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Performance of pacific white leg shrimp fed an open-source marine resource-free diet under commercial conditions

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Abstract
Pacific white leg shrimp were stocked (150 PL m^{-3}) into one of four plastic-lined 1000 m^3 (0.1 ha) ponds. Ponds were randomly fed one of two diets in duplicate: a commercial shrimp feed (Grobest® Vista Eco), or an open formula, experimental, fishmeal and fish oil-free diet (F3). Shrimp were fed by hand and automatic feeders using commercial feed tables for 56 days. Sub-samples of shrimp (n = 50) were weighed weekly from each pond to evaluate growth and adjust feeding rates. Following harvest, no differences were observed between diets for survival. Combined pond biomass was 2045.0±45.96 kg (S.D.) and 2302.5±28.99 kg for commercial and F3 shrimp respectively. FCR were 1.15±0.00 and 1.03±0.01 (P< 0.05). Individual F3 fed shrimp were significantly (P< 0.05) heavier at trial termination with animals fed the commercial diet weighing 18.03±0.24 g versus 23.25±0.84 g (P< 0.05). Total hemocyte counts were 33.26±0.46 and 33.88±0.72, that for semi-granular cells 20.19±1.65 and 18.72±0.81, granular cells, 15.20±1.39 and 15.56±0.15, hyaline cells, 64.62± 3.01 and 65.72±0.96, and phenoloxidase activity, 56.87±6.12 and 50.29±0.55, for commercial and F3 fed shrimp respectively. A basic economic analysis indicated increased profitability for the F3 fed shrimp (US$7362.43 versus US$10665.62) with a return on investment (ROI) of 64.5% for the commercial feed and 89.8% for the F3 ponds. There were no differences between samples for proximate composition, cooked tail color as assessed by a BASF Salmon Color Fan or texture, as determined by a texture analyzer. Results from this trial unconditionally demonstrate the practicality of cultivating white leg shrimp using marine resource-free-based diets without impacting growth performance or consumer acceptability, while returning increasing ROI.

Keywords: *Litopenaeus vannamei*, pond culture, water quality, color, texture

Introduction
Global food production systems must become more sustainable to ensure the continued supply of high-quality foods and avoid ecosystem destabilization. In this regard a significant challenge for aquaculture will be the reduction or elimination of its addiction to marine resources as feed ingredients. Currently the aquaculture industry uses around 86% of global fishmeal (FM), 73% of fish oil (FO) [1] and most commercially caught krill.[2] However, by 2030 the sector is projected to increase harvest by ~30% above 2018 levels while, over the same timeframe, it has been estimated that FM/O production will only increase by 1 and 7% respectively. [3] Accordingly, if aquaculture is to maintain its current upward production trajectory, it will have to severely reduce or eliminate its reliance on marine resources. [4, 5] For the shrimp feed industry to abandon the habit of using marine resources in prepared diets, alternative feedstuffs must not have any negative consequences to the production performance of the animal. Alternative ingredients must, therefore, be readily available and of invariable quality, known digestibility, satisfying the nutritional needs of the target animal, be competitively priced, and suitable for prevailing processing technologies.[6] Moreover, they must not negatively compromise product quality both from processing and consumer standpoints. The feasibility of meeting the foregoing requirements, while being a big ask, has already been accomplished to a certain extent. Thus, since the beginning of the century there has been a continuous and significant reduction in the amount of FM/O used by shrimp feed manufacturers [1, 5].

The potential to eliminate marine resources from shrimp feeds is demonstrated by a growing number of laboratory trials that have employed various animal and vegetable protein substitutes for FM (Table 1). In some of these studies the alternative ingredients employed have negatively impacted shrimp growth when compared against FM/O control feeds, especially when a single alternative protein source has been applied. This experience is perhaps not too surprising given that aquafeeds comprise a multitude of ingredients and that a single alternate foodstuff is unlikely to provide the balance of nutrients required by growing animals – i.e., lacks substitutability. [6] In any case, it has been suggested that the scale-up of a single ingredient source, such as poultry by-product, feather and insect meals, while possible in the near-term, is unlikely to satisfy all future demands of the aquafeed sector. [2] When blends of alternate feedstuffs have been used for shrimp diets improved responses have, however, been observed (Table 1). Application of multiple protein and oil sources thus allows the aquafeed manufacturer to replace specific ingredients and avoid supply or demand shocks and accompanying price hikes while minimizing negative nutritional outcomes. [2]

Comparatively few studies have assessed the value of marine resource-free feeds in pond settings (Table 1) and there is a requirement for such practical studies to corroborate laboratory findings. Here, under farm conditions, we compare an open formula FM/O-free feed (F3), validated for its efficiency in the laboratory,[7] against a closed formula, widely used, commercial diet. The objectives of the trial were to assess differences in growth, survival and profitability between feeds, and the impact of diet on body coloration and product quality.

