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Effects of binders on stability and palatability of formulated dry compounded diets for spiny lobster *Panulirus homarus* (Linnaeus, 1758)

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ABSTRACT

Experimental trials were conducted to formulate a palatable dry compounded diet for subadults of the spiny lobster *Panulirus homarus* in the size range 103-114 mm, using ingredients derived from natural sources in a desirable combination of 54.5% crude protein. Since the diet exhibited low stability, series of experiments were conducted further using different combinations of binders sourced from plant as well as synthetic origin to derive a stable and palatable pellet diet. Among 35 test diets formulated, diet with good palatability and maximum pellet stability (85.55 ± 5.94% for 8 h) were identified as the pellets made with binders in combination of sodium alginate (3%), 'stick on' a commercial phytochemical (1%) and agar agar (3%). This combination of binders was found suitable for artificial pelleted diet prepared for subadults of *P. homarus*.

Keywords: Binders, Palatability, *Panulirus homarus*, Pellet feed, Spiny lobster, Stability

Introduction

Spiny lobsters belonging to the order Decapoda of family Palinuridae are one of the world's most valuable sea foods with high market appeal. One of the major constraints encountered in the large scale commercial aquaculture of spiny lobsters is the lack of a cost effective dry pellet diet (Jeffs and Hooker, 2000; Jeffs and Davis, 2003; Tuan and Mao, 2004; Perera *et al.,* 2005; Barclay *et al.,* 2006; Williams, 2007). Presently there is no standard formulated feed available exclusively for growing spiny lobsters in India. A grow out pelleted feed is very much necessary for sustainability and advancement of lobster culture. Formulated, commercial feeds are preferable for use in aquaculture operations due to their availability, convenience for storage and low microbial contamination compared to live or frozen foods (Conklin *et al.,* 1977, 1983).

According to Conklin *et al.* (1983), there exist a need for focused investigations for the development of formulated diets based on the specific dietary requirements of spiny lobsters. Research on artificial feed for spiny lobsters in India is limited to the works by Ayyappan *et al.* (2002), Lamek Jayakumar *et al.* (2004, 2011) and Margret *et al.* (2009). However, the major problem encountered by them in feed formulation of lobsters is the low water stability of feed pellets. The present study attempted to formulate an artificial pellet diet suitable for sub-adults of *Panulirus homarus* with good water stability.

Materials and methods

Ingredients selection

Initially, a palatable basal pellet diet was prepared for subadults of *Panulirus homarus* in the size range 101-113 mm total length weighing below 100 g, using cheap and readily available ingredients without adding extra binders (Table 1). The ingredients were selected based on earlier reports of lobster's preferences, nutrient status, texture, flavour, cost effectiveness, attractiveness, keeping quality and steady availability. Other than the natural ingredients, di-sodium hydrogen orthophosphate, 'supradin' a commercial multi-vitamin and mineral tablet, sodium chloride and cod liver oil were also incorporated in the diet.

Feed formulation

The ingredients such as fresh and cleaned anchovy, groundnut oil cake, soya bean seeds, boiled mussel

Table 1. Composition of basal diet

Ingredients	Quantity (g)	Protein (%)	Carbohydrate (%)
Casein	22.00	17.30	2.89
Fish meal	24.00	14.00	2.88
Ground nut oil cake	22.00	10.00	3.34
Soya meal	22.00	9.60	7.48
Mussel meal	4.00	3.10	0.54
Sea weed	2.00	0.50	0.47
Vitamin/minerals	1.00	-	-
Sodium chloride	0.50	-	-
Di-sodium hydrogen ortho-phosphate	0.50	-	-
Cod liver oil	2.00	-	-
Total	100.00	54.50	17.6

meat and fresh seaweed (*Sargassum wightii*) were sun-dried under hygienic conditions for 5 h and, subsequently dried in hot air oven at 55˚C. The ingredients were then individually made in to fine powder using electrical mixer, quantified, homogenised well and made into a dough using distilled water. The dough was then steam cooked for 10 min, and subsequently cooled. Casein, code liver oil, sodium chloride di-sodium hydrogen orthophosphate and supradin powder were also added to the dough and pellets of approximately 4 mm diameter were prepared manually. The pellets were then sun dried for 5 h and dried in hot air oven at 55°C till the moisture content of the pellets reduced to 7%. The basal diet was then stored in air-tight containers. The proximate composition of basal feed was estimated as protein 54.5% and carbohydrate 17.6 %.

