Development of a Low-fish Meal Feed Formulation for Commercial Production of *Litopenaeus vannamei*

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Abstract

Fish meal is used in marine shrimp feeds because it is high in protein, highly digestible and is an effective feed attractant. Reasons for current interest in its replacement include irregular availability, variable quality, perceived contribution to deterioration of fisheries, potential for adulteration, contamination with hydrocarbons and biological pathogens, and increasing cost. Growth trials were conducted with juvenile *Litopenaeus vannamei* to evaluate modifications in nutrient supplementation/restriction of a basal fish meal replacement formula at the 50% replacement level. Trials compared growth and survival of shrimp fed a basal control feed to experimental feeds in which crystalline amino acids (CAA), oil and lecithin, blood meal and specific minerals were modified. Improved or similar growth relative to the control feed were shown with reduced levels of CAA, removal of menhaden oil, inclusion of a lower level of lecithin, and maintenance of blood meal and mineral supplements at their basal levels. This information was used to develop a generalized fish meal replacement formulation for marine penaeid shrimp using readily available partially-purified ingredients. Low- and high-marine animal meal feed formulations are compared.

Running title: shrimp fish meal replacement

Introduction

Although the world supply of fish meal has been constant, about 6.2 MMT (Hardy, 2001), current data on Gulf of Mexico menhaden landings indicate a short-fall so far this year (http://www.st.nmfs.gov/stat/market_news). Estimates of the portion of world supply used by aquaculture range between 18 (Barlow, 2000) and 31% (http://www.feap.info), with the remaining portion used for terrestrial production feeds. Compounded commercial shrimp production feeds contain about 25% fish meal (Tacon and Barg, 1998).

The availability of fish meal is largely dependent upon weather patterns (e.g., El Niño) and ability to locate fish in harvest grounds (Pontecorvo, 2001) and perception of over-exploitation, whether real or not, has caused projection of higher future prices (Delgado et al., 2002). According to FAO (2002), only 18% of total fish stocks are over-exploited and these species are typically long-lived, slow-growing ones less able to support high exploitation rates. On the other hand, pelagic fish used for fish meal/oil are characteristically small and bony and not suitable for human consumption.

The use of fish meal in aquaculture feeds has undergone considerable recent scrutiny. One area of concern regards the trophic efficiency associated with aquaculture use of fish meal. An estimated 3.75 kg of fish are required to produce 1.0 kg of shrimp (FCR =1.5, 5 kg fish = 1 kg fish meal, 25% fish meal inclusion rate). Another issue regards bans on use of fish meal in terrestrial feeds. Fish meal has is considered ultimately capable of causing bovine spongiaform encephalopathy (BSE) due to its potential for adulteration with meat meals and other byproducts implicated with this disease. Fish meal has also been verified as contaminated with polychlorinated biphenyls (PCBs). In three independent studies, 37 fish meal and fish feed samples from six countries were tested for PCB contamination: almost all samples were confirmed positive (Jacobs, 2002; Easton, 2002; CFIA, 1999).

World shrimp production has eclipsed 1 MMT and is one of the factors increasing demand for fish meal. With increasing demand and steady supply, price increases are seemingly inevitable. One-third of the fish used to make fish meal inputs, about 10 MT, is converted to aquaculture feeds (Tacon, 1998; Pike, 1998). The remaining two-thirds of the fish, ~22 Mt, is used to make fish meal for chicken, pig and other animal feeds, although the share of aquaculture continues to increase. The proportion of fish meal supplies used for farming fish rose from 10% in 1988 to 17% in 1994 and 33% in 1997 (Pike, 1998; Tacon, 1999). Moreover, fish meal prices have increased substantially in the past three decades and are likely to increase further with continued growth in demand (Naylor et al., 2000). One estimate predicts fish meal and oil in aquaculture will rise by more than 50% by 2010 (Pike, 2000). Increases in fish meal and fish oil prices could undermine the profitability of many aquaculture enterprises. However, some increased availability of fish meal for aquaculture is likely to occur with decreased use for swine or poultry production.

