stimulation by microbial bioactive inclusion

In experiment 1 the addition of the microbial bioactive to the diet was also observed to improve the growth of the shrimp (both in the high and low fishmeal based diets; diets F45:M5, F45:M10, F5:M10 and F0:M10). Indeed if the performance of the shrimp fed diets F5:M10 and F0:M10 which had 5% and 0% fishmeal respectively is compared against their analogous diets (F5:M0 and F0:M0) without the microbial bioactive it can be noted that their performance is 40% to 46% better. This contrasts the diet fortified with microbial bioactive that still had high fishmeal (diets F45:M5 and F45:M10), but the growth improvement was only 11% to 18% better than that of the reference (F45:M0). However, in experiment 2 the inclusion of 10% of the microbial bioactive in to a similar diet with high levels of fishmeal increased growth rates of shrimp in indoor lab conditions from 0.87 g/wk to 1.30 g/wk, an increase of 50%. We postulate that this variability in response may be an effect of shrimp stock used, with different stocks used for each of the different experiments.

Other researchers have also recently reported the use of microbial biomass products in shrimp feeds with varying degrees of success (De Schryver et al., 2008; Ju et al., 2008; 2009; Kuhn et al., 2008; 2009). In the work of Ju et al (2008), the authors included a microbial biomass in at 20% inclusion level and observed an increase in growth rate of *L. vannamei* from 0.85 g/wk to 1.03 g/wk in indoor lab conditions, a 21% increase in growth rate. These authors also went on to fractionate the microbial biomass into various chemical fractions and identified that the prospective bioactive component was in the acetone soluble fraction, possibly a carotenoid of some sort, though none of the fractions produced in their study were significantly different from each other in terms of growth rates achieved.

4.3 Complete replacement of fishmeal and fish oil in clear-water by using microbial bioactive

The use of clear-water experimental systems provides the most stringent manner in which to assess the impact of a feed on shrimp performance because it isolates other exogenous factors other than the feed provided (Cuzon et al., 2004). In the second experiment, consistent with the observations of the first, there was a consistent decline in growth of the shrimp with reduction in fishmeal content of the diet. However, significant improvements were seen to both the 10% fishmeal (diet F10:O2:M0) and 0% fishmeal (diet F0:O2:M0) treatments with the addition of the microbial bioactive. In each case the relative biomass gains for each diet with its "paired' microbial bioactive fortified analogue (diet F10:O2:M0 cf. F10:O2:M10and diet F0:O2:M0 cf. F0:O2:M10) were close to or over 100% better.

The replacement of fish oil with linseed oil was arguably more successful and is consistent with earlier observations and established knowledge on the management of essential fatty acid demands in this species (Deering et al., 1997; Glencross and Smith, 1999; Glencross et al., 2002b). In those studies it was shown that provided the n-3 and n-6 fatty acids were kept in 'balance' then the animal had sufficient capacity to synthesise their own LC-PUFA and in fact that if this optimal

balance was maintained and nominal amounts of LC-PUFA supplied then this actually promoted superior growth. These earlier observations are again confirmed where it can be seen that replacement of the fish oil with linseed oil (diet F10:O0:M0) and addition to the diet with 10% fishmeal (which introduces nominal amounts of LC-PUFA) actually resulted in numerically better growth than that achieved with the comparative diet with fish oil (diet F10:O2:M0).

4.4 Complete replacement of fishmeal and fish oil in green-water by using microbial bioactive

It has been suggested that the culture system used in experimentation with shrimp can have a significant impact on the results achieved (Tacon et al., 2002; Cuzon et al., 2004). Cuzon et al. (2004) proposed that it was more appropriate to conduct shrimp trials in outdoor green-water tanks that better mimicked pond conditions. In the present study there is some indication to support this, in that the observation of reduced growth with shrimp fed no fishery products in their diet from the clear-water trial was not apparent in the green-water trial, suggesting that the shrimp were obtaining some level of nutrition from the green-water system that helped offset those growth losses observed in the clear-water trial. However, the growth stimulatory effect of the microbial bioactive was apparent in both experimental systems. Additionally, the use of this microbial bioactive and the complete replacement of fishery resources had no significant impact on the total LC-PUFA content of the shrimp produced using the green-water system.

