Use of agriculture byproducts in diets for pacific white shrimp *Litopenaeus vannamei*

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Abstract

Eight Hawaii agricultural byproducts were tested for suitability in diets for Pacific white shrimp *Litopenaeus vannamei*. Two growth trials were conducted in a zero-water exchange system and two digestibility trials were conducted in a clear water system. A control growth diet in which fish meal (menhaden) was the single largest protein source (43.5% of total protein) was used in all trials. Ingredients that were rich in protein were incorporated in diets in replacement of fishmeal, while those low in protein replaced wheat. Meat and bone meal (MBM) at four levels of inclusion (7.4, 14.9, 22.3 and 29.7%), macadamia nut grit (MNG) at two levels of inclusion (15 and 30%) and spent fruit fly medium (FFW, 9.0%) were examined in Trial 1, and whole papaya (Whole Pap,10%), papaya flesh (Pap Flesh, 5%), papaya seed (Pap Seed, 10%), wheat mill run (WMR, 44.2%) and okara (soy pulp) (10 and 20%) were examined in Trial 2. All diets were formulated to contain 35% crude protein. Menhaden oil was adjusted to maintain the level of lipid in the diets at 9.5%. In Trial 2, a commercial diet (Rangen 35/2.5) was fed as an additional treatment. The diets were fed using automatic belt feeders on a 24-hour cycle to juvenile shrimp (Trial 1, 1.0 g; Trial 2, 1.5 g initial weight) for eight weeks in zero-water exchange outdoor seawater systems (1300 L volume). The final weight, growth and survival data from each trial were submitted to ANOVA procedures and the MBM data in Trial 1 to quadratic regression procedures, with a 5% error rate for significance.

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In the Trial 1, the shrimp fed the MBM at 50% replacement of fish meal grew significantly slower and achieved a lower final weight than those fed the control diet. There were no other differences in growth. In Trial 2, the shrimp fed the commercial diet grew significantly slower and achieved a lower final weight than those fed the control diet, but there were no other differences among the other treatments. The feed conversion ratio data in Trial 2 did not reveal any differences among treatments, but were more variable, and therefore less powerful as an indicator of nutritional quality, than were the growth data.

In the digestibility trials, for apparent digestibility coefficient for dry matter (ADC-DM), Pap Flesh and Whole Pap had the highest levels of 68.5 and 71.4% respectively. For the crude protein apparent digestibility coefficients (ADC-CP) the FFW and MNG were the highest at 62.8 to 73.0%. Crude lipid apparent digestibility coefficients (ADC-CL) were highest for the MBM and MNG byproducts, 87.4 and 87.9% respectively. The Energy apparent digestibility coefficient (ADC-E) varied the greatest; FFW-first trial and Whole Pap levels were 61.7 and 68% respectively with Okara at the lowest level of 18.2%.

Based on these findings, it was concluded found that these agricultural byproducts can be included in diets for shrimp with little or no effect on growth under conditions of zero-water exchange.

Introduction

With the worldwide increase in aquaculture production over the past few decades, there has been a concomitant increase in demand for ingredients for aquaculture feed production, resulting in upward pressure on the price of key ingredients, notably of fishmeal and fish oil (Hardy 2006; Tacon et al. 2006). Since most fisheries are at or near sustainable harvest limits, there is little scope for increased production of marine protein or oil sources. Most of the increased demand for fishmeal generated by the growth in aquaculture has been met by diversion of fishmeal from poultry and swine feeds, but in future, this will become less of an option (Tacon and Forster 2000; Hardy 2006; Tacon et al. 2006). To support the projected increase in production of aquatic feeds, alternative ingredients need to be identified and developed. For the past several years, substantial research has been directed to this task, with considerable success (Lim and Dominy 1992; Hardy 1999; Storebakken, Refstie & Ruyter 2000; Swick 2002; Davis and Arnold 2000; Forster et al. 2002). While most of this work has concentrated on utilization of inexpensive commodities, such as soybean meal, especially in feeds for fish, comparatively little work has been done on other products for use in shrimp feeds.

Agricultural byproducts are the materials left over from production of animals and plants for food, including, shells and rejects from fruit and nut processing and offal from slaughter houses. In many cases, these byproducts are not utilized and their disposal presents a cost to the processor. Some agriculture byproducts are produced at large scale and are essentially homogenous, but others are generated in relatively small amounts and can be of variable quality. The larger commodities are generally produced at relatively few central locations for wide-scale distribution. The smaller commodities, however, may be produced only regionally and distributed locally. Byproducts produced by island communities, such as those of the Pacific, which are geographically isolated from large mainland markets, are primarily in the latter category. The cost of importation of feeds and ingredients raises the cost of aquaculture production in these locations. Identifying and evaluating alternative ingredients made from byproducts of agriculture production in these island communities will have an even greater impact on the profitability of aquaculture.

Most of the research on alternative ingredients for use in shrimp feeds has been conducted in clear water, under controlled conditions (Davis and Arnold 2000; Forster et al. 2003). In contrast, shrimp are farmed in ponds under conditions of limited water flow, where there is opportunity for endogenous production of material that may provide alternate sources of nutrition to the shrimp (Tacon et al. 2002; Moss et al. 2006; Amaya et al. 2007). Conducting nutrition research under conditions similar to actual commercial conditions provides a more direct indicator of the suitability of various byproducts for inclusion in shrimp feeds than does conducting trials under clear water controlled conditions. To ascertain the utility of various representative agriculture byproducts locally produced in Hawaii, two growth trials were conducted with Pacific white shrimp Litopenaeus vannamei under pond-like conditions. The ingredients tested in the first trial included meat and bone meal (MBM) at four levels, a macadamia nut byproduct (MNG) at two levels, and spent fruit fly culture media (FFW). In the second trial, locally grown wheat mill run, three papaya byproducts and okara (a soy byproduct), were tested. The byproducts with higher protein levels (MBM, MNG, papaya seed and okara) were included in partial replacement of fishmeal, whereas the low protein containing items (FFW, wheat mill run, papaya byproducts) were included in replacement of whole wheat flour.
In addition the apparent digestibility coefficients of the ingredients were determined.