As a renewable biotic resource, fish meal is high in protein (60-75%, dm basis), depending on species and quality of production, and highly digestible (ADP = 81%; Akiyama et al., 1989; Forster and Dominy, 2001). It has relatively high energy content compared to other protein byproducts (14.3 vs. 11.7 mJ DE/kg from meat meal; Pike, 1999). Fish meal is highly palatable and, at typical inclusion levels (e.g., 25%), is an effective feed attractant (Coman, 1996; Hertrampf and Piedad-Pascual, 2000). It also contains significant levels of polyunsaturated fatty acids (PUFA), highly unsaturated fatty acids (HUFA), minerals, unknown growth factors and phospholipids.

In order to use fish meal in commercial production feed formulations for marine penaeid shrimp and other organisms, substantial variation in its nutritional composition due to source must be recognized. Table 1 exemplifies the variation between herring and white fish meal. This comparison shows substantial variation in protein, lipid, glycine, proline, calcium and phosphorus, all of which implies variable use in feed formulation. Further examination of this variation in terms of essential amino acid (EAA) content is shown in Table 2. Comparison of EAA content indicates highest variation in terms of arginine and lysine.

Apparent chemical score (ACS) of protein from various feedstuff proteins in terms of EAA requirement of marine penaeid shrimp is shown in Table 3 and clearly indicates that, in terms of the EAA shown, none of the non-fish meal ingredients shown could serve as a single replacement for fish meal. This indicates that any potential for replacement of fish meal by one novel ingredient could be difficult, but rather possibly accomplished by a combination of ingredients.

Table 1. Variation in biochemical composition of fish meal[1]

Nutrient	Herring meal	Whitefish meal
protein	71%	66%
lipid	9%	5%
water	8%	8%
ash	12%	21%
energy, digestible	11.0 mJ/kg	10.8 mJ/kg
lysine	7.78 g/100 g protein	7.22 g/10 g protein
arginine	6.34 g/100 g protein	6.58 g/10 g protein
methionine	2.94 g/100 g protein	2.72 g/10 g protein
threonine	4.14 g/100 g protein	4.05 g/10 g protein
glycine	6.00 g protein	9.90 g/10 g protein
alanine	6.30 g/100 g protein	6.30 g/10 g protein
proline	4.20 g/100 g protein	5.30 g/10 g protein
calcium	2.04%	7.17%
phosphorus	1.42%	3.80%

[1] http://www.fao.org; NRC, 1993; Cowey, 1999

fats have a much lower ratio (e.g., approximately 0.05-0.19. For consideration, humans require a ratio of 0.02:1 of n-3:n-6 FA for best growth (Pike, 1999).

Table 2. Variation in essential amino acid content of fish meal[1]

Fish meal Source	arginine	methionine	lysine	threonine
anchovy	3.67	1.94	5.08	2.78
herring	4.61	2.14	5.66	3.01
sardine	3.25	1.95	5.55	2.70
menhaden	3.58	1.77	4.70	2.43
white fish	4.16	1.72	4.56	2.56
CV	0.14	0.09	0.10	0.08

[1]percentage as-fed; Cowey, 1990

Table 3. Apparent chemical score of various feedstuff proteins in terms of EAA requirement by marine penaeid shrimp[1]

	lysine	arginine	methionine	threonine
Requirement (% protein)	4.67[a]	5.4[b]	2.0[c]	3.5[c]
herring meal	169	144	153	114
white fish meal	157	124	138	114117
menhaden meal	156	103	137	117108
blood meal	142	44	63	136
meat and bone meal	111	124	67	93
poultry byproduct meal	95	123	90	100
soybean meal	141	145	72	106

[1] Apparent chemical score is determined by dividing the apparent requirement for an EAA by its concentration as percentage of protein within an ingredient.