There are few studies where there has been complete replacement of fish meal and/or fish oil, in green-water or pond conditions. One such study that did attempt complete replacement of fish meals was that by Amaya et al. (2007), who fed diets based on poultry by-product and soybean meals to *L. vannamei* in pond trials with fishmeal levels ranging from 90 g/kg to 0 g/kg. These authors observed after 81 days that there was no significant difference (P=0.072) in growth between those shrimp fed the different treatments, although the one with 0 g/kg fishmeal was the poorest of all treatments. In this same study Amaya et al. (2007) also examined a diet made solely with plant protein sources, but also supplemented with squid meal (10 g/kg). All experimental diets also performed numerically poorer than the commercial reference used. In the green-water trial in the present study, equal or superior growth was achieved with no aquatic animal meals or oils included in the diet. In addition, the inclusion of a microbial bioactive resulted in superior performance of those same fishmeal and fish oil free diets into which it was included. Therefore we contest that the present study is the first reported that has achieved complete replacement of all fishery derived products and demonstrated equal or superior performance and therefore represents a major step forward in terms of complete independence from reliance of fishery products to underpin shrimp production.

4.5 Conclusions

The findings of this study demonstrate that through the use of a microbial bioactive product containing growth promoting properties that it is possible to completely off-set the need for both fish

meal and fish oil and produce diets for shrimp that have no reliance on any fishery products. While other studies have shown the ability replace fish meal or fish oil, in most cases noted this has only been achieved through the use of other fishery resources like squid or crustacean meals (Amaya et al., 2007). The findings from this study are a major progression in the sustainability of the shrimp farming industry in that it demonstrates clear potential for complete independence from fishery resources. This outcome is yet to be achieved with any other marine species.

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Tables and Figures

Table 1. Composition of the key experimental ingredients (all values are g/kg dry basis - unless otherwise specified).

	Fishmeal	Gluten	Wheat	Lupin	POM	MB
Dry matter (g/kg)	912	904	900	921	906	917
Protein	753	807	129	418	680	42
Lipid	102	22	22	55	182	6
Ash	159	8	839	30	151	269
Carbohydrates	0	163	10	497	69	683
Energy (kJ/g DM)	21.5	22.1	18.4	20.0	21.3	13.0
Alanine	45	19	4	16	48	2
Arginine	40	26	6	55	45	1
Aspartic acid	66	25	7	46	54	4
Cystine	9	20	1	7	8	0
Glutamate	92	299	40	82	87	3
Glycine	42	25	5	18	60	2
Histidine	23	13	1	11	12	0
Isoleucine	32	28	4	18	25	2
Leucine	55	53	9	31	46	2
Lysine	55	11	5	18	37	1
Methionine	23	15	2	4	18	1
Phenylalanine	29	43	6	18	26	1
Proline	30	115	25	21	46	6
Serine	30	40	6	23	28	2
Taurine	7	0	0	0	4	0
Threonine	32	21	5	17	28	3
Tyrosine	24	27	4	18	20	1
Valine	37	28	5	16	27	2

POM: Poultry offal meal. MB : Microbial bioactive

Table 2. Formulations and composition of diets from Experiment 1. Data are percent values.

	F45:M0	F45:M5	F45:M10	F20:M0	F15:M0	F10:M0	F5:M0	F0:M0	F5:M10	F0:M10
Fish meal (anchovetta) [a]	45.00	45.00	45.00	20.00	15.00	10.00	5.00	0.00	5.00	0.00
Gluten (wheat) [b]	5.00	5.00	5.00	3.00	4.75	6.50	8.25	10.00	8.25	10.00
Wheat flour [b]	47.03	42.03	37.03	35.03	34.53	34.03	33.53	33.03	23.53	23.03
Lecithin [a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fish Oil [a]	1.50	1.50	1.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Microbial bioactive [c]	0.00	5.00	10.00	0.00	0.00	0.00	0.00	0.00	10.00	10.00
Lupin kernel meal [d]	0.00	0.00	0.00	20.00	22.50	25.00	27.50	30.00	27.50	30.00
Poultry offal meal [a]	0.00	0.00	0.00	20.00	21.25	22.50	23.75	25.00	23.75	25.00
Astanxanthin (10%) [g]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Cholesterol [e]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Banox E [f]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin C (Stay C) [g]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix [h]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Composition (% DM unless otherwise stated)										
Dry matter (% as is)	92.6	93.0	92.0	92.9	93.3	93.5	92.7	92.1	92.2	93.0
Protein	43.8	43.8	44.5	45.0	44.5	44.0	44.0	43.9	44.0	44.1
Lipid	7.0	7.0	7.1	7.9	7.7	7.6	7.6	7.5	7.6	7.5
Ash	7.2	8.4	9.5	6.7	6.1	5.6	5.1	4.6	7.4	6.9
Carbohydrate	41.9	40.9	38.9	40.5	41.7	42.8	43.3	44.0	40.9	41.5
Gross Energy (MJ/kg DM)	20.3	20.1	19.9	20.6	20.7	20.7	20.8	20.8	20.4	20.5