Materials and Methods

Growth Trials

Agriculture byproducts produced in the State of Hawaii were obtained and analyzed for chemical composition (Table 1) and amino acid profile (Table 2). Two series of diets (35% crude protein (N * 6.25%); 9% crude lipid) were prepared using these ingredients. A control diet was used in both trials. In Trial 1, MBM replaced fish meal at 25, 50, 75, and 100%, on an equal crude protein basis, and MNG replaced 9 and 18% of fishmeal (15 and

30% dietary inclusion, respectively) and FFM was included at 9% dietary inclusion in replacement of wheat flour (Table 3). In Trial 2, wheat mill run completely replaced wheat flour (44.82% inclusion), papaya flesh and whole papaya freeze-dried meals were included at 5% and 10%, respectively, in replacement of wheat flour, papaya seed meal was included at 10% in partial replacement of fishmeal and wheat flour, and dried okara (soybean pulp) was included at 10% and 20% inclusion in partial replacement of fish meal and wheat flour (Table 4). A commercial feed (Rangen 35/2.5) was included in Trial 2.

Table 1. Composition of agricultural byproducts evaluated as ingredients for shrimp and menhaden meal used in growth trials. All values are on an air dry basis

Ingredient	Moisture %	Crude Protein %	Ether Extract %	Ash %
Menhaden meal		65.8	10.1	
Meat and Bone meal	2.1	52.8	20.2	15.6
Fruit Fly Waste	2.2	19.4	3.3	6.5
Macadamia nut grit	2.5	21.7	7.9	2.6
Wheat mill run	13.8	15.9	3.8	4.1
Whole papaya	23.6	8.2	3.9	4.4
Papaya flesh	22.5	6.6	0.9	4.3
Papaya seed	9.1	27.0	21.7	6.8
Okara	6.2	27.7	6.6	3.2

Table 2. Amino acid profile of agricultural byproducts evaluated as ingredients for shrimp diets and menhaden meal used in growth trials. All values are on an air dry basis. Cysteine and tryptophan were not analyzed

Amino Acid	Menhaden meal g /100 g	Meat and Bone Meal g /100 g	Fruit Fly Waste g /100 g	Macadamia Nut Grit g /100 g	Wheat Millrun g /100 g	Whole Papaya g /100 g	Papaya Flesh g /100 g	Papaya Seed g /100 g	Okara g /100 g
DAA									
Ala	4.69	1.57	1.46	1.05	1.21	0.49	0.22	0.50	0.99
Asx[1]	4.60	1.87	1.69	2.59	0.12	0.26	0.67	1.13	0.68
Glx [2]	7.16	4.48	3.41	3.92	1.76	0.51	0.30	1.90	1.93
Gly	5.38	5.54	0.98	0.95	1.27	0.52	0.31	0.74	1.73
Pro	6.75	8.16	0.82	1.02	2.11	1.04	0.79	4.93	3.52
Ser	1.53	1.75	0.92	0.93	0.76	0.30	0.16	1.13	1.46
Tyr	1.54	1.82	0.52	0.80	0.77	0.91	0.14	4.61	1.07
IDAA									
Arg	3.72	3.98	0.85	2.79	1.34	0.59	0.23	2.21	2.19
His	1.54	1.50	0.41	0.46	0.52	0.15	0.09	0.38	0.72
Ile	2.27	2.91	0.64	0.59	0.87	0.41	0.16	0.69	1.31
Leu	3.37	3.45	1.22	1.13	1.26	0.49	0.20	0.65	1.34
Lys	4.19	3.01	0.99	0.78	0.42	0.50	0.16	2.20	0.98
Met	1.48	0.95	0.72	0.21	0.30	0.01	0.03	0.15	0.28
Phe	2.53	2.10	0.72	0.61	0.71	0.30	0.14	0.69	1.75
Thr	2.41	1.08	0.52	0.78	0.81	0.41	0.15	1.45	1.08
Val	3.08	3.06	0.88	0.80	1.34	0.50	0.21	1.00	1.51
Sum of AA	56.89	47.30	16.75	19.41	15.71	7.40	3.97	24.44	22.54

[1] The amino acids asparagine and aspartic acid are combined by the method of hydrolysis and their sum is reported at Asx.
[2] The amino acids glutamine and glutamic acid are combined by the method of hydrolysis and their sum is reported at Glx.

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Table 3. Formulation of diets used in Growth Trial 1. Except where noted, all values are on an as fed basis

Ingredient	Control	MBM25	MBM50	MBM75	MBM100	FFW	MNG 15	MNG 30
	%	%	%	%	%	%	%	%
Fish Meal[3]	23.20	17.40	11.60	5.80		23.20	21.00	19.10
M&B Meal[4]		7.40	14.90	22.30	29.70			
Fruit Fly Waste (hammermilled)						9.00		
Macadamia Nut Grit							15.00	30.00
Wheat, whole (15.71/1.92)	44.82	44.12	43.32	42.62	41.92	37.22	32.72	20.42
Vital wheat gluten (80.35/0.13)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Brewers yeast (43.96/0.16)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Squid Meal (70.1/4.22)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Soybean meal[5]	12.30	12.30	12.30	12.30	12.30	11.00	12.30	12.30
Soy lecithin - CSM	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.90
Menhaden oil	4.50	3.60	2.70	1.80	0.90	4.40	3.80	3.10
Potassium phos dibasic	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Sodium phos dibasic	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Magnesium phos dibasic	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Proximate composition								
Dry matter (%)	95.7	95.3	93.7	95.4	95.2	96.6	95.3	96.9
Crude protein (% dry matter)	36.1	36.8	35.0	35.2	35.6	35.1	35.4	35.7
Crude lipid (% dry matter)	9.7	10.1	10.1	10.3	9.3	9.9	9.8	10.0
Ash (% dry matter)	9.1	8.6	10.2	8.2	8.8	8.2	7.8	7.8

[3] Menhaden meal (Omega Protein, Special Select)
[4] Island Commodities
[5] Dehulled, solvent extracted, toasted.