[a] Fox et al. (1995)

[b] Chen et al. (1992)

[c] estimated by authors

A comparison of fatty acids (FA) content of various fish oils to various other oil/fat sources is shown in Table 4. This comparison indicates high variance in FA content among herring oils and menhaden oil. It also shows the obvious lack of marine FA in terrestrial animal- and plant-derived oils. The n-3 marine FA, eicosopentaenoic and docosohexaenoic acid, are essential for normal growth and survival of marine penaeid shrimp. Fish oil has a n-3:n-6 FA ratio of 15.7-16.7; whereas, terrestrial-source

Table 5 compares mineral composition of various fish meals to other ingredient meals sourced from terrestrial animals and plants. Fish meal is typically lower in Ca and P than meat meal and meat and bone meal. It is also lower in Mg and Cu than meat and bone meal. Soybean meal contains less Ca and P than most fish meal sources, but is higher in Mg and Cu content.

Vitamin content in various fish meals is compared to other feed ingredients in Table 6 and shows relatively similar biotin content of fish meal to other ingredients. On the average, fish meal is substantially higher in choline content than blood meal or corn gluten meal and somewhat higher than meat meal, meat and bone meal or soybean meal. It is lower in choline content than poultry byproduct meal. Fish meal typically contains more thiamine than blood meal, meat meal, meat and bone meal, and poultry byproduct meal, but substantially less than soybean meal. Soybean meal and corn gluten meal contain no vitamin B_{12}. Vitamin E content of fish meal is typically higher than that of meat meal, meat and bone meal, poultry byproduct meal and soybean meal. It is lower in vitamin E content than corn gluten meal.

A comparison of energy content between fish meal (64%) and other energy feedstuffs (Table 7) shows somewhat lower gross energy (GE) than that of soybean meal (48%). However, in general, the GE value of fish meal is fairly similar to that of corn, wheat, wheat bran and wheat middlings. Its GE content is somewhat higher than that of alfalfa.

In terms of metabolizable energy (protein), fish meal provides somewhat lower energy via protein than corn, or wheat. As an energy source via protein it shows greater availability than alfalfa, soybean meal, and wheat bran or wheat middlings. About 72% of GE in fish meal is available for metabolic use, which is somewhat lower than that for corn (87.9%), but substantially higher than that for alfalfa (44.1%), soybean meal (55.6%), wheat bran (32.4%) or wheat middlings (46.9%).

Table 4. Fatty acid composition comparison, fish oils vs. other oils[1]

Oil	18:2 n-6	18:3 n-3	20:5 n-3	22:6 n-3
herring-Atlantic	1.1	0.6	8.4	4.9
herring-Pacific	0.6	0.4	8.1	4.8
menhaden	1.3	0.3	11.0	9.1
poultry fat	19.5	1.0		
beef tallow	3.1	0.6		
corn oil	58.0	0.7		
soybean oil	51.0	6.8		

[1] NRC, 1993

Table 5. Mineral composition comparison, fish meals vs. other meals[1]

Ingredient	Ca[2]	P[2]	Mg[2]	Cu[3]	Mn[3]	Zn[3]	Fe[3]
herring	2.20	1.67	0.14	5.60	4.80	125	114
menhaden	5.19	2.88	0.15	10.30	37.00	144	544
white fish	7.31	3.81	0.18	5.90	12.40	90	181
blood meal	0.41	0.30	0.15	8.20	6.40	306	2,769
meat meal	8.27	4.10	0.27	9.70	9.50	80	441
meat/bone	9.40	4.58	1.13	150.00	12.50	89	508
poultry byproduct	3.51	1.83	0.18	14.12	11.00	121	442
corn gluten	0.07	0.44	0.07	26.10	6.30	31	229
Soybean	0.30	0.65	0.29	23.10	30.60	52	140

[1] NRC, 1993
[2] percentage, as-fed
[3] mg/kg, as-fed

Table 6. Vitamin composition comparison, fish meals vs. other meals[1]

Ingredient	Biotin (mg/L)	Choline (mg/L)	Thiamine (mg/L)	B_{12} (µg/L)	E (mg/L)
herring	0.49	5,266	0.4	430	22.1
menhaden	0.18	3,112	0.6	123	12.0
white fish	0.08	3,099	1.7	90	8.9
Blood	0.28	600	0.3	13	
Meat	0.11	1,922	0.2	91	1.0
meat/bone	0.14	2,136	0.2	217	1.1
poultry byproduct	0.09	6,029	0.2	301	2.2
corn gluten	0.19	352	0.3		23.4
Soybean	0.32	2,609	6.0		2.4