Table 1: Example response of white leg shrimp to complete replacement of fishmeal with alternate proteins, when held in various systems.

System	Alternate protein(s)	Feed rate	Age/size @ start	Density (m^{-2})	Trial length (d)	Observations versus experimental or commercial FM-based diets	Reference
Aquaria	SBM	Satiety 6x/d	~1.02 g	16/65 L	56	↓ wt, PER, body protein and moisture and phosphorus v FM diet (*P*< 0.05)	52
Aquaria	PBM	To xs 4x/d	~0.17 g	100	62	↓ wt, (*P*< 0.05), survival, body composition and FCR similar	53
Tanks	eSBPB + egg	Fixed 4/d	~1.13	30	42	No difference in wt gain, FCR or survival.	28
Ponds	NuPro + fert	2x/d	PL_{12-16}	10	84	No difference in wt gain, similar FCR and survival	54
Ponds	eSPBM, SBM + fert	est. FCR 1:1.5<2:1	PL_{10} (~0.82g)	25	90	No difference in wt gain, similar FCR and survival	55
Ponds	SBM, PBM	FCR 1.2	~31.2g	35	126	No differences in wt, FCR, survival (P > 0.05)	22, 23
Tanks	PBM	Satiety 3x/d	~0.22 g	40/ 260 L	60	↓ wt, PER (*P*< 0.05) No difference in body composition	56
Tanks	SBM, CM, WG	Satiety 5x/day	~0.3 g	50	95	↓ wt, SGR and PER (*P*< 0.05) ↑ FCR (*P*< 0.05)	57
Ponds	SBM, PBM, FPM, DG	FCR: 1.2 2x/d	~0.04 g	35	126	No differences in wt, FCR, survival (*P*> 0.05)	58
Concrete raceway	SPC, SM	Satiety 5/d	~7.0 g	8	60	No difference in survival, ↓ wt gain, PER and ADCP (*P*< 0.05), ↑ FCR and FI (*P*< 0.05).	59, 60
Cages in pond	SBM, CM, WM	≤10% biomass 2x/d	~1.08 g	10	35	↑ wt gain (*P*< 0.05)	61
Tanks	PBM, SBM, SCP, WGM	Satiety 4x/d	1.58 g	50/ 250 L	56	No difference in survival, wt or ADG or SGR	7
RAS	CPC, seSBM + krill oil	FCR 1.8 4x/d	~0.15 g	80	42	No difference in survival, wt or FCR v. fish hydrolysate	62
Cages in tanks	CAP/BM	4x/d @ 2-5% body wt	2.78 g	40	56	Negative effect on FCR, wt, FI, survival, PRE, (*P*< 0.05). ↓ meat yields associated with ↑ moisture and ↓ protein (*P*< 0.05)	41
Tanks	SBM, fSBM	5x/d @ 6% body wt	5.53 g	15/ 70 L	56	No difference in survival, wt, SGR or FCR, ↑ protein (*P*< 0.05)	63

Abbreviations: BM = bone meal, CAP = *Clostridium autoethanogenum* protein, CM = corn meal, CPC = corn protein concentrate, DG = distiller's grain, FPM = field pea meal, (f) SBM = (fermented) soybean meal, SCP = single celled protein, SM = shrimp head meal, SPC = soy protein concentrate, PBM = poultry by-product meal, NuPro = yeast extract high in nucleotides, WG = wheat gluten meal, WM = wheat meal.