[a] Fish (Peruvian anchovetta) meal, Poultry offal meal, Lecithin and Fish (Peruvian anchovetta) oil : Ridley Aquafeeds, Narangba, QLD, Australia. [b] Wheat gluten and flour : Manildra, Auburn, NSW, Australia. [c] Microbial bioactive: Novacq™ : CSIRO, Cleveland, QLD, Australia, PCT Patent AU 2008201886. [d]Lupin (*L. angustifolius*) kernel meal: Coorow Seed Cleaners, Coorow, WA, Australia. [e] Cholesterol : MP Bio, Aurora, OH, USA. [f] Banox-E™ : BEC Feed Solutions, Carole Park, QLD, Australia. [g] Astaxanthin (10%) as Carophyll Pink™ and Stay C™: DSM, Wagga Wagga, NSW, Australia. [h] Vitamin and mineral premix : Rabar, Beaudesert, QLD, Australia; includes (IU/kg or g/kg of premix): Vitamin A, 2.5MIU; Vitamin D3, 1.25 MIU; Vitamin E, 100 g; Vitamin K3, 10 g; Vitamin B1, 25 g; Vitamin B2, 20 g; Vitamin B3, 100 g; Vitamin B5, 100; Vitamin B6, 30 g; Vitamin B9, 5; Vitamin B12, 0.05 g; Biotin, 1 g; Vitamin C, 250 g; Banox-E, 13 g; [h]Yttrium oxide: Stanford Materials, Aliso Viejo, CA, USA.

Table 3 Formulations and composition of the diets from Experiments 2 and 3. Data are percent values.

	F50:O2:M0	F50:O2:M10	F10:O2:M0	F0:O2:M0	F10:O0:M0	F0:O0:M0	F10:O0:M10	F0:O0:M10	F10:O2:M10	F0:O2:M10
Fish Meal (anchovetta)	50.00	50.00	10.00	0.00	10.00	0.00	10.00	0.00	10.00	0.00
Gluten	5.00	5.00	10.00	13.00	10.00	13.00	10.00	13.00	10.00	13.00
Wheat Flour	41.23	31.23	34.33	31.53	34.33	31.53	24.33	21.53	24.33	21.53
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fish Oil	2.30	2.30	2.50	2.50	-	-	-	-	2.50	2.50
Microbial bioactive	-	10.00	-	-	-	-	10.00	10.00	10.00	10.00
Linseed Oil	-	-	-	-	2.50	2.50	2.50	2.50	-	-
Lupin kernel meal	-	-	15.00	20.00	15.00	20.00	15.00	20.00	15.00	20.00
Poultry Meal	-	-	26.00	30.00	26.00	30.00	26.00	30.00	26.00	30.00
DL-Methionine	-	-	0.50	1.00	0.50	1.00	0.50	1.00	0.50	1.00
L-Lysine	-	-	0.20	0.50	0.20	0.50	0.20	0.50	0.20	0.50
Carophyll Pink	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Cholesterol	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Banox E	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin C	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin Premix	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Composition (% DM unless otherwise stated)										
Dry matter (% as is)	95.21	94.75	94.79	94.84	93.69	95.37	95.8	95.06	96.1	95.98
Protein	47.26	45.05	47.61	47.45	45.76	48.17	44.62	46.62	43.70	44.54
Lipid	9.87	8.42	10.04	8.58	8.36	10.56	9.52	10.16	10.19	10.38
Ash	10.18	17.47	6.88	5.88	6.92	5.77	13.82	12.60	13.78	12.93
Carbohydrate	32.69	29.06	35.47	38.09	38.96	35.51	32.03	30.63	32.33	32.15
Gross Energy (MJ/kg DM)	20.4	18.6	20.8	21.9	21.3	21.5	19.6	20.3	19.8	20.1
Total SFA (% total FA)	39.6	41.4	29.9	28.6	21.7	20.3	20.6	20.8	29.8	28.9
Total MUFA (% total FA)	15.8	15.6	35.0	37.2	35.4	37.7	36.2	38.8	35.3	37.8
Total PUFA (% total FA)	16.2	14.3	25.0	27.1	39.7	41.6	40.1	40.4	24.7	26.1
Total LC-PUFA (% total FA)	28.4	28.6	10.0	7.1	3.1	0.3	3.1	0.0	10.2	7.3
Total n-3 (% total FA)	30.2	29.9	12.0	9.2	17.7	15.4	18.4	15.2	12.2	9.3
Total n-6(% total FA)	14.5	13.0	23.1	25.0	25.1	26.6	24.8	25.3	22.7	24.1