[1] NRC, 1993

Table 7. Energy content comparison between fish meal and other energy feedstuffs[1]

Ingredient	Gross energy	Metabolizable $energy_{protein}$	% metabolizable energy
fish meal (64%)	4,023	2,899	72.1
alfalfa (17%)	3,747	1,653	44.1
corn	3,913	3,439	87.9
soybean meal (48%)	4,399	2,447	55.6
wheat	3,968	3,086	77.8
wheat bran	4,079	1,323	32.4
wheat middlings	4,156	1,951	46.9

[1]Houser and Akiyama, 1997

The dry matter, protein and energy digestibility of menhaden fish meal is compared to that of other meals in Table 8 and shows lower dry matter and protein digestibility by *Litopenaeus setiferus* than that of wheat gluten, soybean meal or wheat flour. Conversely, the energy it provides to this species is more digestible than that of wheat flour, meat and bone meal or shrimp meal.

Another useful characteristic of fish meal is its relatively high phospholipid content (Hertrampf and Piedad-Pascual, 2000) and the absence of phytate. Phytate or phytic acid is found in high concentration in grain products (e.g., soybean meal) and reduces availability of phosphorus and zinc.

Fishmeal replacement in marine shrimp feeds

The obvious question arises as to why replace fish meal in commercial production feed formulations for marine penaeid shrimp. The following reasons are therefore offered: 1) availability is sometimes irregular due to climate change (e.g., El Niño); 2) protein level, quality and thus, digestibility, among types of fish meal are highly variable; 3) a perception of exploitation of fisheries; 4) because it is commonly adulterated with meat meals (among other things), it has potential to harbor bovine spongiform encephalopathy; 5) it can be contaminated with PCBs, *Salmonella* sp.; and 6) its cost can fluctuate tremendously (e.g., $600-1,400/MT).

Table 8. Percentage dry matter, protein and energy digestibility of various feed ingredients by *Litopenaeus setiferus*[1]

Ingredient	Dry matter	Protein	Energy
menhaden fish meal	57.86	80.81	71.55
Shrimp meal	48.66	75.38	62.23
meat and bone meal	54.09	75.62	67.85
soybean meal	60.26	86.44	72.00
wheat gluten	70.46	90.98	81.17
wheat flour	58.39	82.09	68.97

[1] Brunson et al., 1997

Efforts at replacing fish meal with plant and meat meals have met with reasonable success for various marine finfish and are summarized in Table 9 and show various levels of replacement. In one of the more successful studies, an animal meal mix (e.g., ProPak™) was used to replace up to 50% of the fish meal component of feeds offered *Sciaenops ocellatus* (Meilahn et al., 1996). However, most efforts have focused on the use of soybean meal, lupin or field pea meals.

In terms of marine and freshwater penaeid shrimp, several past and recent studies have evaluated replacement of fish meal with a variety of plant protein meals (Table 10). Most of these studies have involved, as with finfish, the use of soybean meal and have met with variable levels of success. Only one of these studies has indicated potential for 100% replacement and involved use of soybean meal and distillers byproducts included in feeds offered to *Macrobrachium rosenbergii* (Tidwell et al., 1993).

Recently, other studies (Table 11) have focused on replacement of fish meal in marine and freshwater shrimp feeds through the use of meat byproducts or co-extruded meat byproducts with plant meals (e.g., soybean poultry byproduct meal, egg supplement, flash-dried poultry byproduct meal, meat and bone meal, etc.). In only one of these studies, Samocha et al. (2004), was 100% replacement of fish meal shown.

