



## Effect of binder type and concentration on prepared feed stability, feed ingestion and digestibility of *Litopenaeus vannamei* broodstock diets

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### Abstract

The effect of six different binding agents (agar, sodium alginate, cassava starch, gelatin, wheat gluten and kelp meal) in two concentrations (30 g kg<sup>-1</sup> and 50 g kg<sup>-1</sup>) were evaluated with respect to physical quality of *Litopenaeus vannamei* broodstock pelleted feed, after 15, 30, 60, 90, 120, 150 and 180 min of water immersion. The best treatments in terms of water stability, water absorption and protein leaching were obtained with sodium alginate and wheat gluten at 50 g kg<sup>-1</sup>. In a second experiment, the feed ingestion and diet digestibility with these two binders and their combination (1 : 1) were compared against a control diet containing 50 g kg<sup>-1</sup> wheat flour. There were no significant differences ( $P > 0.05$ ) in the daily feed ingestion rate 2.39–3.33% of the biomass. The most representative values of apparent digestibility of protein (ADP) and apparent dry matter digestibility (ADMD) were achieved with diets containing wheat gluten and alginate + gluten mixture as binder. Based on these results, combinations with 50 g kg<sup>-1</sup> wheat gluten is recommended as binder in pelleted feed for broodstock *L. vannamei*.

**KEY WORDS:** binders, digestibility feed, ingestion rate, leaching, *Litopenaeus vannamei*, water absorption

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### Introduction

The nutrition of crustaceans is one of the most studied areas within aquaculture, whose efforts have been focused on the evaluation and preparation of diets with nutritional

levels required by the organism, low cost and characteristics such as: palatability, texture, water stability and optimum digestibility that maximize growth and reproductive functions of commercial species such as 'white shrimp' *Litopenaeus vannamei*. To achieve retention of the physical integrity of feed, with minimal disintegration and nutrients leaching in water is not easy, specially for benthic species like shrimp that display slow consumption habits (Cruz-Suárez *et al.* 2006) and require to nibble the feed before ingestion, not only because the complicated manufacturing processes, storage and transport, but because the feed requires to be compacted by a sufficient time, as to be consumed by the species in culture. According to Cruz-Suárez *et al.* (1999), true nutritional value of feed depends not only on the amount of nutrients, but also on its availability and quality. It has been reported that immersed feed for more than an hour before consumption leach its water-soluble nutrients such as free vitamins and minerals, and amino acids leach and pellets break into smaller particles reducing its nutritional value (Chamberlain 1994). This leaching may cause the eutrophication of water, leading to a poor animal growth, inefficient feed conversion and low survival (Meyers *et al.* 1972; Obaldo *et al.* 2002). In view of these results, factors related to water stability of feed such as particles size of the ingredients (Obaldo *et al.* 2002), raw materials, binding agents and pelleting process are considered during feed manufacture. Additionally, feed stability also depends on the conditions of the culture. High temperatures and low salinity significantly reduce dry matter retention in shrimp feed (Obaldo *et al.* 2002).

One of the factors studied are binders, because they have a strong effect on the physical integrity of pelleted feed (Rosas *et al.* 2008) and the biological availability of nutrients (Forster 1972; Storebakken 1985; Storebakken & Austrong 1987; Koshio *et al.* 1989; Dominy & Lim 1991;



Partridge & Southgate 1999; Cruz-Suárez *et al.* 2001; Pearce *et al.* 2002; Cruz-Suárez *et al.* 2006). The aim of this study was to determine types and concentrations of suitable binding agents, to achieve a good physical stability of pelleted feed in terms of immersion time, a reduced loss of water-soluble nutrients by leaching, accompanied by an improved texturing and acceptability that promote the consumption of feed by *L. vannamei* broodstock.

## Materials and methods

This study was carried out at the National Aquaculture and Marine Research Centre (CENAIM), San Pedro de Manglaralto (Santa Elena Province, Ecuador). Two experiments using a basal diet (CENAIM 54-1) were developed. In first experiment, agar (AG), sodium alginate (SA), cassava starch (CS), gelatin (GL), wheat gluten (WG) and kelp meal (KM) were evaluated as binders in concentrations of 30 and 50 g kg<sup>-1</sup>. In a second experiment, we evaluated individually at 50 g kg<sup>-1</sup> SA and WG and in a combination 1 : 1. A diet containing wheat flour (WF) at 50 g kg<sup>-1</sup> was used as control to determine feed ingestion rate and apparent digestibility coefficients (ADC).

