Meeting the challenge of no fishmeal in practical diets for *Litopenaeus vannamei*: case studies from Labomar, Brazil

Alberto J.P. Nunes*, Marcelo Vinícius do Carmo and Sá and Hassan Sabry Neto

LABOMAR - Instituto de Ciências do Mar, Universidade Federal do Ceará

Avenida da Abolição, 3207 - Meireles, Fortaleza, Ceará, 60.165-081

Tel./Fax: +55-85-32298718, E-mail: alberto.nunes@ufc.br

Abstract

Farm-reared shrimp require highly digestible protein with the correct balance of essential amino acids to achieve maximum growth. In shrimp feeds, much of the animal protein used has been derived from the fisheries of pelagic fish which is transformed into fish meal and fish oil. The problem is that while fisheries production remains stagnant, aquaculture continues to grow at an annual rate of 8.8% since 1970. This work reports the results of three studies in which partial or complete replacement of fish meal was attempted in *Litopenaeus vannamei* diets. In study 1 (MET) 2-hydroxy-4-(methylthio)butanoic acid (HMTBa) was used to meet *L. vannamei* methionine requirements as Anchovy fish meal levels dropped and higher inclusion of soybean meal, soy protein concentrate and fish oil was used in experimental diets. In study 2, performance of juveniles of *L. vannamei* fed on practical diets with increasing levels of Antarctic Krill meal, *Euphausia superba*, and decreasing levels of costly ingredients, namely fish meal (FML), fish oil (FO), cholesterol (CHL) and soybean lecithin (SL) was evaluated. In study 3, soybean protein concentrate (SPC) and soybean oil (SBO) were used to replace fish meal and fish oil following Tacon and Metian's (2008) prediction on their maximum inclusion levels in complete diets for penaeid shrimp for the next 15 years. Results have shown that *L. vannamei* growth, body weight, survival, yield and FCR were supported by HMTBa supplementation when 150 g/kg of fish meal was replaced by vegetable protein ingredients, namely soybean meal, at 50% and 100%. In study 2, Krill meal was also able to partially replace fish meal without unfavorable effects on shrimp growth performance. Study 3 indicated that there was no negative effect on *L. vannamei* performance when fish meal was reduced from 12% to 8.5% using SPC as a substitute

Key words: fish meal, replacement, shrimp, Brazil

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1. Introduction

1.1. Research in Shrimp Nutrition at LABOMAR

Limited applied research has been carried out in shrimp nutrition in Brazil compared to Mexico, for example. As a new emerging industry, government funding in the country for aquaculture studies was scarce and research institutions were deprived of adequate infrastructure and highly trained professionals in the field. This has been rapidly changing, but funding initiatives are still limited and scattered in too many fields of investigation. Unfortunately, many times results can have short-term application to the industry.

In Brazil, most of applied research in shrimp nutrition has been funded by private companies which carry out proof of performance trials in private farms or research and development (R&D) projects in key research centers in the country. However, precise evaluation of feed and ingredient performance at the farm level can be impractical, costly and misleading. Shrimp commercial ponds are large in size and display significant ecological variation, impairing data collection and treatment replication.

LABOMAR, a 50-year old federal research center carries out studies in various fields of marine sciences, including aquaculture. LABOMAR is located in the State of Ceará, NE Brazil, the second largest producer of farm-reared shrimp in the country. With government and private investments, the institution has set up a research laboratory for shrimp nutrition, consisting of two controlled rearing systems of 200 tanks in total. One system operates under outdoor conditions (1.000 l tanks) with green water. The other rearing system operates indoors (500 l tanks) with clear water (Fig. 1). There are also two controlled systems designed to evaluate digestibility and attractability of feeds and ingredients.

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Figure 1. (**A**) Indoor and (**B**) outdoor tanks at LABOMAR´s Laboratory of Aquatic Animal Nutrition.

Most private efforts at LABOMAR aim to improve nutrition technologies and concepts, aligning nutritional products to market needs and validating results under controlled or commercial culture conditions. Regrettably, much of the private research efforts carried out at LABOMAR are proprietary and business-oriented, as such, not allowed to be disclosed publicly due to confidentiality agreements. Results that will be presented here have either been academic research or permission has been granted by private companies for publication.