Materials and methods

Feeds, animals, and husbandry

The formula of the open-source experimental diet is presented in Table 2, while proximate and amino acid composition of the commercial (Grobest® Vista Eco) and experimental feeds are presented in Table 3. Shrimp were stocked into four circular plastic-lined ponds (800 m^3 capacity) at 150 PL m^3 (120,000 pond^{-1}). Ponds were then randomly assigned one of the two diets. Shrimp were fed by hand and automatic feeders using commercial feed tables for 56 days. Sub-samples of shrimp (n = 50) were weighed weekly from each pond to evaluate growth and to adjust feeding rates. Water quality parameters throughout the trial were indistinguishable between ponds and were as follows: temperature: 28.38±0.85 ºC; DO_2: 6.64±0.12 mg ml^{-1}; pH: 7.83±0.17; salinity: 20.31±2.10 g L^{-1}; alkalinity as $CaCO_3$: 113.50±10.25 mg L^{-1}; TAN 0.85±0.91 mg L^{-1}; nitrite: 15.64±41.07 mg L^{-1}; nitrate: 38.06±48.14 mg L^{-1}. Supplements to each pond included lime: 9.76±5.94 kg; minerals: 3.84±0.86 kg. Water exchange rates were the same across all ponds: 12.43±9.49%. Each pond was aerated using 4 x 7 hp blowers connected to 100 well-distributed Aero-Tubes® and a 3 hp paddlewheel of 9 m length (15 paddles). At trial end shrimp were harvested and a sub-sample of 50 animals taken from each pond for further analyses.

Table 2: Ingredients of experimental fish-free feed used to compare the performance of white leg shrimp against a commercial diet.

Ingredient	%
Wheat, hard, grain	28.34
Soybean meal, solvent extracted	26.10
Menon MrFeed Pro50	18.00
Poultry by-product meal, pet food grade	13.00
Wheat gluten meal	5.00
Dicalcium phosphate	2.80
Algae oil, Veramaris	2.10
Lecithin	2.00
Vitamin Premix ARS 702	1.00
Lysine-HCL	0.58
Stay-C	0.50
DL - Methionine	0.38
Trace min premix ARS 1520	0.10
Cholesterol	0.10
Total	100.00

Table 3: Proximate composition, amino acid profile and peroxide values of commercial and experimental feeds.

Composition	Grobest®	F3
Moisture (%)	10.53	9.87
Crude protein (%)	40.83	37.49
Crude fat (%)	5.61	7.45
Crude fiber (%)	2.29	2.08
Ash (%)	12.59	8.5
Total phosphorus (%)	1.40	1.73
Gross energy (Kcal/kg)	3355	3637
Mercury (Hg); (mg/kg)	ND	ND
Peroxide (meq/kg fat)	8.89	0.69 - 1.54
Amino acids (%)		
Cystine (%)	0.46	0.47
Aspatic acid (%)	3.94	3.32
Methionine (%)	0.75	0.89
Threonine (%)	1.53	1.34
Serine (%)	1.89	1.78
Glutamic acid (%)	6.63	7.07
Glycine (%)	2.15	2.06
Alanine (%)	2.02	1.72
Valine (%)	1.79	1.58
Isoleucine (%)	1.51	1.41
Leucine (%)	2.97	2.59
Tyrosine (%)	1.25	1.13
Phenylalanine (%)	1.84	1.67
Histidine (%)	1.02	0.83
Lysine (%)	2.40	2.40
Arginine (%)	2.45	2.31
Proline (%)	1.99	2.21
Tryptophan (%)	0.48	0.42
Total	**37.07**	**35.20**
Taurine (mg/kg)	1196.00	562.60

Hemolymph collection and counts
Hemolymph was collected according to the method described by Hernandez-López *et al.* [8]. The hemolymph was withdrawn from the base of the pleopod of the first abdominal segment near the genital pore. Hemolymph (0.1 mL) was collected into a syringe (26G needle) containing 0.1 mL of precooled (4ºC) anticoagulant solution (30 mM trisodium citrate, 340 mM sodium chloride, 115 mM glucose, and 10 mM EDTA at pH 7.55) [9]. The total volume of the hemolymph-anticoagulant (1/1 v/v) mixture was pooled from 10 animals resulting in a 2+ mL volume. Half of this was used for Total Hemocyte Count (THC), and the other for Differential Hemocyte Counts (DHCs). THC employed the method described by Maftuch *et al.* [10]. While DHC were undertaken according to the method described by Cornick and Stewart [11] as modified by Đặng *et al.* [12]

Phenoloxidase (PO) activity
Measurement of PO followed the procedure of Hernandez-López *et al.* [13]. As modified by Yang and Pan [14]. PO activity was assessed following measurement of dopachorme spectrophotometrically using a Thermo Scientific Multiskan Sky High Microplate reader set at 490 nm. The PO reaction was performed on a 96-well plate.