Lobster collection, packing and transportation

Sub adults of spiny lobster *Panulirus homarus,* each weighing below 100 g in the size range of 100-115 mm (TL) were collected from fishermen operating lobster traps at Kadiapattanam (8º8'4"N; 77º18'31E) and Chinnamuttom (8º5'45"N; 77º33'47"E), Kanyakumari District of Tamil Nadu along the south-west coast of India. The lobsters were selected based on their healthy appearance with all appendages, intact exoskeleton and good pigmentation. Lobsters were then transported to the laboratory of Centre for Marine Science and Technology, Rajakkamangalam under moist conditions.

Experimental trials on P. homarus for feed acceptance

The lobsters were carefully released to 500 l FRP tanks filled with filtered seawater with aeration. After acclimatisation period of 12 h, the basal feed was provided to the lobsters. The basal diet was immediately consumed and thus found palatable as recorded from their consumption behaviour. However, water stability of the pellet was found to be very poor. In order to improve the water stability of the basal diet, eight binders *viz.,* tapioca powder, agar agar, 'stick on' a commercial phytochemical, gum arabic, guar gum, wheat flour, gelatin and sodium alginate were used and 35 combinations of experimental pelleted diets were prepared by adding the above binders at various inclusion levels ranging from a minimum of 4% to a maximum of 12%.

Water stability of all the 35 diets were tested in triplicate for varying exposure periods in seawater by dip, string and pouch methods (Ahamad Ali *et al.,* 2006). Stability of the diets were tested based on endurance in seawater for specified time periods and the palatability was determined based on the diet acceptance in terms of consumption by lobsters. The percentage of dry matter leaching was recorded as an index of water stability. Percentage stability of different feeds were tested based on stability grades as: mild (representing pellet stability ranging from 50 to 59%), moderate (60 to 79%) and good (80 to 89%) based on results recorded upon seawater immersion recorded at specific time intervals at 2 h, 4 h, and 6 h to a maximum of 8 h.

The percentage of nutrient leaching was estimated as:

$$\%\ \text{Nutrient leaching} = \frac{\text{Initial pellet weight - Final pellet weight}}{\text{Initial pellet weight}} \times 100$$

Palatability

After an acclimatisation period of 12 h of the experimental lobsters, feeding experiments were conducted (in duplicate) for ten days to determine the palatability of the test diets. The experiments were conducted using sub adults of *P. homarus* each weighing below 100 g in the size range of 100-115 mm TL. The lobsters were stocked (2 nos. per tank) in serially interconnected rectangular plastic tanks, each holding 75 l filtered seawater and fed with experimental diets. In each tank, two hollow PVC pipe pieces (18 cm length and 9 cm dia) were placed as hide-outs for the lobsters. Aeration was provided continuously in all the tanks throughout the experimental period. The experimental set up was maintained almost in dark condition as the colour of the tanks were deep blue and the tanks were covered with a similar coloured nylon curtain. Since lobsters are mainly nocturnal feeders, feeding was done as a single dose daily at 18.00 hrs. Every day morning, left over feed and faecal strands were siphoned out from each tank followed by 85% water replacement. The lobsters were carefully monitored to record feed acceptance.

Statistical analysis

Results were expressed as mean ± SD and comparison among treatments for various parameters were subjected to one way ANOVA. Duncan's multiple range test (Duncan, 1955) were applied to identify significant differences between individual treatments. Statistical analysis conducted using SPSS 16 for Windows and Microsoft Excel.

