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Manufacturing pellets with different binders: Effect on water stability and feeding response in juvenile *Cherax albidus*

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ABSTRACT

In this study, we used natural polysaccharides (pectin, alginate and chitosan) as binders to generate pellets for crayfish feeding. Pellets were produced by cold extrusion in order to preserve nutrients from degradation and reduce energy consumption. Thereafter, pellets were submitted to a coating procedure, with the aim of improving pellet stability in water. Pellet water stability was analyzed by monitoring the diameter of the released particles in water over progressive time intervals up to 24 h, employing a Low Angle Laser Light Scattering Technique. Alginate containing pellets released particles with a smaller diameter than chitosan and pectin containing pellets, indicating that alginate containing pellets disaggregated more and therefore were less stable in water than the other pellet types. The effects of the different polysaccharide containing pellets were evaluated on crayfish feeding response employing juvenile *Cherax albidus*. The feeding experiment was carried out for 12 weeks, at the end of which growth parameters and the activity of amylase, lipases and proteases in the gastric juice, hepatopancreas and intestine were recorded. Crayfish fed pectin containing pellets exhibited a significant weight gain. Digestive enzyme activities did not statistically show significant differences in the digestive tract except for amylase that was significantly higher in the intestine of animals fed pectin containing pellets. Our data indicate that pectin and chitosan pellets showed the best water stability performances, moreover pectin pellets brought about the highest body weight gain and affected the amylase profile in the intestine of juvenile *Cherax albidus*.

1. Introduction

In aquatic animal feed preparation, stabilizing feed pellets and ensuring minimum nutrient leaching and disintegration appears to be crucial. This holds true especially for crayfish culture, due to the peculiar feeding behavior of such crustaceans, being slow feeders with a characteristic tendency to manipulate food using mouth appendages before ingestion (Holdich, 2002). Much interest has been devoted to the manufacturing of pellets for crayfish farming in order to optimize feed intake and reduce wastage. Numerous natural binders have been employed to manufacture firm pellets with the purpose of increasing their water stability with a concomitant decrease in nutrient loss. Polysaccharides, such as starch, cellulose, pectin, etc. are non-toxic, available in abundance and are gaining in importance as promising biopolymers to be employed as binders (Volpe et al., 2010 for review). The specific macromolecular structures, characterized by the presence of several polar functional groups, allow polysaccharides to retain significant amounts of water or biological fluids, thus providing the formation of hydrogels, i.e. three-dimensional, reliable networks, able to become water resistant throughout chemical or physical phenomena, such as gel formation, retrogradation process, pH-changing and cross linking processes (Farris et al., 2009). Although the potential application of polysaccharides as binders in the feed industry is still in its infancy, agar, alginate, carrageenan, gelatin, carboxymethyl cellulose and starch are among the most tested binders for pellet manufacturing. Due to the ample variability in feed ingredients, percentage of binders included and manufacture technology, it is impossible to come to the conclusion that a certain binder is better than another with respect to its water stability performances. Moreover, experimental outcomes are reported in literature as relative, that is one binder behaving better than another under specific conditions, making it impossible to objectively evaluate binder performances (Paolucci et al., 2011 for review).

The relationship between the availability and composition of feed and the growth rate of a given aquatic species is of a crucial importance for the optimization of their rearing conditions. Since binders often account for a consistent part of pellets, the effect of such

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Table 1
Proximate analysis of the experimental diets formulated with different binders (pectin, alginate, chitosan) (dry matter). Mean ± SD. Three batches for each diet and three samples of pellets for each batch were analyzed ($n = 9$ for each pellet type).

Composition/100 g	Control pellets	Pectin pellets	Alginate pellets	Chitosan pellets
Total fat (g)	7.40 ± 0.28	7.44 ± 0.23	7.03 ± 0.19	7.53 ± 0.15
Saturated fat (g)	2.67 ± 0.04	2.68 ± 0.03	2.52 ± 0.03	2.72 ± 0.04
Unsaturated fat (g)	4.73 ± 0.04	4.76 ± 0.05	4.51 ± 0.05	4.81 ± 0.05
Cholesterol (mg)	128.53 ± 1.01	125.97 ± 0.91	127.85 ± 1.04	127.85 ± 1.03
Sodium (mg)	13.76 ± 3.01	15.07 ± 2.52	14.58 ± 1.73	14.58 ± 1.65
Total carbohydrates (g)	41.38 ± 0.64	43.48 ± 0.54	43.69 ± 0.33	44.00 ± 0.48
Fibers (g)	3.53 ± 0.14	3.92 ± 0.09	3.97 ± 0.07	3.57 ± 0.08
Sugars (mono-disaccharides) (g)	3.98 ± 0.04	3.92 ± 0.04	4.01 ± 0.03	3.98 ± 0.03
Proteins (g)	39.37 ± 0.62	39.02 ± 0.43	39.74 ± 0.55	39.58 ± 0.27
Calcium (mg)	325.36 ± 10.02	326.67 ± 8.32	331.54 ± 7.43	331.04 ± 6.21
Iron (mg)	11.08 ± 1.05	7.21 ± 0.67	12.19 ± 0.95	13.09 ± 0.74