1.2. The Fish Meal Issue

Farm-reared shrimp require highly digestible protein with the correct balance of essential amino acids (EAA) to achieve maximum growth. In order to meet these high protein levels, feeds are manufactured with a combination of feed grade plant and animal protein ingredients. Much of the animal protein used in shrimp diets has been traditionally derived from the capture fisheries of pelagic fish which is transformed into fish meal and fish oil. The problem is that while capture fisheries production remains stagnant, aquaculture continues to grow at an annual rate of 8.8% since 1970 (FAO, 2007). Thus, as the shrimp farming industry moves into more intensive systems and production rises in many

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countries, there is a growing demand for formulated diets dependent on static supplies of fish meal. A global survey conducted by Tacon and Metian (2008) revealed that in 2006 marine shrimp was the leading consumer of fish meal within the aquaculture sector, requiring 0.31 kg of fish meal for every kg of shrimp harvested (3.164 million MT of shrimp harvested for 989.7 thousand MT of fish meal consumed). Fish meal market prices have become more volatile and increased over the years (Figure 1). This has prompted a greater interest in studies that seek to replace fish meal in shrimp diets.

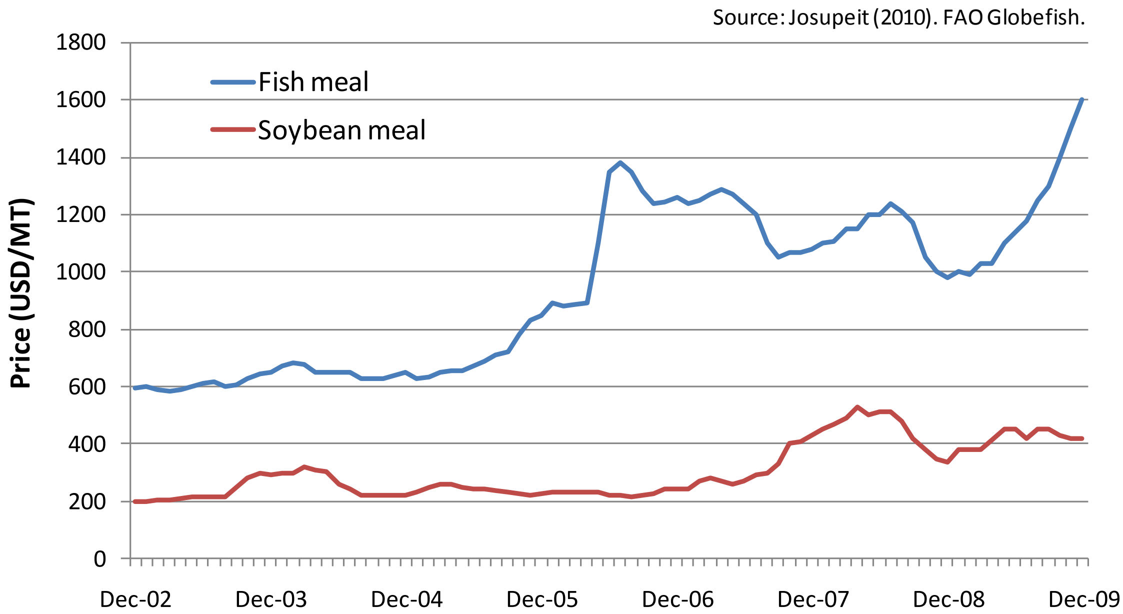

Figure 1. Market price variation (USD/MT) of anchovy fish meal and soybean meal. Source: Josupeit (2010).

This work reports the results of three studies in which partial or complete replacement of fish meal was attempted in *Litopenaeus vannamei* diets. Studies used different formulation approaches to achieve fish meal replacement.