The major components were ground to 212 µ and mixed manually from lowest to highest concentration to achieve a good homogenization. Once all dry ingredients were mixed, vitamin and mineral premixes, soy lecithin and fish oil were added. Finally, water was added gradually (400–500 mL kg<sup>-1</sup>) until the resulting dough could be easily extruded through a 3-mm die of a Lieme (Model 2062) meat grinder. This process was repeated four times to increase ingredients agglomeration. Pellet temperature exiting the die was 60–65 °C. Pellets were tempered at 90 °C for 5 min in an oven ISUZU (Model 2–2132) to simulate a postpellet condition and then dried in a fan-ventilated oven ISUZU (Type MNS 1155) to 60 °C until the moisture content was below 10% in diets. The ingredients used in feed formulation are shown in Table 1.

### Structure and stability of pellet after water immersion

Direct observations of immersed pellets in sea water were made after 60, 120 and 180 min of immersion. After these time periods, the pellets were ranked into five distinct groups displaying the best visual water stability to the least. The subjective criterion for ranking and segregating the pellets in the visual water stability trial was the most intact pellet structure or form to the least (Dominy & Lim 1991).

**Table 1** Feed ingredients used in diet CENAIM 54-1

Ingredients	g kg <sup>-1</sup>	
	Binder 3	Binder 5
Fish meal	300	300
Squid meal	200	200
Soybean meal concentrated	197	197
Fish oil	28	28
Liquid lecithin	10	10
Cholesterol	5	5
Vitamin mixture MAD <sup>1</sup>	20	20
Mineral mixture MAD <sup>2</sup>	20	20
Anti-oxidant	0.02	0.02
Anti-fungal	1	1
Attractant MAD <sup>3</sup>	15	15
Astaxanthin	2.5	2.5
ω-3 highly unsaturated fatty acids (HUFA)	20	20
Docosahexaenoic Acid (DHA)	10	10
Chromic oxide	10	10
Binder	30	50
Corn starch	131	111

<sup>1</sup> (mg kg<sup>-1</sup> of diet): Stay C, 2857; biotin, 5; calcium pantothenate, 500; calciferol, 12.7; choline, 3500; cyanocobalamin, 0.3; folic acid, 15; inositol, 4000; menadione, 40; niacin, 750; *p*-amino benzoic acid, 100; pyridoxine HCl, 120; riboflavin, 200; thiamin, 120; vitamin A-acetate, 41.93; α-tocopherol acetate, 1831.5; α-cellulose, 5906.6.

<sup>2</sup> (mg kg<sup>-1</sup> of diet): Cobalt chloride hexahydrate, 0.456; copper sulphate, 7.7; iron citrate, 681.074; KH<sub>2</sub>PO<sub>4</sub>, 7998; KIO<sub>3</sub>, 0.747; manganese sulphate, 44.768; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 5953.1; sodium selenate, 0.249; zinc sulphate heptahydrate, 577.03; α-cellulose, 4736.88.

<sup>3</sup> (mg kg<sup>-1</sup> of diet): Taurine, 7500; Glycine, 7500.

Pellet stability in water was determined according to the method used by Wouters *et al.* (2001) using three replicates per treatment. For this experiment, 2 g of pellets (length approximately 1.5 and 3 mm of diameter) were weighed and placed into 250-mL glass bottles (bottom area: 28.27 cm<sup>2</sup>), containing 100 mL of sea water (35 g L<sup>-1</sup>) maintained at 28 °C and shaken at 70 revolutions per minute (rpm) in a horizontal shaker EYELA-NTS 1300, by 15, 30, 60, 90, 120, 150 and 180 min. After immersion, pellet was collected within of 600-µ mesh baskets, previously tared. Sea water resulting was used to protein leaching test. The meshes and pellet collected were dried at 60 °C for 24 h and weighed. Feed stability was calculated in terms of Dry Matter Retention (DMR) using the following formula:

$$\text{DMR (\%)} = 100 - \frac{\text{DW}_{\text{bi}} - \text{DW}_{\text{ad}}}{\text{DW}_{\text{bi}}} \times 100$$