Results and discussion

Since lobsters are bottom dwelling animals, high density stable feed is required for their immediate availability when introduced in the culture system. In aquaculture, feed leaching rate is inversely proportional to water stability of pellets. Since crustaceans are slow intermittent feeders, feeds designed for them should be highly stable (Chen and Jenn, 1992; Marchetti *et al*., 1999). The water stability of the pellets mainly depends on the binders used in the feed (Meyers and Zein-Eldin, 1972; Goswamy and Goswamy, 1979; Forman and Lauterio, 1982; Ahamed Ali, 1988).

In the present study, out of the 35 test diets analysed, only 7 diets recorded palatability with stability (Tables 2-5). The stability of the test diets reduced progressively with increasing time period. Stability of the pellets incorporated with different combinations of binders at various inclusion levels showed that binders added in three combinations at 7% level of inclusion resulted in highest stability at a maximum seawater exposure period of 8 h. Among the diets incorporated with two combinations of binders, combination of agar agar (3%) and 'stick on' (1%) recorded better stability. Binders added in combinations of sodium alginate (3%), agar agar (3%) and 'stick on' (1%) recorded highest stability 85.55±5.94%, followed by agar agar (3%), sodium alginate (1%) and 'stick on' (1%) contributed 84.33 ± 3.74 % stability up to 8 h of seawater exposure (Table 3). The diets with agar agar (1%), and sodium alginate (3%) exhibited lowest stability of 55.80±2.21% among the palatable diets (Table 2). Diet with agar at a higher inclusion level of 4%, with 1% gelatin was rejected by the lobsters on the account of non-palatability (Table 2). Though the combination of binders such as agar agar (3%), 'stick on' (1%) and wheat flour (3%) exerted very good stability of 82.21±6.74%, the feed was found unpalatable for lobsters. Similarly binders added in combination of agar agar (5%), gelatin (2.5%), 'stick on' (1%), wheat flour (1%) and guar gum

Table 2. Pellet stability, feed acceptance and feed stability grade of diets prepared using any two combinations of binders

Binder combinations*	Quantity in feed (%)	Feed acceptance by *P. homarus*	Mean pellet stability (%)				F value (comparing binders @ 8 h)	Feed stability grade
			2 h	4 h	6 h	8 h		
A3 S1	4	Accepted	80.35± 2.98	77.55± 4.05	75.69± 2.69	73.68^{c}± 3.74	293.803**	Moderate
A1 SA3	4	Accepted	70.56± 6.92	64.55± 4.99	60.64± 3.98	55.80^{a}± 2.21		Mild
A4 G1	5	Not accepted	88.24± 3.45	84.65± 2.56	73.25± 3.47	63.00^{b}± 4.46		Moderate

**$p < 0.001$

a, b, c - Means with same superscript do not differ from each other (Duncan's multiple range test)

Binders: A - Agar agar; G - Gelatin; S - 'Stick on'; SA - Sodium alginate.

*The numbers denote the percentage of binders in the feed

Table 3. Pellet stability, feed acceptance and feed stability grade of diets prepared using three combinations of binders

