Aquaculture Nutrition **2002 8**; 87–94

Nutritional value of feed peas (*Pisum sativum*) in practical diet formulations for *Litopenaeus vannamei*

D.A. DAVIS[1], C.R. ARNOLD[2] & I. McCALLUM[3]

[1] *Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL, USA;* [2] *The University of Texas at Austin, Marine Science Institute, Port Aransas, TX, USA;* [3]*Pulse Canada, Winnipeg, Manitoba, Canada*

Abstract

Dry peas of mixed Canadian prairie varieties which were commercially obtained and processed to provide a variety of meals were evaluated in practical shrimp feeds. Whole and de-hulled peas were pin milled to produce raw flours. A portion of these meals were processed to produce whole extruded and de-hulled extruded meals. Additionally, a portion of the whole pea meal was processed by infrared cooking to produce a micronized meal. The five meals were evaluated in practical diets for *Litopenaeus vannamei* under controlled laboratory conditions. The first experiment was designed to estimate apparent protein and energy availability of the various meals. Using a practical reference diet, the meals were substituted using a 70:30 ratio to produce the test diets. Based on contrasts, both extruding and micronizing the pea meals resulted in significant improvements in both apparent protein digestibility and apparent energy digestibility values. Apparent energy digestibility values for the various ingredients expressed as percentage ± SD were: whole raw, 72.3 ± 8.1; whole extruded, 86.0 ± 8.9; de-hulled raw, 88.4 ± 4.4; de-hulled extruded, 94.4 ± 10.0; whole micronized, 94.1 ± 10.2. To evaluate the response of shrimp to the diets containing pea meal, two 7-week growth trials were conducted in the laboratory using a practical diet formulated to contain 360 g kg^{-1} protein and 90 g kg^{-1} lipid. In the first growth trial the shrimp had a mean initial weight of 0.66 g and six test diets were evaluated that included the basal diet and five diets for which the pea meals were included in the diet at 250 g kg^{-1} dry weight replacing whole wheat. In the second growth trial the shrimp had a mean initial weight of 1.1 g and only the whole raw and whole extruded meals were evaluated at 50, 100 and 200 g kg^{-1} inclusion in the diet. At the conclusion of the first growth trial weight gain ranged from 718 to 862% and at the conclusion of the second growth trial weight gain ranged from 394 to 502%, with no significant differences or discernible trends observed as a result of the various dietary treatments. Based on the observed results, the continued evaluation of feed peas as a potential ingredient of shrimp feeds is warranted. Additionally, if feed peas are suitably priced, commercial producers are encouraged to evaluate feed peas as an alternative protein and energy source.

KEY WORDS: digestibility, feed pea, *Litopenaeus vannamei*, nutrition, shrimp

Received 21 February 2001, accepted 1 August 2001

Correspondence: D. Allen Davis, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL, USA. E-mail: ddavis@acesag.auburn.edu

Introduction

Grain legumes are the harvested seed of leguminous crops, which include peas and beans and other closely related species within the Fabaceae family. Within this family, soybeans are the most extensively evaluated and most commonly used plant protein source. In addition to soybeans, there are a number of other legumes that have potential value to feed manufacturers. Recently, researchers have begun to evaluate the acceptability of other grain legumes such as lupin (Sudaryono *et al.* 1999), cowpea and rice bean (Eusebio 1991) in shrimp feeds. Another legume with potential is the feed pea, *Pisum sativum*. This crop has long been used in livestock feeds as a source of energy and protein but has only recently been evaluated in feeds for aquatic species. Feed peas have been evaluated as potential feed ingredient for several species of fish including the European sea bass, *Dicentrarchus labrax* (Gouveia & Davies 1998, 2000), Australian silver perch, *Bidyanus bidyanus* (Allan *et al.* 2000), Atlantic salmon, *Salmo salar* (Carter & Hauler 2000), rainbow trout, *Oncorhynchus mykiss* (Gomes *et al.* 1993, 1995; Burel *et al.* 2000) and turbot, *Psetta maxima* (Burel *et al.* 2000).

In general, feed peas have produced a favourable response in fish and they could also serve as an alternative feed ingredient for shrimp feeds. Although data is limited, feed peas have been evaluated in diets for *Penaeus monodon* (Smith *et al.* 1999) and *Litopenaeus stylirostris* (Cruz-Suarez *et al.* 2001). However, prior to evaluation in commercial rations, the biological value of feed peas and the influence of processing should be evaluated for the species of interest. Processing such as de-hulling and thermal treatments to increase starch gelatinization have been demonstrated to increase digestibility of plant protein sources with a number of species and is likely to influence nutrient availability in shrimp. Hence, this research was designed to evaluate the influence of various processing conditions (de-hulling, extrusion and micronization) on the biological availability of feed peas to juvenile pacific white shrimp, *L. vannamei*.

