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Assessing the feasibility of dietary soybean meal replacement for fishmeal to the swimming crab, *Portunus pelagicus*, juveniles

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ABSTRACT

The feasibility of dietary soybean meal (SBM) replacement for fishmeal was evaluated to the swimming crab *Portunus pelagicus* juveniles over six consecutive molts by measuring their growth, development, hemolymph cholesterol, triglycerides, phosphate, whole-body crude protein and cholesterol as well as hepatopancreatic trypsin activity, histopathology and glycogen reserves. A total of six isonitrogenous, isolipidic and isoenergetic diets were formulated with SBM replacing fishmeal at 0 (control), 20, 40, 60, 80 or 100% of total dietary protein. Each treatment consisted of 30 replicate crabs starting from the first juvenile stage and after the crabs molted to the seventh stage, three-day post molt crabs were sampled. Results showed that crabs fed the 20% SBM diet had the best growth, which was significantly higher than the control diet (0% SBM). Growth became significantly lower in the 60% dietary SBM treatment and above, while hemolymph cholesterol, triglycerides and phosphate significantly decreased with increasing dietary SBM. The whole-body moisture, crude protein and cholesterol were unaffected by dietary SBM. Meanwhile, hepatopancreatic trypsin activity significantly decreased in the 20 to 60% SBM treatments, with a further significant decrease in the 80 and 100% SBM treatments. Hepatopancreatic damage, significantly fewer epithelial cells and glycogen reserves occurred at 60% SBM and above. Results indicate that dietary SBM can replace up to 40% of fishmeal in the diets of *P. pelagicus* juveniles without reducing their growth or hepatopancreatic condition.

Statement of relevance: SBM at 40% can be used in the diets of *P. pelagicus* without affecting survival or growth.

1. Introduction

The current use of fishmeal in the diets of fish and crustaceans is largely viewed as both an uneconomical and unsustainable practice, which has prompted research into identifying suitable protein alternatives (Gatlin et al., 2007). Plant protein sources are often the focus of such research, and among these, soybean meal (SBM) is perhaps the most studied and successful largely due to their relatively high protein content and well-balanced amino acid profile. Nevertheless, in general, SBM is unable to completely replace fishmeal without compromising the growth of crustaceans (Alvarez et al., 2007; Rahman et al., 2010; Fuertes et al., 2012; Bulbul et al., 2015, 2016). Moreover, the ability of crustaceans to accept dietary SBM is highly species specific and ranges from 40% for the speckled shrimp *Metapenaeus monoceros* (Rahman et al., 2010) and 76% for the white shrimp *Litopenaeus schmitti* (Alvarez et al., 2007) to relatively low levels of 22% and 25% for the kuruma shrimp, *Marsupenaeus japonics* (Bulbul et al., 2015) and signal crayfish, *Pacifastacus leniusculus* (Fuertes et al., 2012), respectively.

The causes for the limited use of plant-based fishmeal alternatives, including SBM, are often attributed to deficiencies in essential nutrients, such as amino acids, as well as anti-nutritional factors (ANF). For example, when using plant-based proteins, phosphate and cholesterol are often cited as limiting nutrients since these are inherently in lower amounts compared to fishmeal. Moreover, the presence of ANF can further exacerbate this issue that may be reflected by lower amounts in the plasma or hemolymph of aquatic animals (Yue et al., 2011; Prabhu et al., 2014). Cholesterol is well known to be essential for crustaceans, and is the main component of lipoproteins that are responsible for lipid transport and metabolism (Yepiz-Plascencia et al., 2000). Meanwhile, excessive amounts of dietary plant proteins were also shown to decrease trypsin, which is a protease enzyme, within the liver of seabream *Sparus aurata* (Robaina et al., 1995) and hepatopancreas in lobster *Panulirus argus* (Perera et al., 2012) and kuruma shrimp, *Marsupenaeus japonicus* (Bulbul et al., 2016). Recently, saponin from SBM was shown to induce intestinal enteritis in Atlantic salmon *Salmo salar* (Krogdahl et al., 2015).