[a] Fish (Peruvian anchovetta) meal, Poultry offal meal and Fish (Peruvian anchovetta) oil : Skretting Australia, Hobart, TAS, Australia. [b] Wheat gluten and flour : Manildra, Auburn, NSW, Australia. [c] Microbial bioactive: Novacq™, CSIRO, Cleveland, QLD, Australia, PCT Patent AU 2008201886. [d]Lupin (*L. angustifolius*) kernel meal: Coorow Seed Cleaners, Coorow, WA, Australia. [a] Cholesterol : MP Bio, Aurora, OH, USA. [e] Banox-E™ : BEC Feed Solutions, Carole Park, QLD, Australia. [f] Astaxanthin (10%) as Carophyll Pink™ and Stay C™: DSM, Wagga Wagga, NSW, Australia. [f] Vitamin and mineral premix : Rabar, Beaudesert, QLD, Australia; Total FA: total fatty acids.

Table 4 Shrimp growth and feed utilisation parameters over the 42 day period of Experiment 1

	F45:M0	F45:M5	F45:M10	F20:M0	F15:M0	F10:M0	F5:M0	F0:M0	F5:M10	F0:M10	Pooled SEM
Initial weight (g/shrimp)	8.23	8.32	8.17	8.20	8.17	8.15	8.07	8.22	8.16	8.08	0.015
Weight (g/shrimp)	13.68^{b}	14.36^{ab}	14.62^{a}	13.38^{b}	13.14^{bc}	12.43^{c}	12.65^{c}	12.68^{c}	14.59^{a}	14.59^{a}	0.145
Gain (g/shrimp)	5.45^{b}	6.04^{ab}	6.45^{a}	5.18^{b}	4.97^{bc}	4.28^{c}	4.58^{c}	4.46^{c}	6.42^{a}	6.51^{a}	0.142
Gain rate (g/wk)	0.91^{b}	1.01^{ab}	1.08^{a}	0.86^{b}	0.83^{bc}	0.71^{c}	0.76^{c}	0.74^{c}	1.07^{a}	1.09^{a}	0.024
Feed fed (g/shrimp)	20.4^{c}	25.6^{b}	28.1^{ab}	31.8^{a}	28.0^{ab}	27.2^{ab}	26.0^{b}	24.0^{bc}	33.8^{a}	31.7^{a}	0.388
FCR (fed/gain)	3.92^{a}	4.26^{a}	4.44^{ab}	6.23^{cd}	5.72^{c}	6.42^{d}	5.69^{c}	5.46^{bc}	5.27^{bc}	4.87^{b}	0.107
Survival (%)	76.0^{b}	100.0^{a}	92.0^{a}	92.0^{a}	92.0^{a}	96.0^{a}	100.0^{a}	84.0^{ab}	100.0^{a}	96.0^{a}	6.59

FCR : Feed conversion ratio. Different superscripts within rows indicate significant differences ($P<0.05$). An absence of superscripts implies that there were no significant differences ($P>0.05$).