Where DW<sub>bi</sub> = Dry weight of diet before water immersion

DW<sub>ad</sub> = Dry weight of diet after drying



### Water feed absorption

The water absorption percentage was calculated by gravimetric difference between registered weight of feed at each immersion time evaluated and the initial weight of pellet, expressing the result according to the following formula:

$$\text{Water absorption (\%)} = \frac{DW_{ai} - BW - IW}{IW} \times 100$$

Where  $DW_{ai}$  = Diet weight after X minutes of immersion

BW = Basket weight

IW = Initial weight of diet

### Protein leaching

During pellet stability test, sea water collected from each immersion time was filtered through a paper filter Whatman No. 1. Aliquots of 10 mL were used to quantify protein content by the method of Bradford (1976) using kit Dye Reagent (BIO-RAD®, CA, USA). Bovine serum albumin was used as standard.

### Collection and maintenance of shrimp

Broodstock *L. vannamei* (16 males and 16 females with an average weight of  $38.4 \pm 2.9$  g and  $43.0 \pm 4.1$  g, respectively) were selected and placed in four 2-ton tanks each divided into four compartments. The allocation of animals in experiment units was random to 1 : 1 (male–female) ratio. Water temperature was maintained at 28 °C. Each tank received constant aeration, continuous water exchange (200%) and natural photoperiod. Shrimp were fed at 5% of the biomass in each tank. Exuviae and faeces were removed daily.

### Feed ingestion rate

This experiment lasted 15 days, the weights of the animals were recorded before-and-after the experiment. Shrimps were fed at 03:00 h using a lantern with blue light and at 12:00 h. Two hours after each feeding (05:00 and 14:00 h) uneaten feed was collected into 500-µm mesh baskets by syphoning. Pellet collected without faecal matter was dried at 60 °C for 24 h, and after this time, the meshes containing the dry pellet were weighed. Feed consumption was made on a dry basis using the following formula:

$$\text{Ingestion rate} = \frac{(W_{\text{given}} \times DMR_{120}) - W_{\text{remaining}}}{W_{\text{animal}}} \times 100$$

Where  $DMR_{120}$  = % dry matter retention at 120 min of immersion.

### Determination of digestibility

Shrimp were fed on their assigned diets that had been supplemented with 10 g kg<sup>-1</sup> chromic oxide by 10 days once ingestion test ended. After that period, faeces were collected 4 h after (07:00 and 16:00 h) each feeding by syphoning. The faecal material was collected in meshes, gently washed with distilled water and poured in 1.5-mL Eppendorf. Faeces were centrifuged, lyophilized and carefully homogenized and stored at -20 °C until analysis. Diets and faeces were analysed for protein content and digestibility by chromic oxide method according to Forster & Gabbott (1971) and McGinnis & Kasting (1964), respectively. Apparent digestibility coefficients and for test diets were calculated by the indicator method:

$$APD (\%) = 100 - \left[ 100 \times \frac{\% Cr_2 O_3 \text{ diets}}{\% Cr_2 O_3 \text{ feces}} \times \frac{\% \text{ nutrient feces}}{\% \text{ nutrient diets}} \right]$$

Where indicator is chromic oxide, and nutrient is dry matter or protein.

### Statistical analysis

All data were subjected to the Bartlett test to verify homogeneity of variances. If non-homogeneity of variances were detected, data were transformed with arcsine Y (water stability, APD, ADMD), logarithm Y (water absorption) and Y<sup>x</sup> (protein leaching). The results for each type of binding agent were analysed using one-way analysis of variance (ANOVA). When significant differences between treatments were found, a multiple range test (Least Significant Difference) at a level of confidence of 95% was run to establish which treatments were different.