As shown by the previous, most previous fish meal replacement studies have focused on either on single plant protein replacement or preparation of coextruded specialty meals. These approaches to fish meal replacement could be necessary for small mills, mills lacking advanced formulation technology, or be warranted should a novel ingredient substantially decreases ingredient cost of feeds. Our hypothesis is that a low-fish meal commercial production feed for marine penaeid shrimp is possible using readily available partially-purified ingredients. The objectives of this study were to 1) evaluate modifications in nutrient supplementation/restriction of a basal fish meal replacement formula at the 50% replacement level for *L. vannamei* and propose a generalized commercial production formula for this species.Methods and Materials

Table 9. Summary of recent fish meal replacement studies in fin fish

Study	Replacement	Species
Kaushik et al., 2004	Plant proteins; decreased FM protein to 2%	*Dicentrarchus labrax*
Chou et al., 2004	Soybean meal; replaced 40% of FM protein	*Rachycentron canadum*
Williams, 2004	Meat meal, casein were good replacements; soybean and lupin meal, poor	*Lates calcarifer*
Glencross et al., 2003	Lupikn kernel meal; 37.5% replacement at expense of FM	*Oncorhynchus mykiss*
Borlongan et al., 2003	Feed pea meal; 20% of total protein	*Chanos chanos*
Booth et al., 2001	De-hulled field peas; improved digestibility	*Bidyanus bidyanus*
Meilahn et al., 1996	ProPak™ (animal meal mix plus crystalline EAA); replaced 50% FM	*Sciaenops ocellatus*

Table 10. Replacement of fish meal in shrimp feeds using plant protein meals

Study	Replacement	Species
Lim and Dominy, 1990	Soybean meal; 40% replacement of anchovy fish, shrimp head and squid meals	*Litopenaeus vannamei*
Piedad-Pascual et al., 1990	Soybean meal; 55% inclusion level, low stocking density	*P. monodon*
Tidwell et al., 1993	Soybean meal and distillers byproducts; 100% replacement, but at low stocking density	*Macrobrachium rosenbergii*
Lim, 1996	Solvent-extracted cottonseed meal	*L. vannamei*
Sudaryono, 1999	Various lupin meals	*P. monodon*
Eusebio and Coloso, 1998	Cowpea, green mungbean, rice bean, leaf meals	*L. indicus*
Penaflorida, 1995	Papaya, camote leaf meal	*P. monodon*

Table 11. Replacement of fish meal in shrimp feeds using meat byproducts

Study	Replacement	Species
Samocha et al., 2004	Co-extruded soybean poultry byproduct meal and egg supplement; 100% replacement of FM	*Litopenaeus vannamei*
Davis and Arnold, 2000	Co-extruded soybean-poultry byproduct meal, flash-dried poultry byproduct meal; 50% replacement of FM	*L. vannamei*
Yang et al., 2003	Meat and bone meal, poultry byproduct meal; 50% replacement of FM	*Macrobrachium nipponense*
Forster et al., 2003	Meat and bone meal; 25-75% replacement, depending upon source	*L. vannamei*
Cruz-Suarez et al., 2001	Canola meal; replaced a portion of FM, soybean meal and wheat	*L. vannamei*
Sudaryono et al., 1995	Combination of shrimp head meal and scallop waste gave best results	*Penaeus monodon*

Source of shrimp

Postlarval shrimp (*L. vannamei*) were purchased from Shrimp Improvement Systems (Plantation Key, FL) and reared in indoor cylindrical tanks connected to a recirculating seawater treatment system at the Texas Agricultural Experiment Station
(TAES) Shrimp Mariculture Project (Port Aransas, TX) until achieving an appropriate size for stocking of growth trial tanks.

Experimental design

Four optimization trials were undertaken in which shrimp were offered a basal feed containing 12.5% fish meal: Trial 1 (EAA modification); Trial 2 (fish oil and lecithin modification); Trial 3 (blood meal inclusion level); and Trial 4 (mineral modification).