Peroxide value
The peroxide value of the feeds was determined in accordance with the AOAC 965.33/AOCS Cd 8-53 (VF) method. Briefly, samples were dissolved in acetic acid and chloroform solution with the addition of potassium iodide solution in the presence of starch. Iodide was oxidized to iodine by the active oxygen (peroxides) in the product. The liberated iodine was titrated with standardized sodium thiosulfate solution.

Texture and color determinations
Sample preparation: frozen shrimp were thawed in a refrigerator (< 4 °C) for 24 hours. Shrimp were then deheaded and the tails, with shell on, were boiled in municipal water until the internal temperature reached 63 °C, the temperature recommended by the USFDA to safely consume seafood. Shrimp were immediately strained with water and rinsed for 2 minutes with cold water and subsequently stored in the refrigerator (< 4 °C) for 12 hours. Samples were then brought to room temperature (20 °C) over a two-hour period prior to texture analysis. This ensured that all samples were analyzed at the same temperature. Fifteen cooked shrimp tails from each treatment group were deshelled and analyzed on shrimp tail segment two for texture profile analysis (TPA) using a TA.XT Plus Texture Analyzer outfitted with a 45-degree stainless steel conical TA-15 Probe (Texture Technologies Corp., Hamilton, MA, USA). The TPA method [15] was employed to determine hardness, springiness, resilience, cohesiveness, and chewiness. Color of cooked shrimp was assessed using a BASF Salmon Color Fan.

Statistical analyses
Results were assessed for differences using Student's *t*-test. A two-tailed Student's *t*-test was used to determine if any differences between treatments were detected for texture. In both cases a *P*-value of $\leq$0.05 was deemed significant.

Results
Over the 56-day trial, animals fed the commercial feed returned an average weight of 18.02±1.73 g while shrimp presented with the F3 diet were larger (23.25±1.92 g; $P<$ 0.05; Table 4; Figure 1) and illustrated a tighter size distribution (Figure 2). Specific growth rates were 6.63±0.02 and 7.09±0.07% day^{-1} ($P<$ 0.05) and final harvest weights 1293.00±318.20 kg and 1502.00±282.84 kg for the commercial and F3 fed ponds respectively (Table 4). Over the study period, there were no differences in feed intake for either diet with 2278.90±56.57 kg of the commercial diet being consumed, while that for the F3 feed was 2306±7.07 kg, yielding feed conversion ratios that differed ($P<$ 0.05) at 1.15±0.00 and 1.03±0.01 respectively (Table 4). At trial

termination animals were inspected for EMS/AHPND, EHP and WSSV (PCR tests) as an integral component of disease monitoring and ponds 2, 3 and 4 were found to be EMS/AHPND positive. However, there were no clinical signs of disease, and no differences were recorded in mean survival rates between treatment groups (Table 4), phenoloxidase activity, THC or percentage hyaline, semi-granular and granular cells.

Table 5 summarizes textural analyses of shrimp fed the two different feeds. There were no differences recorded between any of the tested parameters when diets were compared. Table 6 provides an overview of key economic parameters from the current trial, and an assessment of the overall return on investment accrued from each of the two diets investigated. There were no differences in costs associated with the preparation of ponds, their amendments with lime, chlorine, and molasses nor addition of minerals or use of disinfectants throughout the production period. The same was also true for labor costs, power/fuel employed to run pumps, aerators, and for security lighting or other inputs such as post-larvae. Differences were apparent in terms of feed contribution and cost, but this was associated with growth rates and yields returned (Table 6). Greater animal size (Figs. 1 and 2), with concomitant reduction in number of shrimps per kg resulted in the F3 fed animals having a higher value, 6.36% greater than the Grobset® fed stock. Together with the higher production yield, sales revenue was 11.8% higher for the F3 fed shrimp which elicited a more favorable return on investment (Table 6).

Table 4: Mean production parameters for Pacific white leg shrimp fed a commercial and practical fish-free feed for 56 days in commercial ponds. Data with different superscripts in a row were significantly different (*P*< 0.05).