### Results

The shape of pellets based on visual examination showed that diets SA3, SA5 and WG5 remained compact after immersion in sea water after 180 min (best), while AG3 and AG5 diets presented more disintegration and increasing

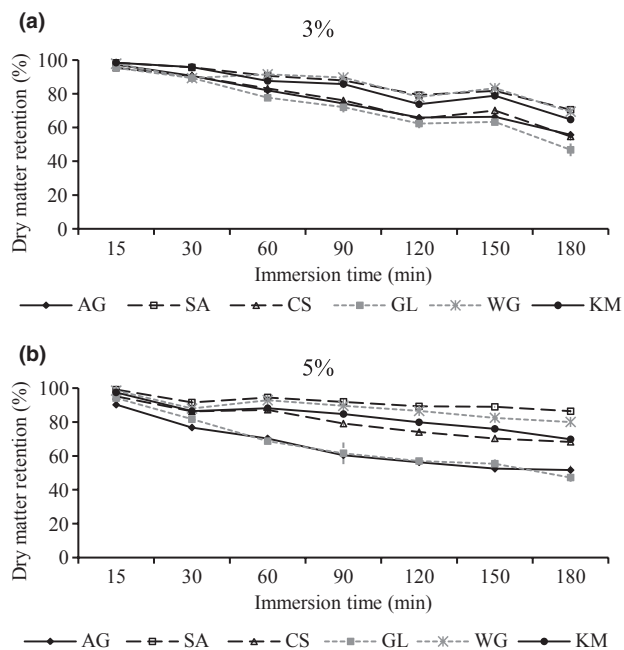


water turbidity (worst). Other diets were classified as good (GL3, WG3, GL5, KM5), moderate (KM3, CS5) and bad (CS3).

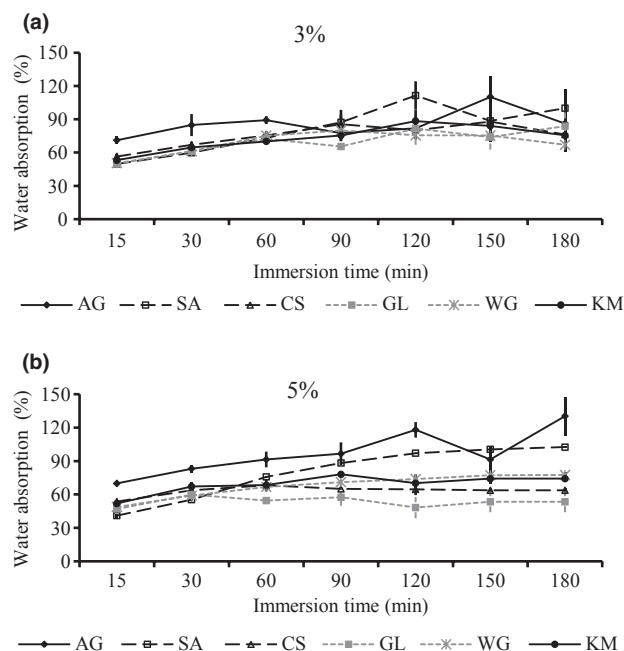
### Diet quality

The increase in the inclusion level from 30 to 50 g kg<sup>-1</sup> of AG, GL and KM did not improve water stability of diets at all evaluated times. AG and GL presented worst dry matter retention in the present study (Fig. 1a,b). In the same way, diet with CS 50 g kg<sup>-1</sup> produced a higher stability after 60 min against diet containing 30 g kg<sup>-1</sup>. There were not significant differences ( $P > 0.05$ ) in level of inclusion of binders SA and WG for the first 15 min. At 30 min, there was a greater loss of dry matter with 50 g kg<sup>-1</sup> of inclusion compared with 30 g kg<sup>-1</sup>, respectively. After 60 min of immersion, these binders (SA5 and WG5) achieved significantly higher ( $P < 0.05$ ) dry matter retention compared with diets at 30 g kg<sup>-1</sup>.

Water absorption of binders ranged from 41 to 130% (Fig. 2a,b) and was time-dependent. In general, agar at either 30 or 50 g kg<sup>-1</sup> absorbs significantly ( $P < 0.05$ ) more water than the other binders tested in the first 60 min. Diets with 30 g kg<sup>-1</sup> of any of binders tested did not show a definite pattern after 90 min. However, 50 g kg<sup>-1</sup> sodium alginate induced greater water absorption after 90 min as compared to diet 50 g kg<sup>-1</sup> agar ( $P < 0.05$ ).



**Figure 1** (a, b) Effect of binder type and concentration on prepared feed stability, based on time of water immersion.



**Figure 2** (a, b) Effect of binder type and concentration on prepared feed water absorption based on time of water immersion.