Experimental system and stocking

Juvenile *L. vannamei* (mean initial weight 0.2-0.5 g) for Trial 1 were stocked into experimental recirculating systems under the following conditions: 32.0 L (0.07 m^2

bottom area) rectangular tanks; five shrimp/tank; water exchange rate of 1,440% per day. For Trials 2,3 and 4, shrimp of similar size were stocked into similar tanks but at a density of 4 shrimp/tank and with 2,770% daily water exchange. Each experimental feed was offered to shrimp at a ten-fold level of replication (i.e., 10 experimental tanks per dietary treatment).*Experimental feeds*

A 35% crude protein basal control diet (Table 12) was formulated to contain 12.5% menhaden fish meal and was slightly modified according to the following as-fed criteria. Trial 1 modifications involved manufacture of six experimental feeds: low methionine (0.10%), high methionine (0.50%), low lysine (0.10%), high lysine (0.50%), low arginine (0.10%) and high arginine (0.50%). Trial 2 modifications to the basal diet called for preparation of no-menhaden oil (0.00%) and low-menhaden oil (1.00%) experimental feeds as well as low- (1.00%) and high-lecithin (5.00%) feeds. For Trial 3, blood meal content of the basal control feed was modified into medium- (9.00%) and high-content (12.00%) experimental feeds. Trial 4 modifications involved preparation of the following experimental feeds: no NaCL (0.0%), 2.5% KCl and 4.0% $CaHPO_4$.

Dry feed ingredients were first mixed in a vertical V-type mixer followed by addition of water to achieve a wet mash consistency appropriate for subsequent cold extrusion through a 3.0 mm die using a Hobart-200 mixer (Troy, OH) with meat chopper attachment. Extruded feed strands were dried in a forced-air convection oven overnight to achieve a "dried" moisture content of 10%. Dried feed strands were hand-ground with a mortar and pestle and screened through #10 and #18 sieves to achieve appropriate-sized feed particles.

Table 12. Basal low fish meal control feed

Ingredient	% as-fed
Wheat flour	37.40
Corn gluten	12.00
Soy protein	7.50
Crab meal	7.00
Fish meal	12.50
$CaHPO_4$	3.00
CMC binder	4.00
Blood meal	6.00
Menhaden oil	1.90
Lecithin	3.00
Fish solubles	2.00
KCl	1.50
NaCl	0.50
Cholestrol	0.20
Vitamin mix 1	0.27
Vitamin mix 2	0.23
L-lysine HCl	0.30
DL-methionine	0.30
L-arginine	0.30
Ascorbic acid (Stay-C vitamin C)	0.10

Feeding trial maintenance

Juvenile *L. vannamei* were offered feeds on a semi-continuous basis (20 feedings per day) using automated wheel-type feeders. Experimental system water quality was evaluated in terms of temperature, salinity and pH (daily) as well as NH_3/NH_4^+-N, NO_2-N and NO_3-N (Clesceri, et al. 1998). All residual/uneaten feed, molts, feces and dead shrimp were removed from experimental tanks on a daily basis. All experimental trials were terminated after 31-33 days.

Results and Discussion

Periodic determination of water quality factors in experimental systems indicated that at no time could any of the factors be considered outside appropriate ranges for normal growth and survival of *L. vannamei*.

Trial 1

Results from Trial 1 are shown in Table 13. No significant difference (P>0.0500) was shown in survival among shrimp fed both control and experimental feeds. Schefe's test (Day and Quinn, 1989) indicated that only in the case of shrimp fed the low-methionine diet was instantaneous growth rate (IGR) of shrimp greater than that for shrimp fed the basal control feed (P=0.0340); 7.87 ± 0.25 vs. 7.58 ± 0.22, respectively). This indicates that a reduction of DL-methionine in the basal formulation from 0.3 to 0.1% could improve growth and reduce overall feed cost in commercial formulations. Supplementation of DL-methionine at >0.88% in research feeds offered to Penaeus monodon resulted in reduced weight gain (Millamena et al., 1996). Results from Trial 1 also suggested that inclusion of L-lysine HCl and L-arginine at levels at least as low or lower than of the basal formulation could be possible.