Materials and methods

Test ingredients

A composite lot of commercial dry peas, *Pisum sativum*, of mixed Canadian prairie varieties, were processed to prepare the various meals. Whole and de-hulled peas were pin milled to produce raw flours. Additionally, solvent extracted canola meal was obtained and hammer milled prior to extrusion. A portion of these flours were then extruded (Werner and Pfeiderer ZSK-57, Vienna, Austria). Each flour was preconditioned (Wenger model 2 DDC, Kansas City, MO, USA) and extruded with a co-rotating twin screw extruder through a 6-mm round hole die at a product temperature reaching 145 °C at the die plate, and pressure ranging between 620 and 740 psi. The extruder screw was 147 cm long with a length to diameter ratio of 24:1. The material was fed into the conditioner at a rate of 90.9 kg h^{-1}, 93.6 kg h^{-1} and 113.4 kg h^{-1} for whole peas, de-hulled peas and canola meal, respectively, and water injected at a rate of 4.5 L h^{-1}. The material was then dried in a fluid bed dryer at 110 °C and pin milled to produce the extruded meals. Another portion of the whole peas was tempered to 140 g kg^{-1} moisture and processed using infrared cooking, reaching a temperature of 120 °C. The heat treated (micronized) peas were subsequently rolled to produce a flake and then milled into a fine powder. Five pea meals were tested that consisted of: whole raw (WRA), whole extruded (WEX), de-hulled raw (DRA), de-hulled extruded (DEX) and whole micronized (WMI) pea meals. Additionally, extruded canola meal (EC) and wheat middlings (WM) were included as references. Proximate composition of the pea meals and canola meal are reported by (McCallum *et al.* 2000). Wheat middlings had the following proximate composition expressed as g kg^{-1} as in: moisture, 136; protein, 166; either extract, 41; crude fibre, 76; total ash, 46.

Digestibility

Voluntary consumption of test diets with chromic oxide as an inert marker was utilized to determine apparent digestibility coefficients for the various ingredients using *L. vannamei* (8–10 g mean weight) as the test species. Each feed ingredient was evaluated utilizing a 30% replacement in the semi-purified reference diet (Table 1). Apparent digestibility coefficients for the reference diet was used to calculate digestion coefficients for the feedstuffs of interest based on the percentage substitution of the test ingredient (Forester 1999). In preparing test diets, the basal diet (BD) (Table 1) was mixed and the dry matter content of the basal mix and test ingredients determined. The test ingredients and basal

Table 1 Composition of the basal diet formulated to contain 360 g kg^{-1} protein and 9 g kg^{-1} lipid

	g kg^{-1} Dry weight
Fish meal[1]	300.0
Soybean meal[2]	253.0
Wheat starch[3]	13.2
Whole wheat[3]	250.0
Nutribinder[4]	100.0
Menhaden fish oil[5]	43.3
Trace mineral premix[6]	5.0
Vitamin premix[7]	20.0
Stay C[8]	1.0
Calcium phosphate[9]	2.0
Lecithin[10]	5.0
Chromic oxide	7.5

[1] Menhaden fish meal, Special Select™, Zapata Protein USA Inc., Randeville, LA, USA.

[2] Solvent extracted, Producers Coop, Bryan,TX, USA.

[3] United States Biochemical Corporation, Cleveland, OH, USA.

[4] Industrial Grain Products Inc., Lubbock,TX, USA.

[5] Omega Protein Inc., Reedville,VA, USA.

[6] g 100 g^{-1} Premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulphate 4.0, magnesium sulphate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 53.428.

[7] g kg^{-1} Premix: thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B_{12} 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20 000 IU g^{-1}) 5.0, vitamin D_3 (400 000 IU g^{-1}) 0002, DL-α-tocopheryl acetate (250 IU g^{-1}) 8.0, α-cellulose 865.266.

[8] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[9] Cefkaphos® (primarily monobasic calcium phosphate) BASF Corporation, Mount Olive, NJ, USA.