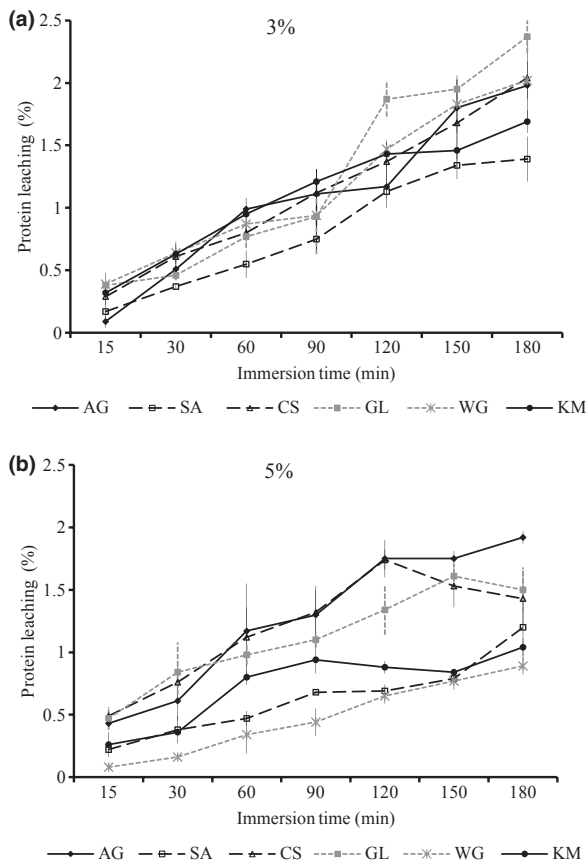
Protein loss was directly proportional to the immersion time (Fig. 3a,b). The inclusion of 50 g kg<sup>-1</sup> WG significantly ( $P < 0.05$ ) lowered leaching at all times evaluated when comparing to 30 g kg<sup>-1</sup> binders, being no more than 0.9% after 180 min of immersion. This significant difference between 50 and 30 g kg<sup>-1</sup> also was evidenced with KM after 90 min and with SA and GL after 120 min.

### Feed ingestion rate and digestibility

Feed consumption varied significantly ( $P < 0.05$ ) depending on the feeding time being higher at 03:00 h as compared to 12:00 h. Diets WG + SA and control had the highest ingestion rate at 03:00 h. Control diet at 12:00 h had the highest feed intake percentage ( $1.31 \pm 0.40\%$ ) of all ( $P < 0.05$ ). There were not significant differences ( $P > 0.05$ ) in the daily ingestion rate (Fig. 4). However, WG5 and control diets presented the highest ingestion rate percentage ( $3.00 \pm 0.53$  and  $3.33 \pm 0.90\%$ , respectively).

The apparent protein digestibility of diets SA5 + WG5 and WG5 was significantly higher ( $P < 0.05$ ) as compared to SA5 and control, with no statistical differences among them (Fig. 5). The lower dry matter digestibility was observed in diet containing sodium alginate. There was no statistical difference ( $P > 0.05$ ) between WG5, WG5 + SA5 and control diets (Fig. 5).



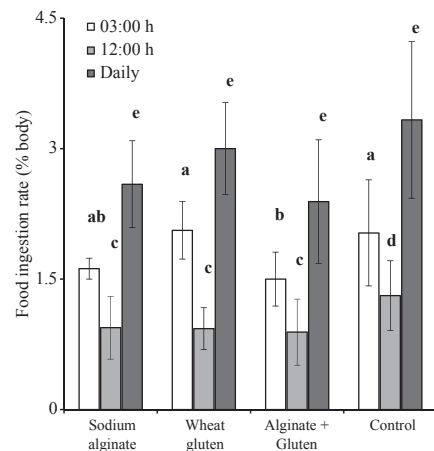


**Figure 3** (a, b) Effect of binder type and concentration on prepared feed protein leaching based on time of water immersion.