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2. Materials and methods

In study 1 (MET) 2-hydroxy-4-(methylthio)butanoic acid (HMTBa) was used to meet *L. vannamei* methionine requirements as Anchovy fish meal levels dropped and higher inclusion of soybean meal, soy protein concentrate and fish oil was used in experimental diets. First, a basal diet with 150.0 g/kg (as fed) of Anchovy fish meal (NV_B) and no supplemental methionine (HMTBa) was designed. Out of this diet, two positive control diets (NV50_C+ and NV100_C+) were formulated to reduce fish meal at 50% and 100%, respectively. The positive control diets were supplemented with HMTBa at 1.0 g/kg and 2.0 g/kg (as is), respectively, in order to target dietary methionine levels similar or slightly above those achieved with the basal diet. Two nearly equivalent diets acted as negative controls (NV50_C- and NV100_C-), without supplementation of HMTBa. In order to maintain crude protein, total fat, gross energy, phospholipids and essential amino acids (EAA) as consistent as possible across all control diets, fish meal was replaced by soybean meal, soy protein concentrate and fish oil (Table 1). Formula cost was reduced when fish meal was progressively replaced by these ingredients. A total of 50 indoor tanks of 500 L operating under continuous clear-water conditions were used in the study. This allowed 10 replicate tanks to be used for each diet. Shrimp were stocked under 40 shrimp/tank (70 animals/m^2). The trial started when shrimp had reached 2.22 ± 0.19 g (n = 50) in wet body weight.

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Table 1. Experimental diets used in study 1 with chemical composition. Chemical values were determined by laboratory analyses

Ingredient (g/kg, as is)	NV_B	NV50_C+	NV50_C-	NV100_C+	NV100_C-
Soybean meal	350.0	457.6	450.0	487.0	485.2
Wheat flour	235.6	217.0	221.7	210.0	210.0
Fish meal, Anchovy	150.0	75.0	75.0	0.0	0.0
Poultry by-product meal	60.0	60.0	65.7	60.0	60.0
Rice, broken	50.0	21.9	21.8	0.0	0.0
Soy protein concentrate	43.1	30.0	30.0	93.3	96.4
Squid meal, whole	0.0	20.0	20.0	20.0	20.0
Fish oil	15.0	30.0	30.0	44.0	44.0
Soybean oil	19.4	8.5	7.9	0.0	0.0
MERA™ Met Ca[1]	0.0	1.0	0.0	2.0	0.0
L-lysine	0.0	0.0	0.0	0.4	0.3
Other micro ingredients	76.8	79.8	77.8	83.3	84.2
Proximate Composition (g/kg, dry matter basis)					
Moisture	92.6	95.7	91.0	88.1	92.9
Crude protein	392.2	383.5	391.8	393.2	406.6
Lipids	70.5	75.9	70.7	78.0	60.7
Total fiber	22.7	26.6	28.3	27.1	31.7
Ash	98.7	97.6	97.9	95.5	88.4
Amino Acids (g/kg, dry matter basis)					
HMTBa	0.0	0.65	0.0	1.14	0.0
Methionine	6.0	5.4	5.2	4.5	4.8
Cystine	5.4	5.3	5.4	5.6	5.7
Methionine + cystine	11.4	10.7	10.6	10.1	10.5
Lysine	19.7	20.4	18.8	19.4	22.4
Formulation Cost[2]	--	11.7%	12.5%	22.2%	23.2%

[1] 84% 2-hydroxy-4-(methylthio)butanoic acid (HMTBa, Novus International, Inc., St. Charles, MO, USA)

[2]% reduction in formula cost in comparison to the control diet NV_B

In study 2, performance of juveniles of *L. vannamei* fed on practical diets with increasing levels of Antarctic Krill meal, *Euphausia superba*, and decreasing levels of costly ingredients, namely fish meal (FML), fish oil (FO), cholesterol (CHL) and soybean lecithin

(SL) was evaluated. The study was conducted in 20 indoor (500 L in volume; 100 shrimp/m^2) and 20 outdoor tanks (1,000 L; 60 shrimp/m^2). Five replicates were designated for each control and treatment feeds, which were randomly assigned in both the clear and green-water systems. Feeds were designed with a least-cost formulation approach to achieve formula cost savings in treatment diets, while keeping a nearly similar amino acid profile as the basal diet. Feeds consisted of a basal diet (**CTL**) designed to fully meet *L. vannamei* nutritional requirements. The **CTL** diet contained 187.5 g/kg FML, 20.0 g/kg FO, 15.0 g/kg SL, and 1.5 g/kg CHL. From the basal diet, three other formulas were designed to progressively replace FML, FO, SL and CHL by Krill meal. Diets KM10, KM50 and KM110 contained Krill meal at 10.0 g/kg, 50.0 g/kg and 110.0 g/kg, respectively, with reduced levels of FML (125.0 g/kg, 62.5 g/kg, and 0 g/kg, respectively), FO (15.2 g/kg, 17.7 g/kg, and 0.1 g/kg), SL (15.0 g/kg, 0 g/kg, and 0 g/kg) and CHL (1.5 g/kg, 0.8 g/kg, and 0 g/kg). As these ingredients were replaced by Krill meal, formula costs in KM10, KM50 and KM110 reduced 8.0%, 12.8% and 8.7%, respectively, compared to CTL.