[10] Aqualipid 95, Central Soya Chemurgy Division, Fort Wayne, IN, USA.

mix were homogenized in a food mixer (Hobart Corporation, Troy, OH, USA) for 30 min. After mixing, boiling water was blended into the mixture until a consistency appropriate for pelleting was reached. Each diet was pelleted utilizing a meat grinder with a 2-mm die and dried by forced ambient air to a moisture content of 80–100 g kg^{-1} and stored at –10 °C.

Shrimp were maintained in a semi-closed recirculating system consisting of a series of 105 L culture chambers, a rapid rate sand filter and a biological filter. Salinity, temperature and dissolved oxygen were maintained at (mean ± SD) 29.7 ± 2.1 g L^{-1}, 27.9 ± 0.7 °C and 6.2 ± 0.4 mg L^{-1}, respectively. Prior to the initiation of the experiments, shrimp (5–6 g) were acclimated to the experimental conditions and reference diet. Each of the test diets were fed to four replicate tanks of shrimp (six shrimp per tank) over a 3-day conditioning period followed by a 4-day collection period. Exuviae and faeces were removed from the tanks before the shrimp were given an initial feeding. To ensure that previously consumed material (faeces and exuviae) was cleared from the digestive system, faeces from the first feeding were discarded. Shrimp were allowed to feed for 40–45 min after which faeces was collected by siphoning from the tank into a 48-μm sieve, and uneaten feed removed from the tanks. Faecal samples were immediately rinsed with distilled water. Once all tanks had been siphoned, the feeding process was repeated, allowing at least five collections per day. Faecal samples collected from the same tank/treatment were pooled, resulting in four samples/treatment. Each sample was oven dried (90 °C to constant weight) and then frozen for subsequent analyses. Chromic oxide was determined by the method of McGinns & Kasting (1964), protein by micro-Kjeldahl analysis (Ma & Zuazago 1942) and gross energy by micro-bomb calorimetry (Parr adiabatic calorimeter, Parr Instrument Co., Moline, IL, USA).

Growth trials

Two 7-week feeding trials were conducted to determine the nutritional value of pea meal relative to growth and survival of *L. vannamei* juveniles. Pea meals tested included: WRA, WEX, WMI, DRA and DEX. Additionally, EC meal and WM were included as reference meals. Both experiments used a practical BD that was formulated to contain 360 g kg^{-1} protein and 90 g kg^{-1} lipid. In the first experiment, whole wheat (250 g kg^{-1} diet) was replaced with the test ingredients on an equal weight basis (Table 2). In the second experiment,

Table 2 Ingredient composition (expressed as g kg^{-1} dry weight) of the basal diet and test diets containing whole raw pea meal (WRA), whole extruded pea meal (WEX), whole micronized pea meal (WMI), de-hulled raw pea meal (DRA), de-hulled extruded pea meal (DEX), extruded canola meal (EC) and wheat middlings (WM) offered to 0.66 g shrimp over a 7-week growth trial

	Basal	WRA	WEX	WMI	DRA	DEX	EC	WM
Fish meal[1]	300	300	300	300	300	300	300	300
Soybean meal[2]	253	213	208	216	193	198	116	222
Test ingredient		250	250	250	250	250	250	250
Whole wheat[3]	250							
Wheat starch[3]	17	57	62	54	77	72	154	47
Nutribinder[4]	100	100	100	100	100	100	100	100
Menhaden fish oil[5]	43	43	43	43	43	43	43	43
Trace mineral premix[6]	5	5	5	5	5	5	5	5
Vitamin premix[7]	20	20	20	20	20	20	20	20
Vitamin C[8]	5	5	5	5	5	5	5	5
Calcium phosphate[9]	2	2	2	2	2	2	2	2
Soya lecithin[10]	5	5	5	5	5	5	5	5

[1] Menhaden fish meal, Special Select™, Zapata Protein USA Inc., Randeville, LA, USA.

[2] Solvent extracted, Producers Coop, Bryan, TX, USA.

[3] United States Biochemical Corporation, Cleveland, OH, USA.

[4] Industrial Grain Products Inc., Lubbock, TX, USA.

[5] Omega Protein Inc., Reedville, VA, USA.

[6] g 100 g^{-1} Premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulphate 4.0, magnesium sulphate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 53.428.

[7] g kg^{-1} Premix: thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B_{12} 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20 000 IU g^{-1}) 5.0, vitamin D_3 (400 000 IU g^{-1}) 0.002, DL-α-tocopheryl acetate (250 IU g^{-1}) 8.0, α-cellulose 865.266.

[8] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[9] Cefkaphos® (primarily monobasic calcium phosphate) BASF Corporation, Mount Olive, NJ, USA.