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One of the ways to assess the nutritive condition of crustaceans is through histopathological analysis of the hepatopancreas and associated glycogen content and prevalence of various cells (Simon and James, 2007; Romano et al., 2015; Sukor et al., 2016). Within the hepatopancreatic tubules, there are four main types of cells that include R-cells and B-cells that are responsible for nutrient storage and absorption, respectively, while the F-cells synthesize digestive enzymes and proteins and the E-cells are undifferentiated (Vogt et al., 1985). It has been shown that higher growth and nutrient utilization in the white shrimp *Litopenaeus vannamei* were linked to increased R-cells and decreased B-cells (Romano et al., 2015). There appears, however, to be a paucity of information on the potential influence of dietary SBM to the hepatopancreatic digestive enzyme activity or condition in crabs.

Despite the potential problems with using dietary SBM, this protein source has a relatively high digestibility to crabs (Tuan et al., 2006; Truong et al., 2009; Luo et al., 2011). For example, among various protein sources, including fishmeal, shrimp meal, blood meal and SBM, the dry matter, energy and protein digestibility of SBM was higher than all other ingredients to the mud crab *Scylla serrata* juveniles (Tuan et al., 2006). Similarly, for *S. paramamosain*, the apparent digestibility of dry matter, crude protein and energy were all higher in SBM-based diets compared to cassava meal or fishmeal-based diets (Truong et al., 2009). These findings indicate that SBM could be a suitable fishmeal alternative to portunid crabs; however, to date, investigations on the feasibility of SBM to the swimming crab *Portunus pelagicus* have not yet been performed.

The commercial aquaculture of *P. pelagicus* aquaculture is still largely in the development phase, although there is high consumer demand for this species as well as crabs from the genus *Portunus* throughout the Indo-Pacific region (Jin et al., 2013; Sukor et al., 2016). Nevertheless, feed formulations for portunid crabs are still underdeveloped (Jin et al., 2013) leading to a high reliance on trash fish or shrimp feeds. To potentially improve the cost-effectiveness of portunid crab farming, the aim of this study was to assess the feasibility of replacing fishmeal with SBM on the survival, growth, hemolymph/body biochemical composition as well as hepatopancreatic trypsin and histopathology.

2. Materials and methods

2.1. Experimental diets

A total of six isonitrogenous, isolipidic and isoenergetic diets were formulated with increasing amounts of SBM, at the expense of fishmeal, at 0, 20, 40, 60, 80 or 100% on a total dietary protein contribution basis. Due to residual fish oil and soybean oil in fishmeal and SBM, respectively, the diets were also formulated to contain equal amounts of fish oil and soybean oil. Phospholipid and cholesterol were supplemented at 2% and 0.8%, respectively, to satisfy the requirements for portunid crabs (Sheen, 2000; Li et al., 2014). Sources for all ingredients are provided as footnotes in Table 1.

After grinding and sieving the fishmeal and SBM, these were thoroughly mixed with the other dry ingredients. Appropriate amounts of fish oil, soybean oil, and cholesterol were then mixed together, followed by adding lecithin. Since lecithin was in a hard gum form, this was placed in an oven at 50 °C for 10 min to help soften and homogenize this ingredient with the other lipids. After adding all the wet ingredients, and mixing for 30 min., distilled water was added at 20% of the ingredient weight.

The resulting dough was further mixed for another 30 min., and then extruded through a 1.0 mm diameter die of a single-screw extruder (Brabender KE19; Brabender GmbH, Germany). The three barrel temperatures were maintained at 60–100–120 °C and the die head temperature was set at 160 °C. The extruded pellets were then oven dried at 55 °C overnight, kept in air-tight plastic bags and stored at −20 °C until use. For the experimental diets, the proximate composition was measured according to standard AOAC (1997) methods while the fatty acid composition was measured according to Ebrahimi et al. (2014). Dietary cholesterol was measured according to Rudel and Morris (1973) with slight modifications, which are described in Section 2.4. Meanwhile, the pellet stability of the experimental diets was measured according to Jiang et al. (2013).