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Table 2. Ingredient composition and proximate chemical analysis of the experimental diets used in study 2. Chemical values were determined by laboratory analyses

Ingredients	Diet composition (g/kg as is)			
	CTL	KM10	KM50	KM110
Fish meal, Anchovy	150.0	100.0	50.0	0.0
Fish meal, by-catch	37.5	25.0	12.5	0.0
Broken rice	130.4	115.6	108.0	113.5
Soybean meal	300.0	340.2	338.6	349.4
Meat and bone meal	23.1	55.0	100.0	99.5
Wheat flour	250.0	250.0	250.0	250.0
Fish oil	20.0	15.2	17.7	0.1
Soybean lecithin	15.0	15.0	0.0	0.0
Krill meal[1]	0.0	10.0	50.0	115.0
Cholesterol	1.5	1.5	0.8	0.0
Others*	322.5	322.5	322.5	322.5
USD/MT** / % Savings	662.8	8.0%	12.8%	8.7%
Proximate composition (g/kg dried matter basis)				
Crude protein	325.5	336.0	327.3	338.6
Total lipids	103.8	103.5	107.5	107.5
Ash	99.5	98.3	99.5	96.7
Crude fiber	1.65	1.49	1.64	2.22
Gross energy (kJ/g)	17.5	17.5	16.9	16.8
Moisture (% as fed)	11.1	10.7	10.2	8.8

*Others included: 1.5 g/kg magnesium sulfate, 4.0 g/kg potassium chloride, 5.0 g/kg synthetic binder, 10.0 g/kg common salt, 10.0 g/kg vitamin-mineral premix, 12.0 g/kg bicalcium phosphate, 30.0 g/kg of corn gluten meal and 250.0 g/kg wheat flour.

FOB prices (Fortaleza, Brazil, Dec. 2007). Formula savings (USD/MT) compared to the **CTL (basal) diet.

[1]Qrill™ meal, Akerbiomarine, Oslo, Norway.

In study 3, soybean protein concentrate (SPC) and soybean oil (SBO) were used to replace fish meal and fish oil following Tacon and Metian's (2008) prediction on their maximum inclusion levels in complete diets for penaeid shrimp for the next 15 years. Eight practical isonitrogenous (38.0% crude protein) and isoenergetic (15.9 MJ/kg; dry matter basis) diets

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were formulated and manufactured with two levels of fish oil, 2.0% (control) and 1.0% (experimental). For each group of three diets with the same inclusion level of fish oil (2% or 1%), fish meal was increasingly substituted by SPC at 0% (control), 30% and 60%. In practical terms, replacement levels meant the use of fish meal at 12%, 8.5% and 5%, respectively, following Tacon and Metian's (2008) forecasts for the years 2010, 2015 and 2020, respectively. Two additional diets were also formulated where 100% fish meal was replaced by SPC. The year "2025" was assigned for these two non-fish meal diets. As SPC inclusion increased in the formulas, the dietary level of SBO increased in order to balance the lipid and energy content of the diets. Whenever necessary, experimental diets were supplemented with synthetic sources of methionine and lysine. The study was conducted in 50 circular tanks of 500 L each (6-7 replicate tanks per diet) that operates under a continuous water recirculation regime. *L. vannamei* of 2.02 ± 0.51 g were stocked at 70 animals/m^2 (40 shrimp/tank).

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Table 3. Ingredient composition and proximate chemical analysis of the experimental diets used in study 2. Chemical values were determined by laboratory analyses.