Parameter	Grobest®	F3
Final weight (g)	18.02±1.74[a]	23.25±1.87[b]
Final yield (kg/pond)	2045.50±45.96[a]	2302.50±28.99[b]
SGR[1]	6.63±0.02[a]	7.09±0.07[b]
Feed consumption (kg)	2278.9±56.57	2306.9±7.07
FCR[2]	1.15±0.00[a]	1.03±0.01[b]
Survival (%)	85.76±16.49	75.48±6.73

1. Specific growth rate = (Ln (Wt)-Ln (W0))*100/t(d).
2. Feed conversion ratio = feed intake/weight gained

Table 5: Comparison of textural parameters of raw and cooked Pacific white leg shrimp fed on a commercial or fishmeal and fish oil-free feed (F3) under practical conditions for 56 days (n = 15 per treatment).

			Raw		
Feed	**Hardness [g]**	**Springiness [%]**	**Resilience [%]**	**Cohesiveness [%]**	**Chewiness**
Grobest®	554 ± 221	100 ± 0.1	18.0 ± 2.3	56 ± 10.3	61 ± 33.5
F3	623 ± 147	100 ± 0.1	18.0 ± 1.6	55 ± 9.1	61 ± 19.1
			Cooked		
Grobest®	593 ± 63	99.9 ± 0.6	18.0 ± 0.6	55.8 ± 2.4	63.6 ± 8.3
F3	743 ± 95	100 ± 0.02	17.8 ± 0.4	58.7 ± 3.0	78.4 ± 10.8

Table 6: Key economic data from the current trial, assessing the return on investment attained following rearing of Pacific white leg shrimp on a commercial and open-source marine resource-free feed (F3) for a period of 56 days [1]. All costs were converted from Vietnamese dong using exchange rates of 6/19/2022. [2]Return on investment = (revenue-production cost/production cost).

Parameter	Grobest®	F3
	US$ equivalent[1]	
Pond preparation	198.02	198.02
Amendments (lime, chlorine, molasses)	494.11	494.11
Minerals, disinfectants, miscellanea	843.33	843.33
Seed/PLs	1239.78	1239.78
Electricity	1076.19	1076.19
Fuel	43.05	43.05
Staff (permanent)	1291.43	1291.43
Staff (temporary)	107.62	107.62
Staff food	90.40	90.40
Feed	6030.74	6500.29
Sum (production cost)	11414.67	11884.22
Production cost per kg	3.09	2.91
Feed (kg)	4663	4719
Production yield	3978.2	4492.2
Size/kg	55	43
Price/kg	4.72	5.02
Yield (tons per hectare)	19.89	22.46
Sales revenue	18777.10	22549.84
Revenue-production cost	7362.43	10665.62
ROI[2]%	64.5	89.8

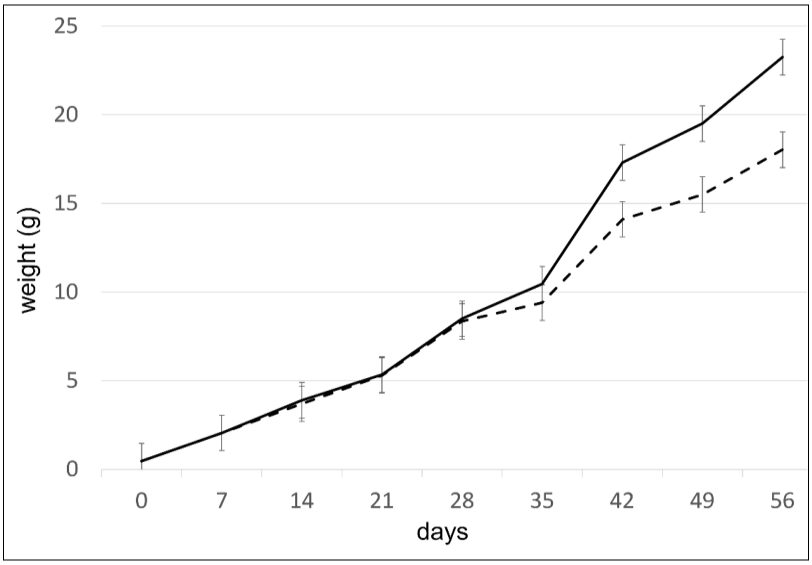

Fig 1: Combined weekly weight measurements (± S.D.) of Pacific white leg shrimp fed either an open-source marine resource-free (F3, solid line) feed or a commercial (Grobest®, dashed line) diet over a period of 56 days (n=100 per point and feed). Differences in weight between groups ($P< 0.05$) commenced from day 42 and continued until the end of the trial.