Table 5. Shrimp growth and feed utilisation parameters over the 42 day period of clear-water study in Experiment 2

	F50:O2:M0	F50:O2:M10	F10:O2:M0	F0:O2:M0	F10:O0:M0	F0:O0:M0	F10:O0:M10	F0:O0:M10	F10:O2:M10	F0:O2:M10	Pooled SEM
Initial weight (g/shrimp)	4.36	4.25	4.41	4.38	4.36	4.37	4.35	4.33	4.36	4.33	0.01
Weight (g/shrimp)	9.57 ^{b}	12.05 ^{d}	8.39 ^{a}	8.16 ^{a}	8.53 ^{ab}	7.35 ^{a}	12.17 ^{d}	10.51 ^{c}	12.79 ^{d}	12.07 ^{d}	0.28
Gain (g/shrimp)	5.21 ^{b}	7.80 ^{d}	3.98 ^{a}	3.78 ^{a}	4.18 ^{ab}	2.98 ^{a}	7.82 ^{d}	6.18 ^{c}	8.43 ^{d}	7.73 ^{d}	0.29
Growth rate (g/shrimp/wk)	0.87 ^{b}	1.30 ^{d}	0.66 ^{a}	0.63 ^{a}	0.70 ^{ab}	0.50 ^{a}	1.30 ^{d}	1.03 ^{c}	1.41 ^{d}	1.29 ^{d}	0.05
Feed fed (g/shrimp)	16.72 ^{a}	19.89 ^{b}	17.51 ^{a}	19.43 ^{b}	19.08 ^{b}	21.26 ^{c}	29.24 ^{d}	19.19 ^{b}	28.25 ^{d}	28.46 ^{d}	0.68
FCR (fed/gain)	3.21 ^{b}	2.55 ^{a}	4.40 ^{c}	5.14 ^{d}	4.57 ^{c}	7.12 ^{e}	3.74 ^{a}	3.11 ^{ab}	3.35 ^{b}	3.68 ^{b}	0.19
Survival (%)	93.3 ^{bc}	100.0 ^{c}	87.5 ^{b}	83.3 ^{ab}	91.7 ^{bc}	70.8 ^{a}	91.7 ^{bc}	91.7 ^{bc}	95.8 ^{c}	91.7 ^{bc}	1.13

Different superscripts within rows indicate significant differences (P<0.05). An absence of superscripts implies that there were no significant differences (P>0.05).

Table 6. Shrimp growth and feed utilisation parameters over the 63 day period of the green-water study in Experiment 3

	F50:O2:M0	F50:O2:M10	F10:O2:M0	F0:O2:M0	F10:O0:M0	F0:O0:M0	F10:O0:M10	F0:O0:M10	F10:O2:M10	F0:O2:M10	Pooled SEM
Initial weight (g/shrimp)	3.84	3.94	3.86	3.94	3.88	3.82	3.90	3.86	3.95	3.88	0.01
Weight (g/shrimp)	9.02 ^{a}	11.67 ^{b}	9.67 ^{a}	9.32 ^{a}	10.33 ^{ab}	9.78 ^{a}	12.24 ^{bc}	11.08 ^{b}	13.20 ^{cd}	13.40 ^{d}	0.23
Gain (g/shrimp)	5.18 ^{a}	7.73 ^{b}	5.81 ^{a}	5.38 ^{a}	6.45 ^{ab}	5.96 ^{a}	8.34 ^{bc}	7.23 ^{b}	9.25 ^{cd}	9.52 ^{d}	0.22
Growth rate (g/shrimp/wk)	0.58 ^{a}	0.86 ^{b}	0.65 ^{a}	0.60 ^{a}	0.72 ^{ab}	0.66 ^{a}	0.93 ^{bc}	0.80 ^{b}	1.03 ^{cd}	1.06 ^{d}	0.02
Feed fed (g/shrimp)	24.44 ^{a}	29.49 ^{b}	24.22 ^{a}	26.79 ^{ab}	27.83 ^{ab}	27.67 ^{ab}	32.20 ^{bc}	27.89 ^{ab}	34.24 ^{c}	30.37 ^{bc}	0.45
FCR (fed/gain)	4.74 ^{bc}	4.16 ^{b}	4.25 ^{b}	4.97 ^{c}	4.45 ^{bc}	4.70 ^{bc}	3.86 ^{ab}	3.89 ^{ab}	3.68 ^{a}	3.18 ^{a}	0.08
Survival (%)	96.7	97.8	82.2	96.7	94.4	96.7	98.9	98.9	94.4	94.4	0.68

Different superscripts within rows indicate significant differences (P<0.05). An absence of superscripts implies that there were no significant differences (P>0.05).