Table 4. Formulation of diets used in Growth Trial 2. Except where noted, all values are on an as fed basis

Ingredient	Control %	Wheat millrun %	Whole Papaya %	Papaya flesh %	Papaya seed %	Okara10 %	Okara20 %
Fish Meal[6]	23.20	23.20	23.20	23.20	20.50	21.00	18.30
Wheat mill run	0.00	44.82	0.00	0.00	0.00	0.00	0.00
Whole papaya	0.00	0.00	10.00	0.00	0.00	0.00	0.00
Papaya flesh	0.00	0.00	0.00	5.00	0.00	0.00	0.00
Papaya seed	0.00	0.00	0.00	0.00	10.00	0.00	0.00
Okara	0.00	0.00	0.00	0.00	0.00	10.00	20.00
Wheat, whole (15.71/1.92)	44.82	0.00	34.82	39.82	39.42	37.02	30.22
Vital wheat gluten (80.35/0.13)	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Brewers yeast (43.96/0.16)	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Squid Meal (70.1/4.22)	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Soybean meal[7]	12.30	12.30	12.30	12.30	12.30	12.30	12.30
Soy lecithin - CSM	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Menhaden Oil	4.50	4.50	4.50	4.50	2.60	4.50	4.00
Potassium phos dibasic	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Sodium phos dibasic	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Magnesium phos dibasic	0.56	0.56	0.56	0.56	0.56	0.56	0.56

[6] Menhaden meal (Omega Protein, Special Select)
[7] Dehulled, solvent extracted, toasted.

Experimental diets were manufactured at the Oceanic Institute (OI), located on the island of Oahu, Hawaii, USA according to standard methodology, as follows: All the major dry feed ingredients were mixed for 15 min in a Hobart food mixer (Model D-300, Hobart Manufacturing Corporation, Troy, Ohio, USA). A warm (approximately 60 °C) aqueous solution of sodium phosphate, potassium phosphate, choline chloride, and trace mineral premix, was then added to the dry ingredient mix, to bring the moisture content of the resulting mash to approximately 34-35%. The mash was then blended for a further 15 min. half the supplemental oil and lecithin were blended in a Kitchen Aid mixer (Model K5SS, Kitchen Aid, St. Joseph, Michigan, USA), added to the mash and mixed for a further 15 min. The resulting mash was then passed through a California Pellet Mill (Model CL5, San Francisco, California, USA) fitted with a 2.4 mm diameter die. No steam was used and the pellet temperature at the die was below 70 °C. The resulting moist pellets were then dried overnight in a drying cabinet using an air blower at approx. 38 °C until the moisture level was below 10%. The vitamin premix and vitamin C source were then emulsified with the remaining oil and lecithin in a Kitchen Aid mixer and this mixture was added to the dry cooled pellets by top coating using a Hobart D300 food mixer with a whisk beater. The finished pellets were then stored in plastic bins at 19 – 20 °C until used. The amino acid profile of the diets was determined (Tables 5 and 6).

Table 5. Trial 1. Amino acid profile of growth trial diets used to evaluate agricultural byproducts as ingredients for shrimp diets used in growth trials. All values are on an air dry basis. Tryptophan was excluded from the analysis

Amino acid	Control		MBM 25		MBM 50		MBM 75		MBM 100		FFW		MNG15		MNG30	
	%	% AA	%	% AA	%	% AA	%	% AA	%	% AA	%	% AA	%	% AA	%	% AA
Ala	2.22	6.76	2.26	6.90	2.33	7.03	2.43	7.34	2.43	7.34	2.11	7.09	2.42	7.41	2.24	6.84
Asx[8]	0.82	2.49	0.88	2.67	0.77	2.31	0.85	2.57	0.85	2.57	0.70	2.36	0.41	1.25	0.85	2.60
Cys	0.09	0.29	0.14	0.42	0.03	0.10	0.03	0.10	0.03	0.10	0.08	0.28	0.17	0.53	0.09	0.27
Glx[9]	4.24	12.93	4.68	14.26	4.56	13.73	4.74	14.33	4.74	14.33	3.83	12.88	3.35	10.23	4.50	13.73
Gly	2.37	7.23	2.60	7.93	2.71	8.16	3.05	9.21	3.05	9.21	2.26	7.59	2.43	7.42	2.49	7.59
Pro	2.99	9.13	3.28	9.99	3.37	10.15	3.59	10.84	3.59	10.84	2.88	9.69	3.41	10.43	3.01	9.19
Ser	1.45	4.42	1.54	4.70	1.54	4.63	1.59	4.79	1.59	4.79	1.42	4.79	1.48	4.51	1.63	4.99
Tyr	1.59	4.85	1.45	4.41	1.59	4.79	1.49	4.50	1.49	4.50	1.42	4.77	1.88	5.75	1.64	5.02
Taurine	*0.35*	*1.07*	*0.42*	*1.28*	*0.43*	*1.31*	*0.49*	*1.49*	*0.49*	*1.49*	*0.35*	*1.17*	*0.38*	*1.18*	*0.32*	*0.99*
Arg	2.30	7.02	2.46	7.51	2.35	7.08	2.50	7.56	2.54	7.58	2.15	7.25	2.73	8.34	2.95	9.01
His	0.96	2.92	0.98	2.99	1.02	3.06	0.97	2.92	1.00	2.98	0.94	3.16	1.08	3.32	0.95	2.89
Ile	2.32	7.07	2.09	6.38	2.08	6.27	1.95	5.91	2.13	6.36	1.97	6.62	2.32	7.09	2.10	6.42
Leu	2.82	8.60	2.52	7.67	2.87	8.66	2.52	7.63	2.62	7.82	2.39	8.05	2.81	8.59	2.55	7.79
Lys	1.72	5.24	1.31	4.00	1.52	4.59	1.22	3.68	1.14	3.41	1.28	4.32	1.27	3.87	1.31	4.00
Met	0.96	2.93	1.03	3.13	0.85	2.55	0.74	2.24	0.80	2.38	1.00	3.37	1.14	3.48	0.81	2.47
Phe	1.67	5.08	1.35	4.13	1.59	4.78	1.40	4.22	1.37	4.09	1.31	4.39	1.42	4.34	1.38	4.22
Thr	1.72	5.25	1.86	5.67	1.67	5.05	1.73	5.24	1.87	5.60	1.72	5.77	1.76	5.38	1.83	5.57
Val	2.55	7.77	2.37	7.21	2.34	7.04	2.29	6.91	2.49	7.44	2.26	7.62	2.64	8.05	2.42	7.38