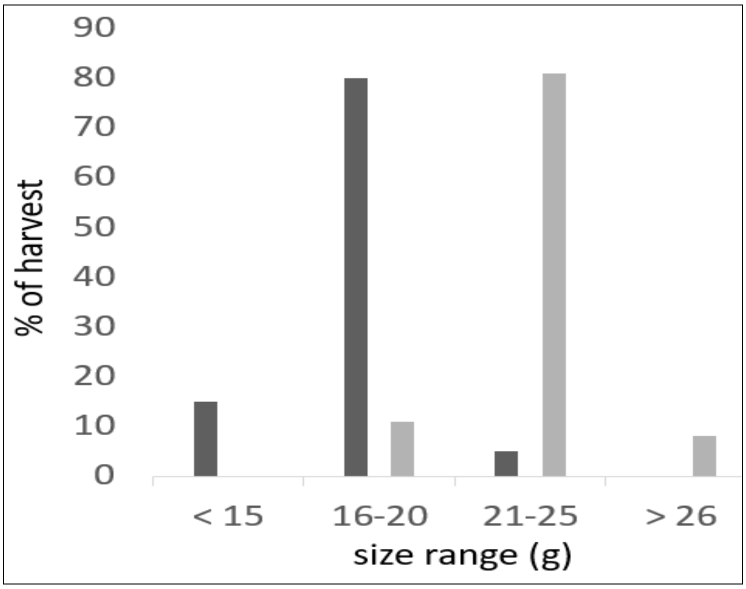

Fig 2: Size distribution of Pacific white leg shrimp following 56 days feeding with either a commercial (Grobest®) or open-source fish-free feed (F3). The dark columns are representative of Grobest® fed animals. F3 shrimp were larger and their size variation less when compared to the Grobest® fed animals.

Discussion

A plethora of studies have examined the potential for replacing FM/O from white leg shrimp diets. Commonly, single ingredient replacements, for example with a plant protein, have resulted in growth declines but, when blends of alternate proteins have been employed, no, or only limited effects on growth have been recorded, especially when animal proteins are incorporated (Table 1). These, generally laboratory-based trials, have contributed robust evidence to establish there is no need to incorporate marine-derived products in shrimp feeds. However, comparatively few published studies report the performance of shrimp fed marine resource-free diets in commercial-sized ponds. Those that exist are difficult to compare due to variations in animal age/size at trial start, differences in stocking densities employed, variable water quality and temperature regimes, the use of in-pond net cages, differences in feeding schemes and procedures (Table 1), and use of diverse animal stocks, not all of which have been specific pathogen free. Finally, different pond management strategies (e.g., fertilization and inoculation) have been used, which impacts the natural productivity of ponds [16, 17] and thus growth potential of stocked animals.

Irrespective of the differences in trial protocols, production cycle-length pond studies with marine resource-free feeds have generally described equivalent growth to experimental and commercial feeds. For example, Davis *et al*. [18]. used various diets substituting FM with poultry by-product and soybean meal, pea meal and others, together with *Schizochytrium* and vegetable oils, and reported weights that were close to those attained by shrimp fed commercial feeds over 62 days at 29 ºC. Even with natural pond productivity, however, weight gain observed was less than half that recorded in the present trial. Reid *et al*. [19] used an organically certifiable yeast-based protein and 1.8 ha ponds inoculated with endemic rotifer and copepods and fertilized with compost. They reported 76-85% survival, and FCRs of ~0.51 over a 12-week period. As observed in the present trial, shrimp fed the yeast-based diet were larger (19 v. 12 g) and expressed less size variability at harvest than control animals.[20] Nevertheless, growth performance was still poorer than observed in the current study. Other pond trials which have evaluated the impact of eliminating dietary marine resources are slightly flawed because they retain FO, or krill oil, squid meal or fish solubles as feed stimulants [21-26]. Despite these drawbacks, each still provides evidence supportive of eliminating marine resources from shrimp feeds. The present trial, therefore, might be considered preeminent since the feeds employed were truly free of marine-derived ingredients, growth performance attained was superior to that reported in other studies, and survival similar. Growth responses to the marine resource-free diet observed here were similar to others who also used diets in which FM/O were substituted by poultry and soybean meal and *Schizochytrium-Mortierella* meal [27, 29]. They reported similar growth of shrimp fed experimental and control feeds but used tanks rather than ponds but still attained poorer growth than measured herein, thereby underlining the need to take laboratory studies into the practical environment.