Table 7. Composition of shrimp from the clear-water study in Experiment 2

	F50:O2:M0	F50:O2:M10	F10:O2:M0	F0:O2:M0	F10:O0:M0	F0:O0:M0	F10:O0:M10	F0:O0:M10	F10:O2:M10	F0:O2:M10	Pooled SEM
Dry matter (%)	25.5	24.9	25.4	25.0	25.2	25.1	25.9	24.7	26.0	26.1	0.1
Protein (%)	20.3	19.7	19.0	21.6	19.4	22.0	21.4	21.3	21.5	22.7	0.2
Lipid (%)	2.1 ^{ab}	1.8 ^{ab}	2.1 ^{ab}	2.0 ^{ab}	1.9 ^{ab}	1.6 ^{a}	1.7 ^{a}	1.7 ^{a}	2.0 ^{ab}	2.2 ^{b}	0.0
Ash (%)	3.1 ^{a}	3.0 ^{a}	3.2 ^{ab}	2.9 ^{a}	3.1 ^{a}	3.1 ^{a}	3.3 ^{ab}	3.4 ^{b}	3.7 ^{b}	3.2 ^{ab}	0.0
Gross energy (kJ/g)	5.0	4.8	5.0	5.0	4.8	4.9	4.9	4.8	5.0	5.0	0.0
C14:0	1.7 ^{c}	1.6 ^{c}	1.1 ^{bc}	0.8 ^{b}	0.7 ^{b}	0.2 ^{a}	0.1 ^{a}	0.3 ^{a}	0.8 ^{b}	1.1 ^{bc}	0.1
C16:0	24.9 ^{b}	24.9 ^{b}	22.1 ^{ab}	22.4 ^{ab}	20.6 ^{ab}	19.9 ^{a}	19.1 ^{a}	19.1 ^{a}	22.9 ^{ab}	23.2 ^{ab}	1.0
C18:0	9.2 ^{bc}	8.7 ^{b}	6.5 ^{b}	2.3 ^{a}	9.6 ^{c}	10.0 ^{c}	9.0 ^{bc}	8.9 ^{b}	4.7 ^{ab}	0.0 ^{a}	0.6
Total SFA	36.9 ^{c}	36.3 ^{bc}	30.2 ^{b}	25.9 ^{a}	31.9 ^{b}	31.4 ^{b}	28.3 ^{ab}	28.8 ^{ab}	28.7 ^{ab}	24.8 ^{a}	1.4
C14:1	0.0	0.0	0.1	0.1	0.2	0.0	0.0	0.1	0.0	0.0	0.0
C16:1	2.3 ^{c}	2.1 ^{c}	1.9 ^{c}	1.6 ^{bc}	1.0 ^{b}	0.5 ^{a}	1.1 ^{b}	1.0 ^{b}	1.7 ^{bc}	1.6 ^{bc}	0.1
C18:1	16.0 ^{a}	19.2 ^{a}	30.1 ^{b}	36.0 ^{cd}	28.5 ^{b}	27.7 ^{b}	30.2 ^{bc}	30.4 ^{bc}	32.8 ^{c}	42.8 ^{d}	1.6
C20:1	0.5 ^{b}	0.8 ^{c}	0.2 ^{ab}	0.0 ^{a}	0.4 ^{b}	0.2 ^{ba}	0.0 ^{a}	0.2 ^{ab}	0.0 ^{a}	0.0 ^{a}	0.0