[8] The amino acids asparagine and aspartic acid are combined by the method of hydrolysis and their sum is reported at Asx.
[9] The amino acids glutamine and glutamic acid are combined by the method of hydrolysis and their sum is reported at Glx.

Table 6. Growth Trial 1. Final weight, growth and survival of shrimp fed a fishmeal based control diet or diets containing agricultural byproducts. Means within a column with a common superscript are not significantly different (SNK test; P<0.05; n=3). SD = treatment standard deviation; SEM = pooled standard error of means; P is the error probability

Treatment	Final Wt (g)	SD	Growth (g/wk)	SD	FCR (g/g)	SD	Survival (%)	SD
Control	8.7a	0.6	0.96a	0.07	1.59	0.08	94.3	2.1
MBM25	7.9a,b	0.9	0.86a,b	0.10	1.66	0.18	95.7	0.6
MBM50	6.6 b	0.8	0.71b	0.10	2.02	0.35	94.0	1.0
MBM75	7.5a,b	0.3	0.81a,b	0.03	1.72	0.15	94.0	3.6
MBM100	7.7a,b	0.4	0.84a,b	0.05	1.84	0.04	90.0	4.6
FFW	7.7a,b	0.8	0.84a,b	0.10	1.64	0.01	98.0	2.0
MNG15	8.0a,b	0.3	0.88a,b	0.05	1.76	0.16	89.0	5.6
MNG30	7.3a,b	0.7	0.79a,b	0.08	1.74	0.03	94.3	3.8
Research	8.2a,b	0.2	0.90a,b	0.02	1.64	0.26	93.3	4.9
Practical	7.2a,b	0.7	0.76a,b	0.08	1.89	0.14	97.7	2.5
SEM	0.352		0.043		0.100		2.00	
P	0.029		0.031		0.131		0.076	

Juvenile shrimp (initial weight 1.0 ± 0.05 g Trial 1; 1.5 ± 0.11 g Trial 2) were obtained from the OI hatchery and randomly stocked into individual conical bottom culture vessels (1,300 liters functional volume) at an initial stocking density of 100 shrimp per tank (approx. 54 shrimp m^{-2}). Each vessel was initially filled with salt water from a well and then maintained on a zero-water exchange regime (only fresh water was added to make up for evaporative loss). Water temperature and dissolved oxygen were measured in each tank twice daily. Culture water levels of ammonia (total ammonia nitrogen) and nitrite were monitored weekly (one replicate per treatment), throughout the trial.

Each diet was fed using a continuous (24 hour) feeder to three (Trial 1) or four (Trial 2) randomly selected vessels according to a feeding table that accounts for individual shrimp weight and water temperature (Tacon et al. 2002). At 2-week intervals the mean weight of a sample of at least 20 animals from each culture vessel was taken and the animals returned to their vessel. These sample weights were used to adjust daily ration size. After 8 weeks of growth, all animals were collected from each vessel, counted and weighed in groups of 10. The final weight was calculated as total weight of the animals recovered from each vessel divided by the number of animals. Growth rate was calculated as final weight minus initial weight divided by number of weeks (8). Feed conversion ratio (FCR) was calculated as feed presented to each vessel divided by the shrimp biomass accumulation (total final weight minus total initial weight). It was not possible to assess actual consumption, nor was mortality accounted for in calculating FCR. Survival was calculated as number of live shrimp recovered from each tank after 8 weeks divided by the initial number (100).

The final weight, growth rate, FCR and survival data were analyzed by one-way ANOVA procedures, according to a completely random experimental design. In addition, the data from the MBM treatments in Trial 1 were subjected to forward step-wise regression analysis (first and second order) with level of MBM inclusion as the independent variable. Survival data were transformed (arc sin square root) prior to analysis (Zar 1999). A 5% error rate was used to determine significance. Where a significant treatment effect was detected by ANOVA, comparison of treatment means was determined using Student-Neumann-Keuls test.

Digestibility Trial

Digestibility Diet Preparation

The control diet was formulated to contain 40% crude protein and 7.5% lipid (Table 9). For the test diets, 30% of the control diet was replaced (as fed basis) by the byproduct ingredient and contained 0.5% chromic oxide as an indigestible marker. Ingredients were mixed in a Hobart Mixer, pelleted on California Pellet Mill, 2.4 mm die, air dried and top coated with 50% of the oil and lecithin.