[10] Aqualipid 95, Central Soya Chemurgy Division, Fort Wayne, IN, USA.

raw pea meal or extruded pea meal was included in the BD at 50, 100 and 200 g kg^{-1} replacing whole wheat (Table 3). Test diets were formulated to be isonitrogenous to the BD by reducing the soybean meal content and using wheat starch as a filler. Prior to use, the fish and soybean meals were ground with a laboratory type hammer mill using a #40 screen (1.02 mm diameter hole). These ingredients, as well as the test meals, were analysed for protein and moisture content prior to the formulation of the diets. After processing, the dry ingredients and oil were mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Hot water was then blended into the mixture to attain a consistency appropriate for pelleting. Each diet was pressure pelleted using a meat grinder and a 2-mm die. After pelleting, the diets were dried to a moisture content of 8–10% and stored at 4 °C. Moisture content and crude protein levels of the diets were determined after processing. Protein content was determined by the micro-Kjeldhal method (Ma & Zuazago 1942).

High health postlarvae, were obtained from a commercial hatchery and held under quarantine conditions for approximately 5 weeks. During this holding period the shrimp were maintained on a commercial shrimp feed (Rangen Inc., Buhl, ID, USA) and evaluated for signs of viral and bacterial pathogens. The shrimp were sorted to a uniform size and stocked in the research system. Each dietary treatment was randomly assigned to replicate groups of shrimp maintained in a common semi-closed recirculating system consisting of a series of rectangular tanks, a circulation pump, rapid-rate sand filter, supplemental aeration and biological filter. For the first growth trial, eight shrimp having a mean weight ± SD of 0.66 ± 0.02 g were stocked into four replicate tanks per dietary treatment. Each tank was designed to contain 68 L of seawater. For the second growth trial, 12 shrimp having a mean weight of 1.1 ± 0.04 g were stocked into three replicate tanks per dietary treatment. Each tank was designed to contain 110 L of seawater. The shrimp were fed according to a fixed feeding rate which was adjusted for expected growth and observed mortalities. In addition to daily mortality checks, the shrimp were enumerated twice weekly. In experiment 1, a total of 13.5–13.8 g (dry weight) of feed per shrimp was offered over the course of the growth trial and 14.3–15.1 g per shrimp was offered in experiment 2. Shrimp were fed four times per day, at approximately 08:00, 11:00, 13:00 and 16:00 hours. Photo-period was set for 12:12 h light:dark cycle. Water quality was maintained by biological filtration, removal of settled solids, and replacement of the systems make-up water with prefiltered ozone-treated seawater at a rate of 4 L min^{-1}. Water

Table 3 Ingredient composition of the test diets (g kg^{-1} dry weight) offered to 1.1 g shrimp over a 7-week growth trial

	Whole raw			Whole extruded		
	5%	10%	20%	5%	10%	20%
Fish meal[1]	300	300	300	300	300	300
Soybean meal[2]	245	237	221	244	235	217
Test ingredient	50	100	200	50	100	200
Whole wheat[3]	200	150	50	200	150	50
Wheat starch[3]	25	33	49	26	35	53
Nutribinder[4]	100	100	100	100	100	100
Menhaden fish oil[5]	43	43	43	43	43	43
Trace mineral premix[6]	5	5	5	5	5	5
Vitamin premix[7]	20	20	20	20	20	20
Vitamin C[8]	5	5	5	5	5	5
Calcium phosphate[9]	2	2	2	2	2	2
Soya lecithin[10]	5	5	5	5	5	5

[1] Menhaden fish meal, Special Select™, Zapata Protein USA Inc., Randeville, LA, USA.

[2] Solvent extracted, Producers Coop, Bryan, TX, USA.

[3] United States Biochemical Corporation, Cleveland, OH, USA.

[4] Industrial Grain Products Inc., Lubbock, TX, USA.

[5] Omega Protein Inc., Reedville, VA, USA.

[6] g 100 g^{-1} Premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulphate 4.0, magnesium sulphate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 53.428.

[7] g kg^{-1} Premix: thiamin HCl 0.5, riboflavin 3.0, pyrodoxine HCl 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B_{12} 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20 000 IU g^{-1}) 5.0, vitamin D_3 (400 000 IU g^{-1}) 0.002, DL-α-tocopheryl acetate (250 IU g^{-1}) 8.0, α-cellulose 865.266.

[8] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

[9] Cefkaphos® (primarily monobasic calcium phosphate) BASF Corporation, Mount Olive, NJ, USA.