Total MUFA	20.1 ^{a}	23.1 ^{a}	33.3 ^{bc}	39.0 ^{c}	31.8 ^{b}	29.2 ^{b}	32.1 ^{b}	32.3 ^{b}	35.7 ^{bc}	44.9 ^{d}	1.7
C18:2n-6	12.0 ^{a}	12.0 ^{a}	16.3 ^{ab}	17.4 ^{ab}	18.4 ^{b}	22.1 ^{b}	21.8 ^{b}	21.4 ^{b}	15.7 ^{ab}	16.3 ^{ab}	0.9
C18:3n-3	0.2 ^{a}	0.3 ^{a}	0.2 ^{a}	0.0 ^{a}	4.1 ^{b}	4.7 ^{b}	6.7 ^{c}	5.9 ^{bc}	0.0 ^{a}	0.8 ^{a}	0.4
Total PUFA	12.2 ^{a}	12.4 ^{a}	16.6 ^{ab}	17.4 ^{ab}	22.7 ^{b}	26.8 ^{b}	28.6 ^{b}	27.2 ^{b}	15.7 ^{ab}	17.1 ^{ab}	1.2
C20:4n-6	2.5 ^{b}	2.3 ^{b}	2.2 ^{ab}	2.3 ^{b}	1.4 ^{a}	3.1 ^{c}	2.4 ^{b}	2.3 ^{b}	1.7 ^{a}	1.7 ^{a}	0.1
C20:5n-3	12.7 ^{c}	12.1 ^{c}	8.5 ^{b}	8.3 ^{b}	5.3 ^{ab}	4.3 ^{a}	3.9 ^{a}	4.3 ^{a}	9.4 ^{b}	6.5 ^{ab}	0.5
C22:5n-3	1.2 ^{b}	1.1 ^{b}	0.3 ^{a}	0.0 ^{a}	0.0 ^{a}	0.0 ^{a}	0.0 ^{a}	0.0 ^{a}	0.0 ^{a}	0.0 ^{a}	0.1
C22:6n-3	14.4 ^{c}	12.9 ^{c}	7.6 ^{ab}	6.4 ^{ab}	5.4 ^{a}	4.6 ^{a}	4.5 ^{a}	4.8 ^{a}	8.8 ^{b}	5.0 ^{a}	0.6
Total LC-PUFA	30.8 ^{c}	28.4 ^{c}	19.9 ^{b}	17.7 ^{b}	13.5 ^{ab}	12.6 ^{a}	11.0 ^{a}	11.7 ^{a}	19.9 ^{b}	13.1 ^{ab}	1.2
Total n-6	14.5 ^{a}	14.3 ^{a}	18.7 ^{ab}	19.7 ^{b}	20.8 ^{b}	25.9 ^{c}	24.5 ^{c}	23.9 ^{bc}	17.4 ^{ab}	18.0 ^{ab}	1.0
Total n-3	28.5 ^{c}	26.5 ^{c}	17.8 ^{b}	15.4 ^{ab}	15.5 ^{ab}	13.6 ^{a}	15.1 ^{ab}	15.0 ^{a}	18.3 ^{b}	12.2 ^{a}	1.1
n-3 : n-6	1.96 ^{c}	1.85 ^{c}	0.95 ^{b}	0.78 ^{ab}	0.75 ^{ab}	0.52 ^{a}	0.62 ^{a}	0.63 ^{a}	1.05 ^{b}	0.68 ^{a}	0.1