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Experimental System

The digestibility trials were carried out at OI in 550 L polyethylene tanks (95 x 97 x 61 cm) with a sloping bottom emptying into a central drain. Water and feces exited the tank via the central drain well to an external sedimentation column. A grating was placed over the drain well to separate the shrimp from exiting feed and feces particulates and molt shells. Water overflowed at the top of the external column and the settled feces were collected through a valve in the bottom the column. Each tank had continuous water flow from a sea water well. A flow rate of 3.2 L min^{-1} (approximately 8.4 tank volumes day^{-1}) was set to maximize fecal recovery in the settling column while maintaining appropriate levels of dissolved oxygen, ammonia, and nitrite in the water. Each tank had one central air-stone with air flowing at 1.8 L min^{-1}. Water quality parameters were monitored weekly using a YSI 650 MDS datalogger connected to a YSI 600QS sonde (Yellow Springs, OH, USA). Total ammonia-nitrogen and nitrite were analyzed on a HACH DR/890 Colorimeter using methods #10031 and 8507, respectively (Loveland, CO, USA).

Digestibility Experimental Protocol:

Two digestibility evaluations for the FFW, MBM, MNG ingredients were run. (FFW 1 & 2, MBM 1 & 2, and MNG 1 & 2). Kona-line post larval shrimp were obtained from the Oceanic Institute shrimp hatchery and grown to size on a commercial feed (CP 40%). Tanks were stocked with shrimp in the initial weight range of 7 to 10 g at a density of 100 shrimp m^{-2}. Shrimp were fed at 5% of biomass a commercial feed (CP 40%) using a 24 h belt feeder and feeding tray (35 x 40 x 12 cm) and held until acclimated to the tank system and significant molting ceased. Replicates of two tanks for the first trial and three tanks for the second trial were randomly assigned to each diet. After the molting cycle was completed, the shrimp were conditioned for 3 days on the treatment diet prior to the start of feces collection.

Tanks were fed 80% of the test diet overnight via a continuous belt feeder. During the day, a routine of cleaning, feeding and feces collection was carried out: at 07:55, amount of excess overnight feed was recorded; at 08:00, tanks were siphoned, the bottom drain area scrubbed, the drain line and sedimentation column flushed, brushed, and the sedimentation column rinsed with fresh water; at 09:00 and 10:00, 20% of the daily feed ration was handfed into a feeding tray; at 11:00, remaining feed and feces were siphoned out and discarded, feeding trays removed, and the tank cleaning procedure repeated; at 11:45, the drain line and sedimentation column were flushed with sea water and closed; at 12:00, collection caps were installed on the bottom valve of the sedimentation column and the valve opened. Feces samples were collected continuously, with collection caps removed and replaced at 13:00, 14:00, 15:00, and 15:30. Feces samples were poured from the collection cap into a strainer, the strainer gently dipped into a container of distilled water several times, the water wicked away through the strainer, transferred to a small board, examined for contamination by diet particles under 7x magnification, and stored refrigerated in a small container. After the last daily fecal collection, the feeding trays were placed into the tank and overnight feeding commenced. Daily fecal samples were frozen at the end of each day's collection and held until trial completion. Samples for each tank were then pooled, freeze-dried and ground prior to analysis. Digestibility trials were run from 10 – 20 days until sufficient quantity was obtained.

Chemical Analysis

Proximate analyses for the ingredients and the diets (moisture, crude protein, crude lipid, and ash) and minerals were conducted by University of Hawaii (UH), Manoa Agricultural Diagnostic Laboratory (AOAC 2000). Minerals, including chromium, were analyzed by UH by inductively coupled plasma atomic emission spectroscopy using a Model Atomscan 16 radial configuration instrument (Thermo Jarrel Ash, TJA Solutions, Franklin, MA, USA). Analyses of the feces (moisture, crude protein, and crude lipid) were conducted at OI following methods described in AOAC (2000). Energy was determined at OI on a Parr

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1261 Isoperibol Bomb Calorimeter (Parr Instruments, St. Moline, IL, 61265, USA). The apparent digestibility coefficients were calculated according to Forster et al. (1999).

Results

The ingredients examined in this study were selected for their potential suitability as ingredients for animal feeds in Pacific island regions, including quantity produced and composition. The proximate composition of the eight by-product ingredients (Table 1 & Table 8) varied widely. MBM, Okara, Pap Seed had the highest levels of crude protein, crude lipid and energy, while Whole pap and Pap Flesh had the lowest levels. The proximate levels ranged from 6.6 to 53.6% crude protein, from 1 to 22% crude lipid and from 3,542 to 5,311 cal/g for gross energy. Intermediate levels were about 20 – 27% CP, 4600 – 4700 cal/g, and 6 – 8 % CL. Several of the ingredients were less than 20% CP, 4% CL and 4000 cal/g. Moisture of the air-dried ingredients used in the diet ranged from 2% (MBM, FFW, MNG) to 24% (Whole Pap and Pap Flesh), and in general, these contained amino acids at levels considerably lower than fishmeal. The MBM sample used in this study contained the highest protein level and for this reason is the only ingredient that could replace significant levels of fishmeal. The indispensable amino acid profile of MBM sample was similar to that of the menhaden meal, except that lysine and methionine levels were about one third lower in the MBM.

Growth Trial 1

Morning water temperatures averaged 26.0 (range 24.3-27.5) °C and afternoon temperatures 27.8 (range 24.5-30.3) °C, with the higher temperatures recorded at the beginning of the trial (October) than at the end (November). These temperatures are slightly below optimum for growth (30-32 °C) for this species. Salinity, dissolved oxygen, total ammonia nitrogen, nitrate levels were within acceptable levels for this species under these experimental conditions.