substances on the ingestion, digestion and assimilation processes should be carefully taken into account in order to maximize the experimental feed efficiency. Among crustaceans, binders have been tested for growth performance in several species, with often conflicting results. Wheat flour, whole cassava meal and dry molasses were effective binders and caused the highest weight gain in crayfish *Macrobrachium rosenbergii* post-larvae, in comparison to agar (Seixas Filho et al., 1997a), although agar containing pellets had the best texture (Seixas Filho et al., 1997b). In contrast, Kovalenko et al. (2002) report that growth of larvae fed alginate containing feed, was only 90% of that achieved for larvae fed newly hatched nauplii of *Artemia*. Both lignosol and agar brought about a significant increase in weight in juvenile shrimp *Palaemonetes varians* and *Palaemon elegans* (Palma et al., 2008). Alginate, agar and pectin caused good growth performance in *Cherax albidus* adults with respect to control animals fed a natural diet, with pectin giving the best results (Volpe et al., 2008). It is commonly agreed that species exhibit a particular suite of digestive enzymes that reflect their different life history (Figueiredo and Anderson, 2009), and consequently it is possible to predict the ability of the species to use different nutrients from the digestive enzyme profile. Since the digestive enzyme activity is high for those substrates that are more common in the diet (Moss et al., 2001) there is a common belief that the activity of digestive enzymes can be boosted by providing certain nutrients into the diet for an adequate amount of time. However, virtually no studies have been carried out with the main purpose of investigating the effect of feed binders on digestive enzyme activity.

In this frame we have undertaken a study in order to set up a simple and sustainable pellet manufacturing methodology on a laboratory pilot scale. Pellets containing the same proximate composition with additional polysaccharides (pectin, alginate and chitosan) employed as binders were cold extruded and coated with the same binder employed to manufacture the pellet. Pellets were tested to assess their water stability and administered to juvenile freshwater crayfish *C. albidus* to examine the potential to respond to different binders.

2. Materials and methods

2.1. Experimental animals

Juvenile crayfish *C. albidus* of 1.0 ± 0.5 g were employed. The crayfish were imported from Mulataga Aquaculture (Perth, Western Australia, P.O. Box 343 Gosnells 6110), as *C. albidus*, and resulted, according to the European Community Law, in good health and disease-free (Health Certificate n. 4436915).

2.2. Installation

The recirculation system used for growth trials consisted of rectangular glass tanks of 20 × 20 × 20 cm. Active carbon filters were installed to eliminate chlorine from the city water. Each tank hosted one animal (the animals were singularly housed). The system was held under L:D 12:12 photoperiod. Temperature and pH (pHmeter GLP 21 Crison) were checked daily. Dissolved oxygen, total ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, phosphorus, phosphate, total chlorine and firmness ($CaCO_3$) were measured twice a week with a Hanna Instruments Photometer C200.

2.3. Pellets manufacturing

Proximate analysis of the experimental diets is shown in Table 1. Pellets were formulated to contain the same amount of proteins, lipids and carbohydrates. 5% of selected polysaccharides (pectin, sodium alginate and chitosan) was added to the pellets. Pectin was from Sigma (Sigma–Aldrich, St. Louis, MO, USA), sodium alginate was supplied by Lianyungang Zhongda Seaweed Industrial Co., Ltd (China) and chitosan was from Fu Zhou Corona Science & Technology Development CO., Ltd (China). Pellets were prepared in the following way: 5 g of each binder was added to 100 ml of water at 70 °C. The solution was stirred until the complete binder dissolution and following cooled. Then 95 g of the ingredients was added and the blend was hand mixed. To attain a strong physical interaction among nutrients and binders, the wet paste was mixed at room temperature in a counter-rotating twin screw mixer (Haake Rheocord Mixer) for 15 min, at a mixing rate of 30 rpm under air flow. The wet paste was then pressed in a single screw extruder (Rheocam, Scamia France), of 20 mm screw diameter, a *L/D* ratio of 11, equipped with a conical shape element, able to provide high shearing, and with a head die of 0.1 cm. The wet extrusion was performed at room temperature to avoid any possible degradation of nutrients, at a constant screw speed of 40 rpm, with a head material pressure of 60 bar and with a powder feed rate of 10 g/min. In this way cylindrical extrudates in the form of "spaghetti" were obtained and at once broken into smaller cylindrical rods (pellets) of about 1 cm length × 0.1 cm diameter and dried for 24 h under the hood. Thereafter, pellets were submitted to a coating procedure as follows: pectin pellets were immersed in a 1% w/v sodium alginate aqueous solution for 5 min; alginate pellets were immersed in a 1% w/v aqueous solution of calcium chloride for 5 min; chitosan pellets were immersed in a 1% w/v chitosan solution for 5 min. Thereafter, pellets were dried for 24 h. Pellets without polysaccharides (control pellets) were made as reported above but did not contain any polysaccharide. After being dried, all pellets were stored at 4 °C. Proximate analysis of pellet ingredients was carried out according to the methods of the AOAC (2007) (Table 1).

2.4. Effect of water immersion time on water stability of pellets

Three different batches were made for each pellet type. Three pellets for each batch were analyzed ($n = 9$ for each pellet type). Pellets were soaked in water for up to 24 h. After the required