Aquaculture 431 (2014) 12–19

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

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

Brett Glencross [a,b,*], Simon Irvin [a,c], Stuart Arnold [a,c], David Blyth [a,c], Nicholas Bourne [a,b], Nigel Preston [a,b]

[a] CSIRO Food Futures Flagship, 41 Boggo Rd, Dutton Park, QLD 4102, Australia
[b] CSIRO Marine and Atmospheric Research, GPO Box 2583, Brisbane, QLD 4001, Australia
[c] CSIRO Marine and Atmospheric Research, PO Box 2066, Woorim, QLD 4507, Australia

ARTICLE INFO

Article history:
Received 15 November 2013
Received in revised form 25 February 2014
Accepted 26 February 2014
Available online 11 March 2014

Keywords:
Prawns
Fishmeal
Alternative ingredients

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 also additional growth. Replication of the clear-water trial in a green-water tank system not only 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 also an improved performance strategy.

1. Introduction

There has been a considerable amount of research in the past few decades to improve the capacity to utilise 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 (Alvarez et al., 2007; Cruz-Suarez et al., 2001, 2007; Davis and Arnold, 2000; Davis et al., 2002; Smith et al., 2007a). 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., 2007a). 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; Glencross and Smith, 1999; Lim et al., 1997).

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%

* Corresponding author. Tel.: +61 7 3826 7236; fax: +61 7 3826 7281.
E-mail address: Brett.Glencross@csiro.au (B. Glencross).

http://dx.doi.org/10.1016/j.aquaculture.2014.02.033
0044-8486/

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 (*P. 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 to 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., 1999). 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 3 mm die and cut to pellet lengths of about 6 mm. The pellets were then steamed at 100 °C for 5 min before being oven dried at 60 °C for 24 h. Diets were kept at −20 °C when not being fed.

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.

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 (*P. 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 the 50 × 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 ± 0.28 °C 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 days 0, 14, 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