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After 8 weeks of growth, the final weight and growth of shrimp fed the diet containing 50% MBM were significantly lower than those fed the control diet, but there were no other significant differences among the treatments (Table 7). FCR and survival over the 8 weeks were not significantly affected by treatment. Regression analysis indicated a significant second order effect of regression of fishmeal replacement by MBM on final weight and growth, with the shrimp fed the 50% fishmeal replacement by MBM having the poorest response. Except for the 50% MBM diet, there was no clear reduction in growth and final weight among the shrimp in any of the MBM treatments. It is unclear why the 50% MBM diet performed poorly, but proximate analysis indicated a slightly elevated ash level in this diet, relative to the other diets, indicating a potential error in diet preparation.

Growth Trial 2

Morning water temperatures averaged 27.8 (range 26.3-30.2) °C and afternoon temperatures 31.6 (range 26.7-34.5) °C, with the temperatures throughout the trial (May – July) being warmer and less variable than in Trial 1. Salinity (32.7 ± 0.9sd g/L) and DO (5.4 ± 0.8 mg/L) were within acceptable limits for this type of trial. Salinity, dissolved oxygen, total ammonia nitrogen, nitrate levels were within acceptable levels for this species under these experimental conditions.

The mean growth rate and weight after 8 weeks of the animals in this trial was approximately twice that of Trial 1 (Table 8). Previous studies (unpublished) have shown a similar effect on growth in shrimp cultured under similar temperature differences. There was a significant difference among the treatments for final weight and growth of shrimp, with lower growth rate exhibited by the shrimp fed the commercial diet relative to those in the treatments fed the diets containing wheat mill run, whole papaya, okara 20% and papaya flesh. While the feed conversion ratio (FCR) was poorest (highest) for the shrimp fed the commercial feed, there was no significant treatment effect.

Digestibility Trials

Water Quality

Water quality parameters were measured weekly through the trial and reflected conditions favorable for shrimp. Through the trial, temperature was 25.6 $\pm$ 0.1 °C, dissolved oxygen 5.9 $\pm$ 0.2 mg L^{-1}, salinity 31.2 $\pm$ 0.1 ppt and a pH of 7.1 $\pm$ 0.1. Total ammonia-Nitrogen was <0.08 mg L^{-1} (non-detectable) and nitrite < 0.01 mg L^{-1}.

Digestibility Diets

The formulations of the diets and the proximate composition are presented in Tables 9 and 10, respectively. The 30% inclusion of the ingredients was reflected in the diets proximate levels of crude protein, crude lipid, energy and ash. The MBM digestibility diet had highest levels of crude protein, crude lipid, ash, higher than the control as well as the other ingredient diets. Whole pap and Pap flesh diets were lowest in crude protein and crude lipid levels.

Apparent Digestibility Coefficients

The apparent digestibility coefficients (ADC) for dry matter (DM), crude protein (CP), crude lipid (CL) and energy (E) are presented in Table 11. Ingredient ADC's ranged for DM from 51.4 to 71.4%, CP from 10.8 to 75.1%, CL from 55.9 to 89.0% and E from 18.1 to 68.0%. The ADC-DM for the reference diet with the pollock meal was higher than those for the byproduct ingredients; among the ingredients, Whole Pap and Pap Flesh diets had the highest ADC-DM of 68.5 and 71.4% respectively; WMR, Okara, and FFW were the lowest. For CP, the ADC was highest for MNG and the FFW, above 60%. Ingredients with crude protein levels below 10% had poor ADC results. It is possible that the low ingredient CP was insufficient at the 30% inclusion level to measure CP digestibility levels.

The ADC CL for all ingredients was >55%, except Pap Flesh, with a proximate CL of <1% and gave poor CL digestibility results (NA). Ingredients with the high CL levels MBM, Pap Seed, and MNG, had high ADC-CLs of 79.6 to 89.0%. For E, the ADC results had the widest range of ADC, with Whole Pap and FFW 1 having the highest levels, above 60%. WMR and Okara had the lowest ADC-E results, below 20%. Overall, MNG, MBM and Whole Pap had the highest ADC, while Okara and WMR had the lowest.

Table 7. Growth Trial 2. Final weight, growth and survival of shrimp fed a fishmeal based control diet or diets containing agricultural byproducts. Means within a column with a common superscript are not significantly different (SNK test; P<0.05; n=4). SD = treatment standard deviation; SEM = pooled standard error of means; P is the error probability

Treatment	Final Wt (g)	sd	Growth (g/week)	sd	FCR	sd	Survival (%)	sd
Control	16.2a	0.55	1.83a	0.05	1.42	0.11	0.903	0.054
Okara 10	16.3a	0.15	1.85a	0.02	1.36	0.05	0.950	0.022
Okara 20	16.5a	0.74	1.88a	0.10	1.54	0.42	0.843	0.143
Papaya Flesh	16.5a	0.19	1.88a	0.01	1.31	0.02	0.938	0.025
Papaya Seeds	16.3a	0.62	1.84a	0.09	1.34	0.06	0.960	0.027
Rangen 35/2.5	15.0b	0.64	1.68b	0.09	1.64	0.39	0.880	0.135
Wheat Millrun	17.2a	0.41	1.96a	0.05	1.29	0.09	0.917	0.057
Whole Papaya	16.6a	0.83	1.88a	0.11	1.31	0.06	0.950	0.042
SEM	0.282		0.037		0.105		0.039	
P	0.002		0.002		0.220		0.315	

Table 8. Proximate composition of Hawaii byproduct ingredients evaluated for apparent digestibility