[10] Aqualipid 95, Central Soya Chemurgy Division, Fort Wayne, IN, USA.

temperature, dissolved oxygen and salinity were maintained at 27.7 ± 0.8 °C, 6.0 ± 0.8 mg L^{-1} and 33.2 ± 3.2 g L^{-1} for the first growth trial and 28.0 ± 0.8 °C, 5.8 ± 0.6 mg L^{-1} and 31.7 ± 2.8 g L^{-1} for the second growth trial, respectively. Total ammonia-nitrogen, nitrite-nitrogen and pH were measured twice weekly following the methods of Spotte (1979) and were maintained at 0.007 ± 0.011 mg L^{-1}, 0.018 ± 0.006 mg L^{-1} and 7.8 ± 0.11 in the first growth trial and 0.008 ± 0.010 mg L^{-1}, 0.014 ± 0.006 mg L^{-1} and 7.8 ± 0.09 in the second growth trial, respectively.

At the conclusion of the growth trials, final mean weights, percentage weight gain and survival for each dietary treatment were determined. Based on feed inputs feed efficiency (FE = weight gain × 100/feed offered) values were estimated. Data were analysed using an ANOVA to determine if significant ($P < 0.05$) differences existed among treatment means. The Student–Newman–Keuls multiple comparison test was used to determine where significant differences existed between treatment means (Steel & Torrie 1980). All statistical analyses were conducted using the SAS Systems for Windows V7 (SAS Institute Inc., Cary, NC, USA).

Results

Apparent digestibility values for protein (APD) and energy (AED) are presented in Table 4. Digestibility values for the seven test ingredients ranged from 90.4 to 117% for protein and 26.1 to 94.4% for energy. Apparent energy digestibility values for the various pea meals expressed as percentage ± SD were: WRA, 72.3 ± 8.1; WEX, 86.0 ± 8.9; DRA, 88.4 ± 4.4; DEX, 94.4 ± 10.0; WMI, 94.1 ± 10.2. Apparent protein digestibility values for the various pea meals were: WRA, 90.4 ± 12.2; WEX, 111.3 ± 13.1; DRA, 93.5 ± 7.2; DEX, 96.7 ± 17.9; WMI, 117 ± 8.0. The data pertaining to pea digestibility was subjected to one-way ANOVA and contrasts were utilized to determine the influence of processing on the digestibility coefficients. Based on contrasts (Table 5), both extruding and micronizing the whole pea meals resulted in significant improvements in APD and AED values over those obtained with the raw meals. No significant differences in APD or AED values were found when comparing WEX to DEX or WEX to WMI. Indicating that if the process includes treatment with high levels of heat and moisture (extrusion processing or micronization) that there is no clear need to de-hull the peas and that the micronization process was not significantly better than extrusion processing.

To evaluate the biological response of the shrimp to the various meals two 7-week growth trials were conducted with juvenile shrimp. The first trial evaluated the replacement of whole wheat with the seven test ingredients at 250 g kg^{-1} of the diet (Table 2). At the conclusion of this growth trial, survival ranged from 96.9 to 100%, weight gain from 718.3 to 862.8% and FE values from 39.6 to 46.0% (Table 6). The second growth trial evaluated the inclusion of 50, 100 and 200 g kg^{-1} of either WRA pea meal or WEX pea meal replacing whole wheat (Table 3). At the conclusion of the second growth trial, survival ranged from 77.8 to 94.4%, weight gain from 406.4 to 502.3% and FE values from 28.8 and 36.1% (Table 7). For both growth trials, there were no significant differences in growth, survival or FE values as a result of the various dietary treatments.

Discussion

Terrestrial plants are often inexpensive sources of protein and energy for commercial feed formulations. Cereal grains such as wheat are good energy sources as they are high in carbohydrates and low in protein. Oil seeds such as soybean meal, are good protein sources as they are high in protein and contain lower levels of carbohydrates. Meals made from feed peas are intermediate in terms of both

Table 4 Apparent protein digestibility (APD) and apparent energy digestibility (AED) values for the basal diet (BD), test diets (70:30, basal diet:test ingredient) and test ingredients. Values represent the mean ± SD based on four replicates