Between-feed differences in growth commenced at week 5 of the trial and to verify whether this resulted due to differences in feed quality, the level of lipid oxidation was examined. FM/O-based feeds can be stored under ambient tropical conditions for up to three months without negative impact on shrimp growth, [30] and since both diets used in the current trial were refrigerated, it would appear unlikely that lipid peroxidation influenced this outcome. Nonetheless, although the F3 diet had a higher lipid content, peroxide value was elevated in the commercial diet, suggesting the onset of oxidation. This may have reflected differences in the lipid type used in each feed. Fish oils, for example, are known to be very sensitive to oxidation due to the presence of high levels of poly-unsaturated fatty acids [31]. In contrast, oils derived from traustochytrids, such as *Schizochytrium* sp., as used in the F3 feed, are assumed more stable [32]. Feeding shrimp rancid diets can result in increased disease outbreaks and mortality, and reduced growth, poorer feed conversion, and profitability [33]. Reduced growth and inferior feed conversion in shrimp fed the commercial diet used in the present trial might, therefore, have been due to oxidative rancidity influencing palatability and feed conversion. Other studies, however, suggest that the peroxide value of < 9 meq/kg fat recorded for the Grobest® diet was negligible and would be unlikely to impact shrimp performance [34, 35].

Color is an important sensory attribute above that of the purely aesthetic and affects consumer attitudes towards quality and food safety. [36, 37] Indeed, color represents an important facet in setting price, especially when product is sold cooked, fresh, or frozen. [38, 39] Accordingly, given that the food industry is extremely competitive, it is essential that shrimp farmers provide products with minimal color variation. At harvest, shrimp from the present production trial, irrespective of dietary treatment, presented as a typical dark blue/slate, reflecting the color of their rearing environment [40]. In previous studies, replacement of dietary FM has been associated with diminished flesh color following cooking, an occurrence attributed to decreased dietary carotenoid intake. Thus, Yao *et al*. [41] reported an adverse effect on cooked color when replacing FM with *Clostridium autoethanogenum* protein. In the present study, however, no differences in color were recorded between the two dietary groups as determined by the salmon color fan. Thus, upon cooking, flesh color transformed into the conventional reddish-orange hue, with no variations in intensity between diets, with samples expressing a dorsal to ventral paling. A null effect on cooked color was also observed when FM was replaced by mealworm *Tenebrio molitor*. [42] In the current study, the observed lack of effect may have occurred due to additional traces of dietary carotenoids being available from the ponds. As well, the higher dietary lipid levels of the FM/O-free feed might have influenced perceived red coloration [43].

Another important sensory characteristic that influences overall consumer delight, purchasing and repurchasing decisions, is muscle texture. [44] Shrimp are generally described as conveying a tender and delicate texture; a feature that may differ due to diet, rearing environment, and storage. For example, texture measurements increase in western blue shrimp (*Litopenaeus stylirostris*) as dietary crude protein increases and lipid levels decline [45], observations corroborated by a study in which water boatmen (*Trichocorixa* sp.) were used as an alternate protein. [46] Storage on ice also changes texture, an effect that may be enhanced due to differences in feed protein quality. [47] Using the same model texture analyzer used here, shrimp have been observed to become dry and rigid following freezing (-18 ºC), [48] but these results may reflect incorrect handling since freezing can encourage protein aggregation and dehydration after thawing. Other studies report either no change in texture, or muscle softening after freezing at -20 ºC. [49, 50] Textural defects can also occur because of elapsed storage conditions, time between harvest and freezing [51] and method of freezing employed. In the present trial animals were carefully frozen immediately following harvest and it is thus unlikely that samples experienced any serious storage-related degradation.

Conclusion

The present study demonstrates emphatically that Pacific white leg shrimp can be reared on marine resource-free feeds. Moreover, feeds, such as the open-source diet used herein, have no negative effects on shrimp health or quality characteristics. Indeed, superior growth and increased return on investment was realized in this trial, executed under commercial conditions. Further large-scale trials are warranted, with more comprehensive organoleptic assessments, in distinct markets since regional differences in consumer delight for shrimp are evident. Application of our findings, which are supported by a growing body of scientific evidence, provide the shrimp farming sector the means to become environmentally sustainable while nourishing industry growth.

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