Proximate (%)	Pollock Meal	Fruit Fly Spent Media	Meat & Bone Meal	Macadamia Nut Grit	Okara	Whole Papaya	Papaya Flesh	Papaya Seeds	Wheat Mill Run
Dry Matter (%)	91.99	97.80	97.91	97.50	94.84	76.38	77.48	90.93	86.16
Crude Protein (%)	69.25	19.82	56.27	21.70	27.65	8.23	6.58	27.04	15.87
Crude Lipid (%)	6.40	3.34	20.95	7.90	6.64	3.88	0.91	21.65	3.82
Ash (%)	17.08	6.67	15.68	2.6	3.19	4.41	4.27	6.83	4.07
NDF (%)	54.44				38.96	11.22	9.89	54.13	36.40
ADF (%)	1.49				24.12	9.95	10.95	47.84	10.54
Lignin (%)	0.50				18.28	7.03	10.39	14.49	7.28
Cellulose (%)	0.91				5.73	3.02	0.51	3.31	3.38
Energy (cal/g)		4344	5311	4738	4790	3751	3543	4657	3876
Minerals									
P %	3.13	0.98	2.84	12.98	0.31	0.18	0.14	0.49	0.85
K %	0.47	1.67	0.67	21.76	1.23	1.94	1.95	2.16	1.1
Ca %	5.31	0.14	5.06	6.2	0.31	0.17	0.13	0.78	0.08
Mg %	0.21	0.27	0.15	8.89	0.13	0.19	0.14	0.54	0.44
Na %	1.07	0.03	0.58	1.03	0.05	0.04	0.06	0.03	0.02
B ppm	6	7	4	421	39	11	12	11	3
Cu ppm	4	10	12	341	4	18	26	18	9
Fe ppm	371	182	995	12880	101	129	63	91	116
Mn ppm	12	89	10	2602	17	13	11	47	136
Mo ppm	1	3							
Zn ppm	54	62	93	1065	19	18	13	55	52

Table 9. Formulation of diets used in diets for apparent digestibility evaluation

Ingredient	Control	FFW	MBM	MNG	Okara	Whole Pap.	Pap. Flesh	Pap. Seed	WMR
	%	%	%	%	%	%	%	%	%
Pollock meal (66.81/7.52)	34.30	24.01	24.01	24.01	24.01	24.01	24.01	24.01	24.01
Wheat, whole (15.71/1.92)	40.62	28.28	28.28	28.28	28.28	28.28	28.28	28.28	28.28
Vital wheat gluten (74.27/1.05)	6.00	4.20	4.20	4.20	4.20	4.20	4.20	4.20	4.20
Brewers yeast (43.96/0.16)	5.00	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Squid Meal (70.1/4.22)	2.50	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Soybean meal (45.49/1.90)	5.00	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Soy lecithin - CSM	2.00	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Menhaden oil	1.50	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Cholesterol	0.24	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Min Px LV99.2	0.06	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Vitamin Px LV99.1	0.40	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Choline chloride	0.12	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Stay C-35 (35% AA potency)	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Potassium phosphate, dibasic	0.56	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Sodium phosphate, dibasic	0.56	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Magnesium phosphate, dibasic	0.56	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Fruit Fly Waste		30.00							
Meat and Bone Meal			30.00						
Macadamia Nut Grit				30.00					
Okara					30.00				
Whole Papaya						30.00			
Papaya Flesh							30.00		
Papaya seed								30.00	
Wheat Mill Run									30.00

Table 10. Proximate composition of diets used in the evaluation for apparent digestibility

	Control 1	Control 2	FFW 1	FFW 2	MBM 1	MBM 2	MNG 1	MNG 2	Okara	Whole Pap	Pap Flesh	Pap Seed	WMR
Proximate (%)													
Dry Matter (%)	94.12	92.23	93.28	91.09	94.33	91.47	93.73	91.41	93.30	85.83	93.14	84.87	93.04
Crude Protein (%)	40.91	41.75	34.78	34.88	46.21	41.25	34.59	33.50	36.84	30.50	29.70	36.31	33.66
Crude Lipid (%)	6.71	7.28	6.50	6.36	9.73	10.90	6.94	7.52	5.41	5.45	4.48	12.52	7.41
Ash (%)	9.48	10.70	8.39	9.54	12.11	11.64	7.63	8.37	7.68	7.80	8.95	7.91	8.08
NDF (%)	71.02		60.77		75.60		70.14		71.06	49.31	44.94	63.47	71.37
ADF (%)	15.71		25.37		51.65		55.49		25.84	24.77	26.89	31.40	27.67
Lignin (%)	12.51		15.73		26.67		27.26		18.85	13.96	12.70	15.48	15.65
Cellulose (%)	2.74		8.90		22.17		25.92		6.42	10.06	13.31	15.19	11.27
Crude Fiber (%)		1.95		3.26		2.67		8.85					
Energy (cal/g)	4374	4376	4274	4260	4472	4481	4405	4349	4420	4229	4084	4503	4335
Minerals													
P %	1.67	1.77	1.45	1.50	1.93	1.83	1.17	1.25	1.17	1.20	1.12	1.35	1.33
K %	0.94	0.78	1.19	1.08	0.90	0.73	0.90	0.80	1.02	1.17	1.32	1.20	0.96
Ca %	1.85	2.14	1.34	1.59	2.57	2.63	1.20	1.48	1.32	1.31	1.22	1.54	1.25
Mg %	0.25	0.27	0.27	0.27	0.28	0.21	0.23	0.24	0.21	0.23	0.22	0.35	0.29
Na %	0.72	0.62	0.46	0.46	0.69	0.60	0.42	0.45	0.47	0.47	0.44	0.46	0.44
B ppm	2	3	2	4	2	4	3	7	4	3	3	3	1
Cu ppm	29	36	36	31	27	30	28	23	25	37	41	10	28
Fe ppm	274	176	204	148	452	396	246	146	199	214	206	174	185
Mn ppm	45	40	66	64	39	38	54	51	38	44	43	51	72
Mo ppm													
Zn ppm	101	128	135	113	121	112	100	84	92	121	130	136	104