	Diet		Ingredient	
	APD	AED	APD	AED
Basal diet	74.1 ± 1.9	72.7 ± 2.7		
BD + whole raw	77.4 ± 2.4	72.6 ± 2.4	90.4 ± 12.2	72.3 ± 8.1
BD + whole extruded	81.6 ± 2.6	76.7 ± 2.7	111.3 ± 13.1	86.0 ± 8.9
BD + de-hulled raw	78.1 ± 1.5	77.3 ± 1.3	93.5 ± 7.2	88.4 ± 4.4
BD + de-hulled extruded	79.0 ± 3.9	79.1 ± 2.9	96.7 ± 17.9	94.4 ± 10.0
BD + whole micronized	83.3 ± 1.7	78.9 ± 2.9	117.0 ± 8.0	94.1 ± 10.2
BD + extruded canola	81.3 ± 2.1	72.9 ± 2.8	96.7 ± 6.5	73.4 ± 9.2
BD + wheat middlings	77.9 ± 4.2	67.8 ± 5.7	96.0 ± 24.3	26.1 ± 19.5

Table 5 Results (Pr > F) from the analyses of the five pea meals using ANOVA analyses and contrast statements to determine the effects of processing on apparent protein digestibility (APD) and apparent energy digestibility (AED) values

Contrast	APD	AED
Whole pea meal: raw vs. extruded	0.0291	0.0395
De-hulled pea meal: raw vs. extruded	0.7144	0.3371
Whole pea meal: raw vs. micronized	0.0078	0.0026
Extruded whole vs. extruded de-hulled	0.1121	0.1864
Extruded whole vs. micronized	0.4371	0.1978

Table 6 Response of *Litopenaeus vannamei* juveniles (mean initial weight of 0.66 g) offered the basal diet and test diets containing whole raw pea meal (WRA), whole extruded pea meal (WEX), whole micronized pea meal (WMI), de-hulled raw pea meal (DRA), de-hulled extruded pea meal (DEX), extruded canola meal (EC) and wheat middlings (WM) over a 7-week growth trial[1]

Diet	Mean weight (g)	Weight gain (%)	Survival (%)	FE[2] (%)
Basal	5.6	718.3	96.9	40.8
WRA	5.5	761.5	96.9	40.4
WEX	6.0	815.4	93.8	44.0
WMI	6.0	789.3	100	43.4
DRA	5.4	718.3	100	39.6
DEX	5.9	808.1	96.9	43.0
EC	6.0	790.0	93.8	43.6
WM	6.3	862.8	93.8	46.0
PSE[3]	0.23	41.89	8.54	1.67

[1] Means of four replicates. No significant differences ($P < 0.05$) were observed between dietary treatments.
[2] Feed efficiency (FE) = (weight gain × 100/feed fed).
[3] Pooled SE.

Table 7 Response of *Litopenaeus vannamei* juveniles (mean initial weight of 1.1 g) offered the test diets containing raw pea meal or extruded pea meal replacing whole wheat over a 7-week growth trial[1]

	Mean weight (g)	Weight gain (%)	Survival (%)	FE[2] (%)
Raw pea meal				
5%	5.2	405.2	94.4	29.1
10%	6.4	502.3	88.9	36.1
20%	5.3	406.4	86.1	29.1
PSE[3]	0.28	33.14	7.35	2.19
Extruded pea meal				
5%	5.3	394.7	86.1	28.8
10%	5.9	463.0	77.8	33.2
20%	5.6	416.2	91.7	30.6
PSE	0.24	22.54	6.00	1.43

[1] Means of three replicates. No significant differences ($P < 0.05$) were observed due to inclusion level for either of the test ingredients.
[2] Feed efficiency (FE) = (weight gain × 100/feed fed).
[3] Pooled SE.

protein and carbohydrate content and consequently have the potential to provide both a good energy source and a moderate amount of protein to the ration. However, inclusion levels in feed formulations must be tempered by cost, processing considerations, nutrient availability as well as palatability of the meal to the target species. Consequently, information pertaining to nutrient availability and the biological response of the target species is critical to the successful utilization of a given ingredient.

Extrusion processing, micronization and de-hulling are common processing techniques that are known to influence nutrient availability in a variety of species; however, there is limited data with respect to aquatic species and particularly shrimp. Under the reported conditions, extrusion processing and micronization of the whole pea meals significantly improved digestibility values for both protein and energy (Table 5). This response is similar to the response reported by Cruz-Suarez *et al.* (2001) using the same pea meals and juvenile *L. stylirstris*. Extrusion processing has also been demonstrated to influence energy availability in several cereal grains offered to *L. vannamei* (Davis & Arnold 1995). Although, there is little data with aquatic species regarding micronization, Gomes *et al.* (1995) reported that it increased the energy and protein digestibility values for full fat soybean meal offered to rainbow trout. These results as well as those conducted with other species, confirm that extrusion and micronization can enhance nutrient availability presumably by elimination of anti-nutritional factors and enhancing starch gelatinization.