Ingredients	Diet composition (g/kg as is)							
	’10-2	’15-2	’20-2	’25-2	’10-1	’15-1	’20-1	’25-1
Fish meal, Anchovy	120.0	85.0	50.0	0.0	120.0	85.0	50.0	0.0
Soy protein concentrate	0.0	38.5	77.5	133.4	0.0	38.4	77.5	133.2
Soybean oil	10.5	13.3	18.0	25.1	20.4	23.0	27.9	34.5
Fish oil	20.0	20.0	20.0	20.0	10.0	10.0	10.0	10.0
Broken rice	41.5	35.1	25.7	11.9	41.5	35.4	25.9	12.7
L-lysine	1.2	1.3	1.5	1.7	1.2	1.3	1.5	1.7
DL-methionine	0.00	0.4	0.8	1.4	0.0	0.4	0.8	1.4
Magnesium sulfate	1.1	0.7	0.7	0.8	1.2	0.7	0.7	0.8
Others*	805.7	805.7	805.7	805.7	805.7	805.7	805.7	805.7
Proximate composition (g/kg dried matter basis)								
Crude protein	388.1	384.1	393.9	390.8	393.5	384.9	385.9	388.4
Total lipids	99.8	89.5	94.8	97.0	93.0	89.3	93.7	97.8
Ash	104.7	97.6	96.1	88.9	105.6	97.1	94.6	91.3
Crude fiber	14.7	17.3	17.0	19.2	17.9	15.5	13.4	17.4
Gross energy (kJ/g)	19.7	19.6	19.9	20.1	19.6	19.8	19.9	20.1

*Others included: 0.7 g/kg Rovimix Stay-C 35%, 7.0 g/kg synthetic binder, 10.0 g/kg potassium chloride, 13.0 g/kg bicalcium phosphate, 15.0 g/kg soy lecithin, 10.0 g/kg vitamin-mineral premix, 150.0 g/kg poultry meal by-product, 250.0 g/kg wheat flour and 330.0 g/kg soybean meal.

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3. Results and discussion

In study 1, shrimp grew continuously over the culture period reaching over 9 g and 11 g in 72 and 96 days of rearing, respectively. Shrimp survival (92.3 ± 5.1% and 81.4 ± 8.0%) yield (461 ± 49 g/tank and 539 ± 70 g/tank) and FCR (2.17 ± 0.19 and 3.12 ± 0.37) showed no differences among experimental diets after 72 or 96 days of rearing ($P > 0.05$), respectively. On the other hand, there were statistical differences in final body weight and weekly growth. A higher body weight was observed when shrimp were fed the basal diet with 150 g/kg of fish meal (NV_B) or when diets were supplemented with HMTBa. There was a detriment to shrimp final body weight when fish meal was reduced without HMTBa supplementation (negative control diets NV50_C- and NOV100_C-). Similarly, a higher weekly growth rate was found for shrimp fed either the basal diet, NV_B, or diets NV50_C+ and NOV100_C+. After the 14-week rearing period, growth rates averaged 0.74 g/week with HMTBa supplementation compared to 0.70 g/week without supplementation (diets NV50_C- and NOV100_C-). A trend towards higher feed consumption was apparent at each time point for shrimp fed both supplemented feeds. Economic analysis indicated that fish meal replacement by vegetable protein ingredients was more advantageous when HMTBa was used. Gross profit increased from 22.8% to as much as 41.9% in diets with HMTBa.

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Table 4: Growth performance (mean ± standard deviation) of *L. vannamei* juveniles fed on diets supplemented with L with 2-hydroxy-4-(methylthio)butanoic acid (HMTBa) at two harvest periods. Common letters denote non-significant differences between feeding treatments according to Turkey's HSD Multiple Range Test at the α = 0.05 level.