Binder combinations	Quantity in feed (%)	Feed acceptance by *P. homarus*	Mean pellet stability (%)				F value (comparing binders @ 8 h)	Feed stability grade
			2 h	4 h	6 h	8 h		
A3 T1 S1	5	Accepted	93.54± 4.56	89.45± 3.85	80.65± 3.09	78.58^{c}± 2.84	652.654**	Moderate
A3 T2 S1	6	Accepted	85.54± 1.88	80.21± 4.95	75.04± 6.69	63.36^{a}± 5.94		Moderate
A3 T3 S1	7	Accepted	76.61± 5.78	74.33± 6.05	65.25± 3.69	60.55^{a}± 2.84		Moderate
A3 SA1 S1	5	Accepted	94.34± 2.98	90.65± 1.05	87.87± 2.69	84.33^{d}± 3.74		Good
A1 SA3 S1	5	Accepted	88.54± 7.08	83.78± 3.05	70.69± 4.89	65.65^{ab}± 5.14		Moderate
A3 SA3 S1	7	Accepted	97.36± 5.88	92.21± 4.95	88.01± 6.69	85.55^{d}± 5.94		Good
A3 G1 S1	5	Not accepted	90.65± 4.56	87.98± 3.85	70.21± 3.09	60.01^{a}± 2.84		Moderate
A3 G2 S1	6	Not accepted	91.11± 2.98	83.12± 1.05	79.21± 2.69	65.01^{ab}± 3.74		Moderate
A3 G3 S1	7	Not accepted	88.51± 5.88	81.24± 4.95	79.35± 6.69	68.32^{b}± 5.94		Moderate
A4 G1 S1	6	Not accepted	89.32± 5.45	70.32± 2.56	65.14± 3.47	61.21^{a}± 4.46		Moderate
A3 S1 W3	7	Not Accepted	96.54± 6.08	94.31± 3.05	88.65± 4.89	81.01^{d}± 5.14		Good
A3 S1 W2	6	Not accepted	97.54± 4.98	95.35± 2.05	90.32± 2.49	82.21^{d}± 6.74		Good
A3 S1 W1	5	Not accepted	92.51± 5.98	90.55± 3.05	85.25± 3.69	78.87^{c}± 5.75		Moderate

** $p < 0.001$

a, b, c, d - Means with same superscript do not differ each other (Duncan's multiple range test)

Binders: A - Agar agar; G - Gelatin; S -'Stick on'; SA - Sodium alginate; T – Tapioca flour; W – Wheat flour.

*The numbers denote the percentage of binders in the feed

Table 4. Pellet stability, feed acceptance and feed stability grade of diets prepared using any four combinations of binders

Binder combinations	Quantity in feed (%)	Feed acceptance by *P. homarus*	Mean pellet stability (%)				F value (comparing binders @ 8 h)	Feed stability grade
			2 h	4 h	6 h	8 h		
A4 W3 SA1 S1	9	Not accepted	91.45± 5.45	84.33± 2.56	75.58± 3.47	63.47^{b}± 4.46	4587.365**	Moderate
A1 T1 G1 S3	6	Not accepted	93.31± 5.88	80.33± 4.95	78.54± 6.69	77.44^{d}± 5.94		Moderate
A3 GA2 S1 W5	11	Not accepted	85.16± 3.45	77.77± 2.56	67.67± 3.47	63.11^{b}± 4.46		Moderate
A5 GA1 S1 W3	10	Not accepted	88.99± 4.56	84.23± 3.85	74.66± 3.09	63.15^{b}± 2.84		Moderate
A3 GG1 S1 W3	8	Not accepted	93.58± 3.85	84.98± 5.95	80.64± 4.01	79.47^{d}± 3.94		Moderate
A3 G3 S1 W3	10	Not accepted	80.68± 6.48	76.57± 4.15	74.47± 6.69	72.47^{c}± 5.04		Moderate
A3 G1 W1 SA1	6	Not accepted	85.65± 2.98	80.65± 4.05	78.58± 2.69	70.65^{c}± 3.74		Moderate
A5 G2.5 GG2.5 W1	11	Not accepted	89.88± 2.98	85.86± 4.05	72.65± 2.69	55.35^{a}± 3.74		Mild
A3 GG2 W3 S1	9	Not accepted	96.47± 4.56	84.65± 3.85	82.28± 3.09	68.37^{bc}± 2.84		Moderate
A2 T1 GG2 S2	7	Not accepted	87.15± 5.15	84.56± 3.56	74.87± 4.44	74.87^{cd}± 4.46		Moderate

** $p < 0.001$
a, b, c, d - Means with same superscript do not differ each other (Duncan's multiple range test)
*The numbers denote the percentage of binders in the feed

Table 5. Pellet stability, feed acceptance and feed stability grade of diet prepared using any five combinations of binders