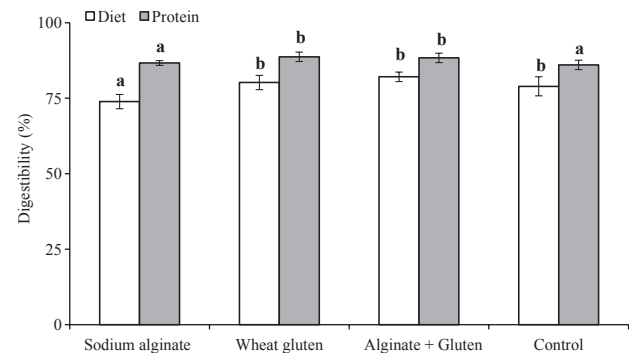
## Discussion

The stability of shrimp feeds in water can be achieved either by extrusion or pelletization processes, triggering the gelatinizing activity of starches, especially those of plant origin. However, if stability in water is insufficient, the inclusion of a binding agent during manufacturing is necessary to reduce dry matter loss not to exceed 10% after 60 min of immersion (Cuzon *et al.* 1994). Binders SA3, CS3, WG3, SA5, WG5 and KM5 evaluated in this study had dry matter retention of about 90%, after 60 min of immersion. Although in this study diets were processed with a meat grinder, diets SA5 and WG5 provided the best stability and low particulate matter disintegration after 3 h immersion with dry matter loss of only 12 and 14%, respectively. These values are much lower than those reported by Kumar & Bandyopadhyay (1999) who observed approximately 70% of dry matter loss in a feed (containing 28% WF) obtained from mincer meat.

Difference observed in the visual evaluation test might be explained due to the formation of an 'inner skeleton'



**Figure 4** Ingestion rate of diets recorded for broodstock shrimp *Litopenaeus vannamei* fed at 03:00 and 12:00 h. Same letters over bars with same colour are not significantly different ( $P > 0.05$ ).



**Figure 5** Apparent protein and dry matter digestibility of *Litopenaeus vannamei* broodstock diets. Same letters over bars with same colour are not significantly different ( $P > 0.05$ ).

produced by binders that reached its maximum compaction (SA3, SA5 and WG5) forming a rigid matrix (Dominy & Lim 1991). The role of a binder in the diet is critical; selection should consider their gelling power to produce stable pellets and acceptance by the animal. A diet containing a poor binder may cause deterioration of water quality and the loss of valuable dietary nutrients (Meyers *et al.* 1972).

Alginate has been used extensively in *Macrobrachium rosenbergi* larviculture to provide excellent water stability (AQUACOP 1976). Similarly, binders such as wheat flour, sodium alginate and wheat gluten are successfully used to stabilize shrimp diets (Akiyama *et al.* 1992; Cuzon *et al.* 1994; Peñaflorida & Golez 1996; Mendoza *et al.* 2001; Terrazas-Fierro *et al.* 2010). However, strong pellet agglutination could affect its use. Therefore, it is necessary to assess binder ability to absorb water providing a soft, easy



to nibble pellet for the shrimp. Cruz-Suárez *et al.* (2001) indicate that pellets significantly increased their ability to absorb water when kelp meal was used as a binder (2–4% inclusion). Kelp meal (30 or 50 g kg<sup>-1</sup>) in our study showed a significant difference in water absorption against AG5 and SA5 diets that showed the highest rates of water absorption (> 100% after 180-min immersion), unlike what was found by Cruz-Suárez *et al.* (2006) in their study with experimental feeds with 40 g kg<sup>-1</sup> kelp meal, water absorption capacity was 153.5%. Gelatin has been also used in fish diets due to its water absorption capacity. However, the limitation of gelatin in feed formulation is due to its low binding power and its rapid leaching process (Partridge & Southgate 1999). The inclusion of gelatin (100 g kg<sup>-1</sup>) in octopus diets resulted in better growth, feed conversion efficiency and survival (Quintana *et al.* 2008; Rosas *et al.* 2008). In this study, water absorption at two concentrations evaluated was low. In general, it has been shown that the water absorption capacity of pelletized feed after 60 min with different binders have values ranging from 58 to 132%, with an average of 94% (Cruz-Suárez *et al.* 2006).

The protein loss in our study indicates that the lowest leaching was achieved with 50 g kg<sup>-1</sup> wheat gluten and 50 g kg<sup>-1</sup> sodium alginate. This might be explained by their better stability (approximately 14%).