Perform.	Day	Experimental Diets					
		NV_B	NV50_C+	NV50_C-	NV100_C+	NV100_C-	Mean ± SD
Initial Weight (g)	---	2.10 ± 0.47	2.25 ± 0.53	2.28 ± 0.50	2.24 ± 0.50	2.25 ± 0.51	2.22 ± 0.19
Final Weight (g)	72	9.60 ± 0.41 a	9.91 ± 0.45 b	9.23 ± 0.58 C	9.62 ± 0.15 ab	9.23± 0.15 c	---
	94	12.29 ± 0.69 ac	12.43 ± 0.54 c	11.85 ± 0.76 B	11.99 ± 0.47 ac	12.35 ± 0.52 ab	---
Growth (g/week)	72	0.73 ± 0.05 ab	0.74 ± 0.05 a	0.68 ± 0.06 b	0.72 ± 0.05 b	0.68 ± 0.02 ab	---
	94	0.74 ± 0.05	0.74 ± 0.05	0.70 ± 0.06	0.74 ± 0.04	0.71 ± 0.03	0.73 ± 0.03
Survival (%)	72	93.8 ± 6.7	91.5 ± 5.3	92.3 ± 4.9	92.5 ± 5.3	91.5 ± 3.6	92.3 ± 5.1
	94	78.3 ± 12.1	85.5 ± 7.1	82.0 ± 9.6	82.3 ± 5.3	82.0 ± 4.0	81.4 ± 8.0
Yield	72	0.48 ± 0.04	0.48 ± 0.07	0.44 ± 0.05	0.46 ± 0.05	0.44 ± 0.04	0.46 ± 0.05
(kg/m^2)	94	0.53 ± 0.10	0.56 ± 0.09	0.52 ± 0.06	0.55 ± 0.05	0.53 ± 0.03	0.54 ± 0.07
AFI*	72	1.03 ± 0.11	1.08 ± 0.07	0.93 ± 0.06	0.96 ± 0.04	0.97 ± 0.07	0.99 ± 0.09
(kg/m^2)	94	1.68 ± 0.21	1.80 ± 0.18	1.60 ± 0.11	1.61 ± 0.09	1.64 ± 0.08	1.67 ± 0.14
FCR*	72	2.13 ± 0.14	2.27 ± 0.23	2.13 ± 0.20	2.11 ± 0.17	2.21 ± 0.17	2.17 ± 0.19
	94	3.26 ± 0.52	3.23 ± 0.33	3.11 ± 0.45	3.09 ± 0.22	2.94 ± 0.20	3.12 ± 0.37

*AFI (apparent feed intake per area of culture); FCR (food conversion ratio).

In study 2, after 72 days of rearing, shrimp weekly growth rates in the indoor and outdoor systems, reached 1.00 ± 0.05 g and 1.04 ± 0.09 g, respectively. At harvest, shrimp reared under both systems showed no statistical differences in performance among treatment feeds ($P > 0.05$). In indoor tanks, animals attained 13.2 ± 0.20 g body weight, 81.4 ± 7.1% survival, 0.78 ± 0.12 kg/m^2 yield and 2.16 ± 0.28 FCR. In outdoor tanks, animals attained 14.2 ± 2.3 g body weight, 91.3 ± 5.7% survival, 0.57 ± 0.07 kg/m^2 yield and 2.15 ± 0.34 FCR. In this study, shrimp biological performance varied more strongly as a result of the rearing system than in relation to feed type. At first it appeared the green-water system imposed a greater challenge to shrimp growth when compared to the clear-water system. However, clear-water tanks were intentionally deprived of natural food items and operated under a stocking density 1.7 times higher than the green-water tanks. At harvest, there was a slower growth and survival for shrimp reared in clear water than in green water. Under clear-water tanks, shrimp yield and apparent feed intake (AFI, Table 5) was also higher which probably led to a greater build-up of nitrogen in the rearing system. These results and observations suggest that clear-water tanks promoted a more challenging culture environment for *L. vannamei*, despite the greater fluctuations in water quality observed in green-water conditions. In the present study, there were only few differences in shrimp biological performance as a result of diet type. The major variation was observed in final body weight when shrimp fed diets containing from 10 to 50 g/kg of Krill meal inclusion were compared with those fed the basal (CLT) and KM110 diets under the green-water system. As these differences were not replicated under a more controlled and challenging rearing condition, as found in clear-water tanks, it can be assumed they were partially the result of external factors (*e.g.*, water quality) other than Krill meal use.

Table 5: Growth response (mean ± standard deviation) of *L. vannamei* juveniles fed diets containing Krill meal for 72 days in 500-L indoor (clear water) and 1,000-L outdoor (green water) polyethylene tanks. No statistical differences were observed between feeding treatments according to ANOVA ($P > 0.05$).