Table 13. Survival and growth (IGR)[1] of juvenile *L. vannamei* offered feeds containing various levels of crystalline amino acids

Dietary treatment	Mean survival (%)	Mean IGR (SD)[2]
Basal feed	98[a]	7.58 (0.22)[b]
Low methionine (0.10%)	98[a]	7.87 (0.25)[a]
High methionine (0.50%)	98[a]	7.66 (0.24)[b]
Low lysine (0.10%)	98[a]	7.72 (0.24)[b]
High lysine (0.50%)	92[a]	7.78 (0.18)[b]
Low arginine (0.10%)	100[a]	7.69 (0.19)[b]
High arginine (0.50%)	100[a]	7.94 (0.52)[b]

[1] Instantaneous growth rate (IGR) is defined as the natural log of the square root of the difference in initial vs. final mean weight of shrimp divided by length in days of the trial.

[2] Means having similar superscripts are not significantly different at *P*=0.0500.

Trial 2

Results from Trial 2 are shown in Table 14. There was no significant difference in survival or IGR between shrimp offered the basal feed and any of the experimental feeds (*P*>0.0500). An indication that menhaden oil (basal formulation level 1.90%) could be reduced or removed from the basal feed formulation was presented. This is probably due to non-limiting availability of n-3 HUFA from other sources in the feed (e.g., crab meal, fish meal, fish solubles, etc.). It is likely that a lower level of lecithin supplementation is (from 3 to 1%) is also recommended, although numerically lower growth was shown.by shrimp fed the low lecithin feed. Improved growth was shown by Gong et al. (2002) for juvenile *L. vannamei* offered feeds containing 0.2 and 1.5% cholestrol and lecithin, respectively, vs. 3% lecithin.

Trial 3

Results from Trial 3 are shown in Table 15. There was no significant difference in survival or IGR between shrimp offered the basal feed and any of the experimental feeds (P>0.0500). However, both survival and IGR were numerically lower for shrimp fed the high blood meal feed (12%). This suggests that, for the basal formulation, supplementation with blood meal above the basal (6%) level could be harmful to shrimp.

Replacement of FM by blood meal in excess of 10% decreased growth in *L. paulensis* and *L. californiensis* (Hertrampf and Piedad-Pascual, 2000). Also, as previously mentioned, several countries have forbidden use of blood meal in terrestrial feeds (especially those fed to ruminants) due to potential for transmission of BSE.

Table 14. Survival and growth (IGR)[1] of juvenile *L. vannamei* offered feeds containing various levels of fish oil and lecithin

Dietary treatment	Mean survival (%)	Mean IGR (SD)[2]
Basal feed (1.90% menhaden oil and 3.00% lecithin)	93.75[a]	8.14 (0.22)[a]
No-oil (0.00%)	97.91[a]	7.88 (0.25)[a]
Low oil (1.00%)	95.83[a]	8.28 (0.24)[a]
Low lecithin (1.00%)	85.41[a]	7.57 (0.24)[a]
High lecithin (5.00%)	91.66[a]	8.02 (0.18)[a]

[1] Instantaneous growth rate (IGR) is defined as the natural log of the square root of the difference in initial vs. final mean weight of shrimp divided by length in days of the trial.
[2] Means having similar superscripts are not significantly different at P=0.0500.

Table 15. Survival and growth (IGR)[1] of juvenile *L. vannamei* offered feeds containing various levels of blood meal

Dietary treatment	Mean survival (%)	Mean IGR (SD)[2]
Basal feed (6% blood meal)	87.50[a]	9.56 (0.72)[a]
Med blood meal (9%)	87.50[a]	9.67 (0.91)[a]
High blood meal (12%)	79.16[a]	9.14 (1.02)[a]

[1] Instantaneous growth rate (IGR) is defined as the natural log of the square root of the difference in initial vs. final mean weight of shrimp divided by length in days of the trial.
[2] Means having similar superscripts are not significantly different at *P*=0.0500.