Another method to potentially increase the nutritive value of legumes is to de-dull them to reduce the fibre content of the meal. Eusebio (1991) concluded that de-hulling cowpea and rice bean enhanced the nutritive value of the meals to *P. monodon*. As pea hulls are high in poorly digestible fibre, de-hulling may be beneficial. Based on the results of this experiment, when pea meals are extruded, de-hulling did not significantly improve the digestibility of the pea meal. Similarly, Cruz-Suarez *et al.* (2001) reported APD values of 87.2 and 82.2% for WEX and DEX pea meals, respectively. In both studies, digestibility values were very similar and probably indicate that there is no need to de-hull peas if they are to be extruded during feed processing.

In commercial rations, pea meal and canola meal are often included together as they complement each other's amino acid profiles. Gomes *et al.* (1993) also noted that co-inclusion may improve extrusion conditions resulting in further enhancements in nutrient availability in aquatic species such as trout. Consequently, canola meal was also included in this trial as a reference protein source. Apparent protein digestibility values of the canola meal were similar to those of the pea meal (Table 4). However, AED values for EC (73.4%) were considerably lower than the 86–94.4% observed for the extruded pea meals. These results and those of Cruz-Suarez *et al.* (2001) indicate that protein digestibility is similar for these meals but pea meal is a better energy source.

As pea meal could also serve as an energy source in commercial rations, replacement of cereal grains with pea meals should be considered. Although ADE values will vary with processing, ADE values for whole wheat (77.1–87.0%), corn flour (65.9–82.8%), rice flour (74.1–94.4%) and milo (66.4–83.4%) have been reported for *L. vannamei* (Davis & Arnold 1995) and are comparable with those of the processed pea meals. Wheat middlings are another potential energy source that consist of wheat bran, shorts, germ and flour recovered from milling wheat grain. However, Brunson *et al.* (1997) using *L. setiferus* as the test species reported an ADE value of 51.9% for WM. Results from this experiment also confirm that WM are a poor energy source and that only about 26% of the energy is available to the shrimp.

Based on the results of the digestibility trials, pea meal could serve as a high quality protein and energy source that is equivalent or superior in terms of apparent digestibility values to other commonly used feed ingredients. However, plant protein sources potentially contain anti-nutritional factors such as oligosaccharides, phytate and tannins. Although peas are considered to contain relatively low levels of these factors (Peterson *et al.* 1994), performance of the animals could be impaired by such factors. Hence, the second component of this research evaluated the biological response of juvenile shrimp to test diets containing the various ingredients. Two independent experiments were conducted with juvenile *L. vannamei* using experimental protocols that have been employed in a wide variety of nutritional studies. Because pea meal would enter into commercial feed formulations as a source of energy and protein, whole wheat and a small portion of the soybean meal was replaced with the pea meal in each test diet. In the first growth trial, each test ingredient was included at 250 g kg^{-1} and in the second growth trial raw pea meal or extruded pea meal was tested at 50, 100 and 200 g kg^{-1} diet. The inclusion of pea meal had no significant effect on survival, growth or FE values. Cruz-Suarez *et al.* (2001) also evaluated various pea meal using *L. stylirostris* as the model species and found only minor differences in growth, which were presumably the result of an increased consumption of shrimp offered feeds containing the micronized pea meals.

Conclusion

Based on the results of this study as well as studies conducted with *L. stylirostris* (Cruz-Suarez *et al.* 2001), pea meal has potential as an alternative feed ingredient in shrimp feeds. When properly processed (extruded or micronized) protein and energy is highly digestible and there appear to be no adverse effects on shrimp growth, survival or FE values at the inclusion levels tested. Based on the positive results of these studies the industry is encouraged to evaluate the use of pea meals in trial formulations and long-term growth trials.

Acknowledgements

The authors would like to extend their thanks to those who have taken the time to critically review this manuscript as well as students and staff who helped support this research. This research was supported in part by the Sid W. Richardson Foundation, Saskatchewan Pulse Growers and the Canadian International Grains Institute. We thank Paul Adelizi with the Saskatchewan Wheat Pool for providing the peas, Francis Gaudet of Belle Pulses Ltd. for de-hulling the peas, Connie Phillips and Kevin Swallow of the Alberta Food Processing Center for extruding the meals and Mark Pickard and Gordon Sellar of Infraready Products for micronizing the peas. Mention of a trademark or proprietary product does not constitute an endorsement of the product by The University of Texas at Austin or Auburn University and does not imply its approval to the exclusion of other products that may also be suitable.