Performance	Water	Experimental Diets				
		CLT	KM10	KM50	KM110	Mean ± SD
Initial Weight (g)	Clear	2.8 ± 0.7	2.9 ± 0.7	2.8 ± 0.6	3.0 ± 0.8	2.9 ± 0.7
	Green	3.5 ± 0.8	3.5 ± 0.7	3.5 ± 0.7	3.4 ± 0.8	3.5 ± 0.8
Final Weight (g)	Clear	13.1 ± 2.2	13.3 ± 1.9	12.9 ± 1.8	13.3 ± 2.1	13.2 ± 2.0
	Green	14.7 ± 2.1	13.9 ± 2.1	14.0 ± 2.5	14.2 ± 2.4	14.2 ± 2.3
Growth (g/week)	Clear	0.98 ± 0.02	1.01 ± 0.07	0.98 ± 0.07	1.01 ± 0.08	1.0 ± 0.05
	Green	1.06 ± 0.08	1.01 ± 0.08	1.02 ± 0.14	1.05 ± 0.07	1.04 ± 0.09
Survival (%)	Clear	83.9 ± 10.1	80.0 ± 6.2	81.4 ± 6.9	80.7 ± 6.0	81.4 ± 7.1
	Green	91.8 ± 4.6	94.4 ± 1.9	91.5 ± 3.6	85.9 ± 8.7	91.3 ± 5.7
Yield (kg/m^2)	Clear	0.80 ± 0.13	0.77 ± 0.14	0.77 ± 0.13	0.78 ± 0.04	0.78 ± 0.12
	Green	0.58 ± 0.05	0.58 ± 0.04	0.56 ± 0.09	0.53 ± 0.09	0.57 ± 0.07
AFI* (kg/m^2)	Clear	1.58 ± 0.18	1.65 ± 0.21	1.66 ± 0.22	1.76 ± 0.14	0.95 ± 0.10
	Green	1.28 ± 0.23	1.12 ± 0.24	1.17 ± 0.12	1.25 ± 0.14	1.23 ± 0.15
FCR*	Clear	1.99 ± 0.25	2.18 ± 0.37	2.18 ± 0.33	2.27 ± 0.18	2.16 ± 0.28
	Green	2.47 ± 0.26	1.96 ± 0.10	2.13 ± 0.18	2.44 ± 0.54	2.15 ± 0.34

*AFI, apparent feed intake per area of culture (kg/m^2); FCR, food conversion ratio.

In study 3, there were no significant differences in shrimp survival among the different feed treatments ($P > 0.05$). Final shrimp survival rates ranged from 89.6 to 94.6%. On the other hand, there were differences in weekly growth rates and final shrimp body weight between shrimp fed the experimental diets ($P < 0.05$, Fig. 2). Shrimp growth decreased as fish meal was replaced by SPC from 0.69 to 0.58 g/week in shrimp fed diets with 2% fish oil and from 0.74 to 0.53 g/week in shrimp fed diets with 1% fish oil. The slowest growth was found for diets deprived of fish meal (year 2025), regardless of fish oil inclusion (1 or 2%). On the other hand, there was no difference in shrimp final body weight when diets with 12 or 8.5% were compared at both levels of fish oil use (1 and 2%). However, Tacon and Metian's (2008) forecast of 5% inclusion of fish meal in shrimp feeds for 2020 was only feasible if fish oil remained at 2% inclusion. In addition, the present work has shown that the minimum fish oil: soybean oil ratio possible without significant growth decline was 1.1:1. Under the conditions of the present study, the lowest possible combinations of dietary inclusion levels of fish meal (FM) and fish oil (FO) level were 5%FM-2%FO (diet 2020) and 8.5% FM-1% FO (diet 2015). It remains to be known which one is more economically advantageous.