Table 1
Ingredient formulation and proximate composition (% dry matter) of the experimental diets with increasing soybean meal (SBM).

Ingredients	Experimental diets (% replacement of fish meal by soybean meal)					
	0% SBM	20% SBM	40% SBM	60% SBM	80% SBM	100% SBM
Fishmeal[a]	61.27	49.02	36.76	24.51	12.25	0.00
Soybean meal[b]	0.00	14.68	29.37	44.05	58.74	73.42
Fish oil[c]	0.01	1.00	2.00	3.00	4.00	5.00
Soybean oil[d]	3.00	2.74	2.49	2.23	1.97	1.72
Corn starch	28.70	24.94	21.18	17.42	13.66	9.90
Lecithin[e]	2.00	2.00	2.00	2.00	2.00	2.00
Choline chloride[f]	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol[g]	0.80	0.80	0.80	0.80	0.80	0.80
Vitamin premix[h1]	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[h2]	1.00	1.00	1.00	1.00	1.00	1.00
α-Cellulose[i]	0.22	0.81	1.40	1.99	2.58	3.17
Proximate composition						
Dry matter	96.82	97.11	96.76	97.49	95.81	93.01
Crude protein	41.84	41.75	42.33	41.45	40.88	41.12
Crude lipid	9.25	9.34	8.75	9.01	9.20	9.43
Crude ash	17.75	15.07	11.93	11.16	7.95	6.99
Crude fiber	0.53	0.85	2.49	2.89	5.12	5.67
Cholesterol[j]	4.07	4.48	4.22	4.89	4.94	4.97

[a] Local fishmeal (mixed species) (moisture, crude protein, crude lipid, and crude ash of 9.10, 65.28, 8.15, 17.92, respectively, on as is basis).
[b] Soybean meal (moisture, crude protein, crude lipid, crude ash and crude fiber of 11.67, 54.48, 1.75, 6.72 and 4.22, respectively, on as is basis).
[c] From menhaden source (Sigma F8020).
[d] Purchased from a local grocery store.
[e] 30% phosphatidylcholine (Sigma P364).
[f] 98% powder (Sigma C7527).
[g] 92.5% powder (Sigma C8503).
[h1] Same composition as Sukor et al. (2016).
[h2] Same composition as Sukor et al. (2016).
[i] α-Cellulose (Sigma C8002).
[j] Expressed at g kg^{-1}.

The crude protein, crude lipid and cholesterol were generally similar among the diets, although the ash and fiber contents decreased and increased, respectively, with increasing dietary SBM (Table 1). The fatty acid composition was relatively similar among the diets, with the dominant fatty acids being 18:2n-6 (mean of 27.4%) followed by 16:0 (mean of 17.34%). With increasing dietary SBM, 20:4n-6 and 20:5n-3 tended to decrease and increase from 3.68 to 1.15% and 7.26 to 11.15%, respectively, while 22:6n-3 was similar across all diets (7.33–8.75%). The pellet stability for the 0–80% SBM diets ranged from 83.9 to 88.3%, but decreased to 77.9% for the 100% SBM diet.

2.2. Source of experimental animals and set-up

The juvenile crabs used in this experiment were larvicultured according to Romano and Zeng (2006), with the exception that 1000 l tanks were used. Once the crabs settled to the first juvenile stage, which was within 12 h of metamorphosis, a total of 180 apparently healthy crabs were moved to individual containers filled with 500 l of natural and pre-filtered (5 μm and UV sterilized) seawater with a salinity of 32 ± 2. This led to 30 replicated crabs in each treatment and all culture units were organized in a random block design that were held under a plastic lined room and an air heater maintained the temperature at 29 ± 2 °C.

In the morning, all containers were checked for mortalities or molts and any uneaten feed was siphoned out. This led to an approximate 30% reduction in the water volume, and the water was then replenished