Table 16. Survival and growth (IGR)[1] of juvenile *L. vannamei* offered feeds containing various levels of minerals

Dietary treatment	Mean survival (%)	Mean IGR (SD)[2]
Basal feed (0.5% NaCl, 1.50% KCl, 3.0% $CaHPO_4$)	86.36	10.00 (0.73)[a]
No NaCl (0.0%)	97.73	9.35 (0.44)[b]
2.5% KCl	83.33	9.22 (0.79)[b]
4.0% $CaHPO_4$	93.75	9.57 (0.53)[a]

[1] Instantaneous growth rate (IGR) is defined as the natural log of the square root of the difference in initial vs. final mean weight of shrimp divided by length in days of the trial.
[2] Means having similar superscripts are not significantly different at *P*=0.0500.

Trial 4

Results from Trial 4 are shown in Table 16. Although there was no significant difference in survival between shrimp offered the basal feed and any of the experimental feeds (P>0.0500), a significant effect on IGR was shown by level of NaCl and KCl supplementation. Reducing NaCl to 0.00% and increasing KCl to 2.5% significantly decreased IGR of shrimp relative to those offered the basal feed. Dietary deficiencies of Na and Cl have not been demonstrated in marine shrimp or fish (NRC, 1993); however, these minerals could be required in grow-out feeds for low-salinity culture conditions. No dietary requirement for K has been shown (He et al., 1992), despite a normal formulation inclusion level is 0.9%. Increasing dietary $CaHPO_4$ from 3 to 4% did not increase IGR. This could have been due to reduced digestibility at basic gut pH (Guillaume et al., 2001).

Conclusions from feeding trials

The previous feeding trials indicated that the base low fish meal feed (Table 12) could be improved by decreasing concentration of DL-methione and that L-lysine HCl and L-arginine content could also be reduced. Trial 2 showed that the basal low fish meal formulation probably does not require addition of menhaden oil and that lecithin to level could be reduced to 1%, albeit probably not without supplementation of dietary cholestrol. There was no need to increase blood meal above 6%, although investigation into a lower dietary inclusion level is warranted. No real change in mineral salt content of the low fish meal basal feed was warranted.

Low- vs. High-Marine Animal Meal Formulation

Based upon the results of Trials 1-4, a low-marine animal meal feed is shown in Tables 17 (ingredient composition) and 18 (proximate analysis). A complete formulation is not provided due to the proprietary nature of the formulation. It should be noted that the proximate analysis, Ca and P content of this feed formulation, when compared to that of a high-marine animal meal commercial feed are similar. A slightly higher level of fiber is shown in the low-marine animal meal formulation, largely due to higher inclusion level of plant meals. Major differences in ingredients between the low- and high-marine meal formulations are as follows: decreased inclusion of marine animal meals (15.0 vs .25.0%), increased level of plant protein meals (66.0 vs. 60.2%), slightly higher overall inclusion of oils (e.g., reduction in fish oil, but higher soybean and corn oil; 8.2 vs. 7.2%), increased minerals (e.g., $CaCO_3$, $CaHPO_4$, NaCl, KCl and MgO; 9.8 vs. 6.6%). A similar levels of vitamin/mineral premix and binder (0.5% for both) are proposed.

Table 17. Generalized feed formulation for a low-marine animal meal feed for marine penaeid shrimp[1]

Ingredients	Low-marine animal meal feed	High-marine animal meal feed
Marine animal meal	15.0	25.0
Plant meal	66.0	60.2
Fish, soybean, corn oil	8.2	7.2
$CaCO_3$, $CaHPO_4$, NaCl, KCl, MgO	9.8	6.6
Vitamin-mineral mix	0.5	0.5
Binder	0.5	0.5

[1] percentage, as-fed

Table 18. Proximate analysis, Ca and P composition of a low-marine animal meal commercial feed formulation for marine penaeid shrimp[1]

Nutrient	Low-marine animal meal feed	High-marine animal meal feed
Crude protein	35.0	35.0
Crude fat	9.0	9.0
Total ash	16.0	16.0
Crude fiber	2.9	2.5

Ca	2.4	2.4
P	1.5	1.5
Carbohydrate-calculated	25.2	25.2

[1] percentage, as-fed

Future research obviously requires commercial verification of the low fish meal//marine animal meal-based feed under controlled conditions and in conjunction with a standard commercial marine penaeid shrimp production feed.

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