Different superscripts within rows indicate significant differences ($P<0.05$). An absence of superscripts implies that there were no significant differences ($P>0.05$).

Table 8. Whole body composition of shrimp from the green-water study in Experiment 3

	F50:O2:M0	F50:O2:M10	F10:O2:M0	F0:O2:M0	F10:O0:M0	F0:O0:M0	F10:O0:M10	F0:O0:M10	F10:O2:M10	F0:O2:M10	Pooled SEM
Dry matter (%)	22.2 [a]	23.9 [ab]	25.1 [ab]	25.0 [ab]	24.2 [ab]	24.7 [ab]	26.6 [b]	25.4 [b]	26.2 [b]	25.7 [b]	0.35
Protein (%)	16.4 [a]	18.8 [ab]	18.1 [ab]	19.1 [ab]	17.2 [ab]	17.9 [ab]	20.6 [b]	19.5 [ab]	19.4 [ab]	19.9 [ab]	0.30
Lipid (%)	1.0 [a]	1.1 [a]	1.5 [b]	1.3 [ab]	1.4 [ab]	1.3 [ab]	1.6 [b]	1.3 [ab]	1.5 [b]	1.4 [ab]	0.06
Ash (%)	3.3 [ab]	3.6 [ab]	3.4 [ab]	3.6 [ab]	3.1 [a]	3.6 [ab]	3.6 [ab]	3.9 [ab]	4.0 [b]	3.7 [ab]	0.09
Gross energy (kJ/g)	3.8 [a]	4.5 [ab]	4.5 [ab]	4.6 [ab]	4.6 [ab]	4.5 [ab]	4.8 [ab]	4.4 [ab]	5.0 [b]	4.7 [ab]	0.07
C14:0	0.4 [ab]	0.5 [b]	0.4 [ab]	0.2 [a]	0.2 [a]	0.2 [a]	0.1 [a]	0.1 [a]	0.3 [ab]	0.3 [ab]	0.02
C16:0	12.0 [ab]	13.0 [b]	12.2 [b]	11.3 [ab]	10.9 [ab]	10.4 [ab]	10.2 [a]	9.8 [a]	11.6 [ab]	11.3 [ab]	0.19
C18:0	6.6 [b]	5.9 [ab]	5.6 [ab]	6.0 [ab]	5.3 [a]	5.4 [a]	5.4 [a]	5.7 [ab]	5.7 [ab]	5.8 [ab]	0.09
Total SFA	22.8 [a]	23.0 [a]	21.3	20.9	19.3 [a]	19.4 [a]	18.8 [a]	19.2 [a]	20.8 [a]	20.3 [a]	0.47
C14:1	0.2	0.3	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.01
C16:1	0.5 [b]	0.4 [ab]	0.2 [a]	0.3 [ab]	0.2 [a]	0.1 [a]	0.2 [a]	0.2 [a]	0.2 [a]	0.2 [a]	0.02
C18:1	9.8 [a]	10.6 [ab]	13.6 [b]	13.0 [ab]	13.5 [b]	13.4 [ab]	13.8 [b]	13.5 [b]	13.7 [b]	13.6 [b]	0.35
C20:1	0.7 [ab]	0.8 [b]	0.5 [ab]	0.5 [ab]	0.4 [a]	0.5 [ab]	0.4 [a]	0.3 [a]	0.5 [ab]	0.4 [a]	0.04
Total MUFA	12.7 [a]	13.6 [ab]	15.6 [ab]	15.2 [ab]	15.7 [ab]	15.9 [b]	16.1 [b]	16.1 [b]	16.0 [b]	15.6 [ab]	0.51
C18:2n-6	6.0 [a]	6.0 [a]	8.3 [ab]	8.5 [ab]	10.0 [ab]	10.8 [b]	10.1 [ab]	10.7 [b]	8.0 [ab]	8.6 [ab]	0.32
C18:3n-3	0.2 [a]	0.3 [a]	0.4 [a]	0.5 [a]	2.5 [b]	2.5 [b]	3.0 [b]	2.7 [b]	0.4 [a]	0.4 [a]	0.22
Total PUFA	6.2 [a]	6.3 [a]	8.7 [ab]	9.0 [ab]	12.5 [b]	13.3 [b]	13.1 [b]	13.5 [b]	8.4 [ab]	9.1 [ab]	0.54
C20:4n-6	10.1 [ab]	8.8 [a]	8.7 [a]	9.5 [ab]	11.1 [ab]	13.1 [b]	11.3 [ab]	13.1 [b]	8.1 [a]	9.1 [a]	0.33
C20:5n-3	18.0 [b]	15.8 [ab]	18.2 [b]	20.3 [b]	14.2 [a]	15.7 [ab]	13.0 [a]	15.1 [a]	18.2 [b]	19.7 [b]	0.45
C22:5n-3	1.6	1.8	2.1	2.1	1.6	1.9	1.7	2.0	2.1	2.2	0.05
C22:6n-3	24.7 [bc]	27.0 [c]	22.2 [b]	19.5 [ab]	21.2 [b]	15.9 [a]	21.4 [b]	16.2 [a]	23.1 [b]	20.8 [ab]	0.62
Total LC-PUFA	56.4	55.7	53.0	53.0	51.0	49.6	50.4	49.3	53.2	53.2	1.59
Total n-6	17.9 [ab]	16.8 [a]	18.5 [ab]	19.5 [ab]	23.2 [ab]	26.2 [b]	23.4 [b]	25.9 [b]	17.6 [a]	19.2 [ab]	0.72
Total n-3	44.8 [a]	45.2 [a]	43.1 [a]	42.5 [a]	40.3 [a]	36.7 [a]	40.1 [a]	36.9 [a]	44.0 [a]	43.2 [a]	1.41
n-3 : n-6	2.5 [b]	2.7 [b]	2.3 [ab]	2.2 [ab]	1.7 [ab]	1.4 [a]	1.7 [ab]	1.4 [a]	2.5 [b]	2.3 [ab]	0.24

. Different superscripts within rows indicate significant differences (P<0.05). An absence of superscripts implies that there were no significant differences (P>0.05).

Figure 1. Effect of fishmeal reduction in formulation on growth rate of shrimp in experiment 1. Shown are the linear regression through the means ± SEM of the data.