References

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J. & Warner-Smith, R. (2000) Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture*, **186,** 293–310.

Brunson, J.F., Romaire, R.P. & Reigh, R.C. (1997) Apparent digestibility of selected ingredients in diets for white shrimp *Penaeus setiferus* L. *Aquacult. Nutr.*, **3,** 9–16.

Burel, C., Boujard, T., Tulli, F. & Kaushik, S.J. (2000) Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). *Aquaculture*, **188,** 285–298.

Carter, C.G. & Hauler, R.C. (2000) Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture*, **185,** 299–311.

Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., McCallum, I.M. & Hickling, D. (2001) Assessment of differently processed feed pea (*Pisum sativum*) meals and canola meal (*Brassica* sp.) in diets for blue shrimp (*Litopenaeus stylirostris*). *Aquaculture*, **196,** 87–104.

Davis, D.A. & Arnold, C.R. (1995) Effects of two extrusion processing conditions on the digestibility of four cereal grains for *Penaeus vannamei*. *Aquaculture*, **133,** 287–294.

Eusebio, P.S. (1991) Effect of de-hulling on the nutritive value of some leguminous seeds as protein sources for tiger prawn, *Penaeus monodon*, juveniles. *Aquaculture*, **99,** 297–308.

Forester, I. (1999) A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquacult. Nutr.*, **5,** 143–145.

Gomes, E.F., Corroze, G. & Kaushik, S. (1993) Effects of dietary incorporation of a co-extruded plant protein (rapeseed and peas) on growth, nutrient utilization and muscle fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **113,** 339–353.

Gomes, E.F., Rema, P. & Kaushik, S.J. (1995) Replacement of fish meal by plant proteins in the diets of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **130,** 177–186.

Gouvei, A. & Davies, S.J. (1998) Preliminary nutritional evaluation of pea seed meal (*Pisum sativum*) for juvenile European sea bass (*Dicentrarchus labrax*). *Aquaculture*, **166,** 311–320.

Gouvei, A. & Davies, S.J. (2000) Inclusion of an extruded de-hulled pea seed meal in diets for juvenile European sea bass (*Dicentrarchus labrax*). *Aquaculture*, **182,** 183–193.

Ma, T.S. & Zuazago, G. (1942) Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. *Ind. Eng. Chem.*, **14,** 280–282.

McCallum, I.W., Newell, L.E., Cruz-Suarez, D. *et al.* (2000) Uso de arvejón (feed pea, chicharo) *Pisum sativum* en alimentos para camarones (*Litopenaeus stylirostris* y *L. vannamei*), tilapia (*Oreochromis niloticus*) y trucha (*Orcorhynchus mykiss*). *Advances en Nutrición Acuicola V, Memorias del Quinto Symposium, Internatcional de Nutrición*. Mérida, Yucatán, Mexico.

McGinns, A.J. & Kasting, R. (1964) Colorimetric analysis of chromic oxide used to study food utilization by phytophagous insects. *Food Chem.*, **12,** 259–262.

Peterson, D.S., Sipsas, S. & Mackintosh, J.B. (1994) *The Chemical Composition and Nutritive Values of Australian Pulses*, p. 64. Acuicola, 19–22 Noviembre, 2000, Mérida, Yucatán, México. Grains Research and Development Corporation, Kingston, Australia.

Smith, D.M., Tabrett, S.J. & Sarac, H.Z. (1999) *Fish Meal Replacement in the Diet of the Prawn Penaeus monodon*. Book of abstracts of the World Aquaculture Society Annual Meeting '99. 26 April–2 May, 1999, Sydney, Australia. WAS, Baton Rouge, LA, USA.

Spotte, S. (1979) *Fish and invertebrate culture: water management in closed systems*, 2nd ed. Wiley, New York, USA.

Steel, R.G.D. & Torrie, J.H. (1980) Principles and procedures of statistics. *A Biometrics Approach*. McGraw-Hill, New York, NY, USA.

Sudaryono, A.E., Tsvetnenko, J., Hutabarat, Supriharyono & Evans, L.H. (1999) Lupin ingredients in shrimp (*Penaeus monodon*) diets: influence of lupin species and types of meals. *Aquaculture*, **171,** 121–133.