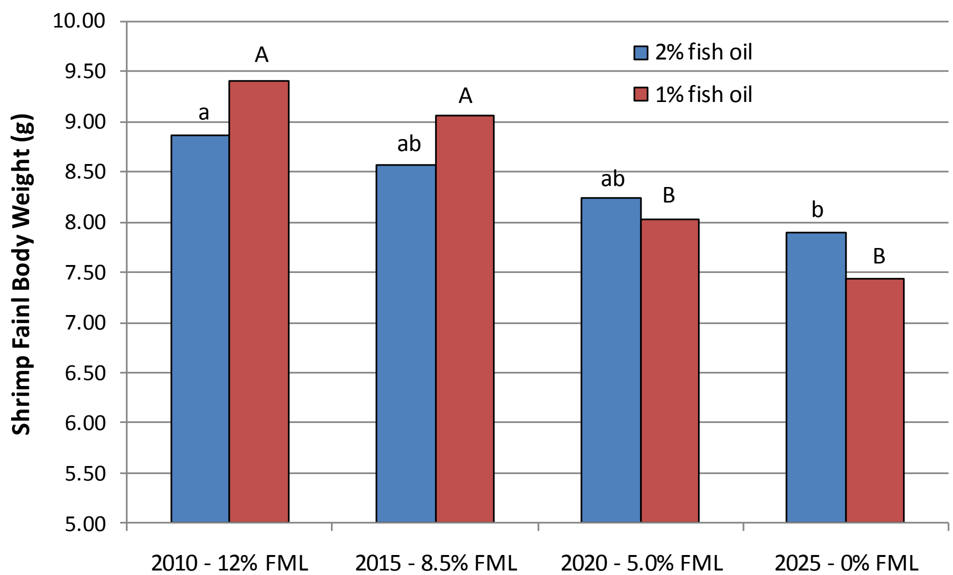

Figure 2. Final body weight of *L. vannamei* reared for 10 weeks in an experimental clear-water system. Shrimp were fed diets with progressive replacements of Anchovy fish meal (FML) by soy protein concentrate, at two fish oil inclusion levels, 2% (standard) and 1% (experimental). Bars with common letters denote non-statistically significant differences (lowercase for 2% and capital letters for 1% fish oil inclusion) at $\alpha = 0.05$ according to Tukey's HSD. The year indicates the Tacon and Metian's (2008) forecasts for the FML in marine shrimp practical diets, except 2025.

Nunes, A. *et al*. 2010. Meeting the challenge of no fishmeal in practical diets for *Litopenaeus vannamei*: case studies from Labomar, Brazil. En: Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0. Universidad Autónoma de Nuevo León, Monterrey, México, pp. 485-501.

4. Conclusions

Results from the present study have shown that *L. vannamei* growth, body weight, survival, yield and FCR were supported by HMTBa supplementation when 150 g/kg of fish meal was replaced by vegetable protein ingredients, namely soybean meal, at 50% and 100%. Trends towards improvements in feed intake were observed for diets with reduced fish meal and HMTBa supplementation. The addition of poultry by-product meal, squid meal and HMTBa may have helped prevent reductions in palatability typically associated with reductions in fish meal content. Further research will be necessary to empirically determine the optimal methionine level in pratical diets for *L. vannamei*. Overall, results indicated that under experimental conditions, it was possible to obtain an improvement in shrimp growth and greater economic returns when diets with lower fish meal levels were supplemented with HTMBa.

Results from study 2 is in agreement with previous investigations with fish which also concluded that Krill meal can partially replace fish meal without unfavorable effects on growth performance and health. Although Krill meal is still more costly than fish meal, its inclusion allowed replacing of expensive ingredients, namely fish oil, soybean lecithin and cholesterol. Consequently, it was possible to achieve formula savings when Krill meal was used without detrimental effects to shrimp growth performance.

Finally, study 3 indicated that there was no negative effect on *L. vannamei* performance when fish meal was reduced from 12% to 8.5% using SPC as a substitute. At these fish meal levels, there was also no loss in shrimp growth when fish oil was reduced from 2 to 1%. A further reduction in fish meal to 5% without loss in shrimp growth performance was only possible when fish oil was kept at a standard level of 2%. On the other hand, under the conditions of the present study, a complete removal of fish meal from *L. vannamei* diet caused a detriment in shrimp body weight, regardless of fish oil inclusion level. Therefore, this work confirms feasibility of the forecasts of Tacon and Metian (2008) regarding fish meal use in 2020 as long as fish oil is kept at 2%.

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