Although the diet containing 50 g kg<sup>-1</sup> of sodium alginate showed the best water stability performance, water absorption and protein leaching as compared to the WG5 diet, inherent problems associated with its digestibility and relative higher cost restricted its use. The inclusion of 10 g kg<sup>-1</sup> alginate in juvenile octopus diets negatively affected the growth and survival probably due to the reduction of nutrients absorption from the diet (Rosas *et al.* 2008). Sodium alginate reacts to the presence of polyvalent cations such as calcium making strong viscous solutions (Meyers *et al.* 1972; Akiyama *et al.* 1992). The high binding effect of alginate can prevent the release of amino acids and other attractants thus reducing feed intake, which is of particular importance for shrimp that locates its feed by chemoreception. Nevertheless, the intake rate has been reported higher in fish when compared to gelatin (Partridge & Southgate 1999). Similarly, wheat gluten is well known for its positive effects on the water stability of feed, its high nutritional value, and complete digestibility (Terrazas-Fierro *et al.* 2010; Brinker & Reiter 2011). Its relatively lower cost provides another advantage over alginate, whose use in a commercial scale has been limited (Cruz-Suárez *et al.* 2001). In addition, the palatability of wheat products has proven to be higher. This was demonstrated by our results,

where WG5 and control diets registered the highest ingestion rates, especially at times when shrimp increased its feeding (03:00 h) reaching values of  $2.06 \pm 0.33\%$  and  $2.03 \pm 0.61\%$ , respectively, although there were no significant differences with sodium alginate.

Slightly higher percentage of feed intake was obtained with the diet control, this might be due not only to the palatability of wheat flour, but its low stability ( $81.82 \pm 0.35\%$ ) resulted in a higher degree of dry matter dissolution after 2 h. Precisely, the importance of a binder is based on ensuring that pellet retains its shape and nutrients to be ingested by the animal.

Although protein content of a diet might be good, it still requires to be digested. The determination of APD and ADMD showed that the best values were achieved with WG5 and control diets, being different to SA5 diet and slightly similar to the SA5 + WG5 diet. Typically, alginate does not affect the labile components of a diet; however, they have been proven to affect digestibility (Cuzon *et al.* 1994). High concentrations of binders can cause reduced digestibility of diet due to poor acceptance by the shrimp (Partridge & Southgate 1999). In studies with rainbow trout, sodium alginate reduced the ingestion and APD. The ADMD obtained with SA was significantly lower in our study. Dry matter content in faeces was reduced with the inclusion of alginate in diets for rainbow trout (Storebakken 1985). The presence of  $\beta$ -D mannuronic acid in sodium alginate is responsible for the reduction in digestibility and dry matter content in faeces (Storebakken & Austrong 1987).

The apparent digestibility of protein and energy digestibility of wheat starch and integral wheat have been found to be higher in *L. vannamei*, and therefore, the most widely carbohydrate ingredient used for energy and agglutination purposes (Davis & Arnold 1995). The digestibility of carbohydrates in shrimp varies with the type of flour, botanical starch origin and level of inclusion (Cuzon *et al.* 2000). In *L. vannamei*, wheat gluten reported APD and ADMD values of  $98.0 \pm 0.4\%$  and  $85.4 \pm 0.4\%$ , respectively (Akiyama 1988). Another study by Terrazas-Fierro *et al.* (2010) found APD and ADMD coefficients in wheat gluten of  $103.1 \pm 0.7$  and  $109.2 \pm 3.8\%$ , respectively. These authors also found digestibility values > 100% in other studies but could not determine why it was irregularly produced. In our study, APD and ADMD coefficients ranged between 86.05–88.73% and 73.89–82.10%, respectively. Differences in APD can be attributed to inequality of amino acid or protein content in diets (Sudaryono *et al.* 1996). The proximate analysis of wheat gluten presented the highest protein concentration (570 g kg<sup>-1</sup>) in our study.



The combination of SA5 + WG5 in the diet resulted in good performance, which suggests that the combination of binders could improve feed conversion. Coated diets (microbound) with alginate + zein or alginate + gelatin (Partridge & Southgate 1999) have been successfully used in diets for fish. On the other hand, Ahamad Ali *et al.* (2005) demonstrated that shrimp feed pellets containing wheat flour as a source of starch and 20 g kg<sup>-1</sup> guar gum showed good water stability and suggest that wheat flour has additional advantage of gluten present in it, which also contributes to the binding. We recommend evaluating the use of combinations of binders including wheat gluten as a binder for shrimp broodstock feed due to its gelling and attractant property, as well as to its lower cost when compared to sodium alginate.

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