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Table 11. Apparent digestibility coefficients of dry matter, crude protein, crude lipid, and energy of byproduct ingredients. Ingredient designated by “1” or “2” are from Trial 1 or Trial 2. Values are mean of two replicates for Trial 1 and means of three replicates for Trial 2

	Dry Matter (%)			Crude Protein (%)			Crude Lipid (%)			Energy (%)		
FFW 1	56.40	±	5.2	60.05	±	6.8	61.24	+	2.41	61.74	±	13.1
FFW 2	53.38	±	1.7	65.47	±	3.6	55.90	±	8.2	32.53	±	5.8
MBM 1	62.55	±	2.1	44.26	±	5.2	87.40	+	4.37	40.27	±	3.7
MBM 2	52.61	±	1.5	30.23	±	3.3	79.67	±	3.32	50.83	±	1.7
MNG 1	59.81	±	2.1	75.09	±	2.1	89.02	+	4.07	35.98	±	4.1
MNG 2	56.34	±	4.0	70.82	±	16.5	86.8	±	10.0	49.42	±	12.3
Okara	53.67	±	1.2	64.91	±	0.5	73.78	+	4.11	18.25	±	6.3
Whole Pap.	71.37	±	1.2	NA			73.83	+	9.50	67.99	±	10.6
Pap. Flesh	68.47	±	4.7	NA			NA			53.32	±	17.7
Pap. Seed	58.57	±	2.2	NA			79.82	+	0.46	47.95	±	7.4
WMR	51.40	±	0.5	39.53	±	2.2	60.98	+	0.72	18.06	±	2.2

FFW = spent fruit fly media waste; MBM = meat & bone meal; MNG = macadamia nut grit; Pap. Flesh = papaya flesh; Pap. Seed = papaya seed; Whole Pap. = whole papaya; WMR = wheat meal run

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Discussion

Continued growth of the shrimp culture industry requires development of cost-effective feeds. To develop such feeds, and to be protected from price fluctuations, formulators need to have a wide selection of ingredients of known nutritional quality to choose from.

Agricultural byproducts are available in a wide range of locations and represent a significant opportunity for nutrient supply (Tacon et al. 2006). Considerable research has been conducted on various byproducts in shrimp feeds (Lim and Dominy 1992; Swick 2002; Davis and Arnold 2000; Forster et al. 2002, 2003). Most of this work has concentrated on protein replacement, primarily on fishmeal replacement, because this is typically the largest single expense in aquatic feed production. This is not the only area of potential significance, however. Incorporation of inexpensive sources of lipids and carbohydrates also may be used to reduce the cost of feeds.

Fishmeals of the quality used in aquatic feeds generally contain protein at levels greater than 60%, and preferably higher than 65%, and commercial shrimp feeds contain at least 30% crude protein. It is to be expected then, that, when assessing the utility of an ingredient for inclusion in a feed, the higher its protein content the more effective as a fishmeal replacement it will be (assuming adequate digestibility and amino acid balance). In this trial, the crude protein level of the fishmeal was 65% and its inclusion in the control diet was 23.2%. Among the ingredients tested, the MBM sample is the most likely to function as a fishmeal replacement because of its relatively high protein level (52.8% crude protein). The lower protein content of the MBM required its inclusion at almost 30% to completely replace fishmeal. The levels of some of the indispensable amino acids were lower in the MBM than in the fishmeal. The requirement for lysine for this shrimp species is 4.49% of protein (Fox et al., 1995). The lysine levels in the diets in which MBM replaced 75% and 100% of the fishmeal contained lysine at less than the required level. There was a general trend to lower growth in the diets with fishmeal replaced by MBM (significant second order regression), and even though this was not significant, it is possible that lysine and perhaps other amino acids or fatty acids are limiting with high inclusions of MBM, unless remedial nutrients are supplemented. MBM of different origins have been shown to be able to replace up to 50% of fishmeal in diets for this species (Forster et al. 2003) under clean water conditions.

The MBM used in the growth trial contained a relatively high oil level, necessitating a reduction in the supplemental fish oil in the high MBM diets to compensate. In this way, the MBM can be seen as an alternative to both fishmeal and fish oil. The highly unsaturated fatty acids of the n-3 series (EPA and DHA) are not generally present to any extent in the MBM, however, and high dietary inclusion levels of MBM will reduce the HUFA n-3 fatty acid content, perhaps leading to a deficiency condition of HUFA.

Although fishmeal is commonly the most costly ingredient in shrimp feeds, and therefore receives the most attention in searching for alternative sources, identifying alternatives for other components of feeds will broaden the scope of selection for feed formulators. This is especially important in locations where access to commodities may be limited, for example in the island communities of the Pacific. Wheat mill run (44.82%), whole papaya (10%), papaya flesh (5%) were incorporated into the diets in replacement of whole wheat flour with no change in fishmeal inclusion, while papaya seed (10% inclusion) and okara (10 and 20% inclusion), which have somewhat higher levels of protein, were added in partial replacement of fishmeal and wheat flour. The fruit fly spent media obtained for this work was used to partially replace the wheat flour (and some soybean meal), because the protein levels in these ingredients were similar. The lack of an effect on shrimp growth, final weight, survival indicates that these agricultural byproducts may be included in diets at these levels for shrimp grown in pond-like conditions. Use of zero-exchange systems allows determination of nutritive quality under conditions reflective of actual farming conditions. It remains for feed formulators to locate sources of these and similar ingredients for inclusion in shrimp feed on a least cost basis.

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