Binder combinations	Quantity in feed (%)	Feed acceptance by *P. homarus*	Mean Pellet Stability (%)				F value (Comparing binders @ 8h)	Feed stability grade
			2 h	4 h	6 h	8 h		
A3 G1 S1 W1 SA1	7	Not accepted	88.54± 4.88	79.36± 4.44	72.54± 3.69	70.26^{c}± 7.94	565.584**	Moderate
A3 G1 S1 W3 SA3	11	Not accepted	83.12± 5.08	68.54± 4.95	67.63± 6.09	62.78^{b}± 5.94		Moderate
A1 G1 S1 W1 SA3	7	Not accepted	90.52± 3.45	81.25± 5.56	74.54± 3.47	72.47^{c}± 4.46		Moderate
A3 G1 S1 W1 SA3	9	Not accepted	90.36± 4.56	85.24± 3.85	75.64± 3.09	73.55^{c}± 2.84		Moderate
A5 GA1 S1 W3 GG1	11	Not accepted	88.87± 5.88	76.47± 4.95	70.68± 6.69	58.54^{a}± 5.94		Mild
A5 G2.5 S1 W1 GG2.5	12	Not accepted	87.87± 5.45	85.85± 2.56	83.64± 3.47	80.65^{d}± 4.46		Good
A3 G1 S1 W2 SA1	8	Not accepted	88.87± 3.65	75.65± 5.56	70.76 ± 3.33	70.65^{c}± 5.55		Moderate
A2 GG2 G1 SA1 S2	8	Not accepted	87.36± 4.56	81.88± 3.85	80.56± 3.09	72.32^{c}± 2.66		Moderate
A4 G1 S1 W3 SA1 GG2	12	Not accepted	94.85± 5.11	82.66± 4.77	77.67± 6.22	74.91^{c}± 5.94		Moderate

** $p < 0.001$
a, b, c, d - Means with same superscript do not differ each other (Duncan's multiple range test)
*The numbers denote the percentage of binders in the feed.

(2.5%), even though exhibited reasonably good pellet stability (80.65 ±4.46%), lobsters were found not attracted to the feed, indicating unsuitability of gelatin, wheat flour and guar gum in the feed for *P. homarus*. The results of the experimental studies further revealed that leaching rates of feed containing tapioca flour added @ 3% were significantly higher than those of the remaining feeds in which tapioca flour was either not included or added @ 1% level. The present study clearly showed that inclusion levels and combinations of the binders are important factors contributing to the stability and palatability of the diets.

Meyers and Zen-Eldin (1972) reported that rough and water soluble ingredients added in the feed exert tendency for faster disintegration. Ahamed Ali (1998) and Mayers *et al.* (1972 a, b) discussed about the range of substances available as binders. Ahamad Ali (1982, 1988, 2010) verified the use of tapioca flour and other binders *viz.*, agar agar, gelatin, poly vinyl alcohol, guar gum, polymethylocarbamide, wheat gluten and sodium alginate in shrimp feed. Lamek-Jayakumar *et al. (*2011) reported rapid deterioration of pellet stability at fourth hour onwards even at a higher inclusion level of binders as much as 33.93% in the feed of spiny lobster *P. homarus*. Lobsters preferred non-soaked diet than soaked diet and were also capable of detecting their diet regardless of prolonged exposure in water and selectively consume 'fresh' rather than a "stale' diet (Tolomei *et al.,* 2003). Ryther *et al.* (1988) and Kanazawa (1994) reported that feed pellets for lobsters should be hard and should remain stable in water for several hours till they are consumed. Ahamad Ali *et al.* (2010) reported guar gum as a binder in feed formulation of shrimps. However in the present study, inclusion of guar gum in the pelleted feed was found unsuitable on account of non-palatability by lobsters. The results of present study recommends binder combination of 3% sodium alginate, 3% agar agar and 1% 'stick on' in formulation of artificial diets for subadults of *P. homarus*.

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