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TECHNICAL REPORT

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Project Manager: Alberto J.P. Nunes
To:
AKVAFORSK

Growth performance of *Litopenaeus vannamei*, fed diets containing Krill meal and Krill oil from the Antarctic Krill, *Euphausia superba*, in replacement of fishmeal, fish oil, soy lecithin and cholesterol

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1 – RESEARCH PROJECT IDENTIFICATION

Contract Research ID	NUT25052007-A
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Project Title	Growth performance of the white shrimp, *Litopenaeus vannamei*, fed diets containing Krill meal and Krill oil from the Antarctic Krill, *Euphausia superba*, in replacement of fishmeal, fish oil, soy lecithin and cholesterol
Project Manager	Alberto J.P. Nunes E-mail: albertojpn@uol.com.br
Trial Location	Laboratory of Shrimp Nutrition Center for Studies on Coastal Aquaculture LABOMAR - Instituto de Ciências do Mar Eusébio, State of Ceará, BRAZIL
Start Date	September 21, 2007
End of Trial[1]	December 16, 2007
Report Date of Submission	January 30, 2008

2 – PROPOSED RESEARCH PROJECT

OBJECTIVES

(1) To evaluate the growth performance of juveniles of *L. vannamei* when fed diets containing Krill meal and Krill oil under partial or full replacement of fishmeal, fish oil, soy lecithin and cholesterol.

(2) To determine optimum inclusion levels of Krill meal in diets for the white shrimp in regards its growth and economical performance.

[1]Refers to the day of shrimp harvest in experimental culture tanks

3 – MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE AND SOURCE OF JUVENILES

The study was conducted in the indoor and outdoor tank facilities of the Laboratory of Shrimp Nutrition (3°50'01.55"S and 38°25'22.74"W) located in the city of Eusébio, State of Ceará, Brazil (Fig. 1). The laboratory is part of the Instituto de Ciências do Mar (Labomar) of the Federal University of the State of Ceará (city of Fortaleza, Brazil).

Juvenile shrimp (1.93 ± 0.56 g; mean ± standard deviation; n = 100) of *Litopenaeus vannamei* were collected from one commercial grow-out of the Le Crevette farm, located 18 km away from the experimental facilities. Post-larvae had been produced in the Sealife® hatchery, located in Cajueiro da Praia, State of Piauí. On September 21-22, 2007, 12,000 juveniles were transported in plastic bags containing seawater and pure oxygen (120 shrimp for every 20 l of water).

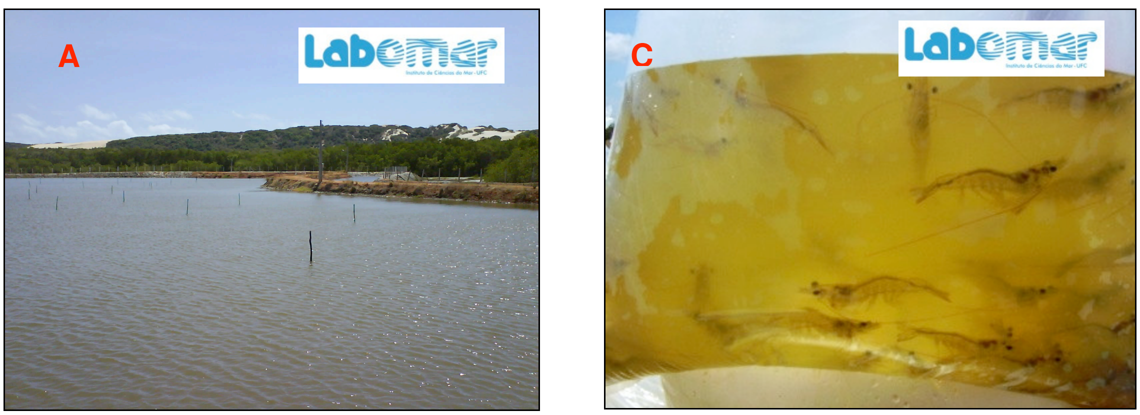

FIGURE 1.
(**A**) Le Crevette shrimp farm where juvenile shrimp was collected. (**B**) shrimp capture with a cast net. (**C**) specimens of juvenile *L. vannamei* used in the study.

3.2 SHRIMP STOCKING AND CULTURE SYSTEM

Immediately after transportation, shrimp were individually counted and stocked in rearing tanks. Shrimp originated from the same farm batch and were stocked over two consecutive days, first in indoor tanks at 57 shrimp/tank (100 shrimp/m^2), and in the next day, in outdoor tanks at 61 shrimp/tank (60 animals/m^2).

A total of 25 outdoor and 25 indoor tanks were assigned for this trial (Fig. 2). Outdoor and indoor tanks are round with a total bottom area of 1.02 m^2 and 0.57 m^2, respectively. The outdoor system have green water conditions and is continually exposed to weather changes, reflecting in a more dynamic nature of water quality. Comparatively, indoor tanks have clear water conditions (no availability of natural food), are sheltered from significant weather changes and water is continually sand filtered overnight for removal of solid wastes. Under indoor conditions, shrimp were kept under a 12-h artificial light cycle during the complete trial. Both systems are provided with constant aeration supplied from mechanical blowers. A back-up supply of pure oxygen is used in case of power failure.

FIGURE 2. (**A**) Indoor and (**B**) outdoor tanks where rearing of *L. vannamei* for evaluation of Krill meal and Krill oil was conducted.

3.3 EXPERIMENTAL DESIGN

The work consisted of four treatment feeds (**N1**, **N2**, **N3** and **N4**) containing either Eco Krill meal® or Krill oil provided by Aker Biomarine ASA (Oslo, Norway) and a basal diet (**N5**) deprived of these ingredients. Treatment feeds were designed to provide a significant formula cost reduction and result in a similar or higher nutritional composition as the basal diet. Five replicate tanks were designated for each treatment and control diet, which were randomly assigned in both the indoor and outdoor systems (Fig. 3).

Shrimp were reared for 72 days in both the indoor and outdoor tanks. On a 3.5-weekly basis, 10 animals of each tank were captured to determine their individual wet body weight in an electronic scale (Fig. 4). After weighing, all animals were returned to their respective rearing tank. No sampling was carried out three days before or after the full and new moon. Over these periods, shrimp are less active in feeding and more prone to shedding of their exoskeleton. At harvest, shrimp were evaluated in regards to their final wet body weight (g), weekly growth rate (g/week), final survival (%), yield (g/m^2) and food conversion ratio (FCR).

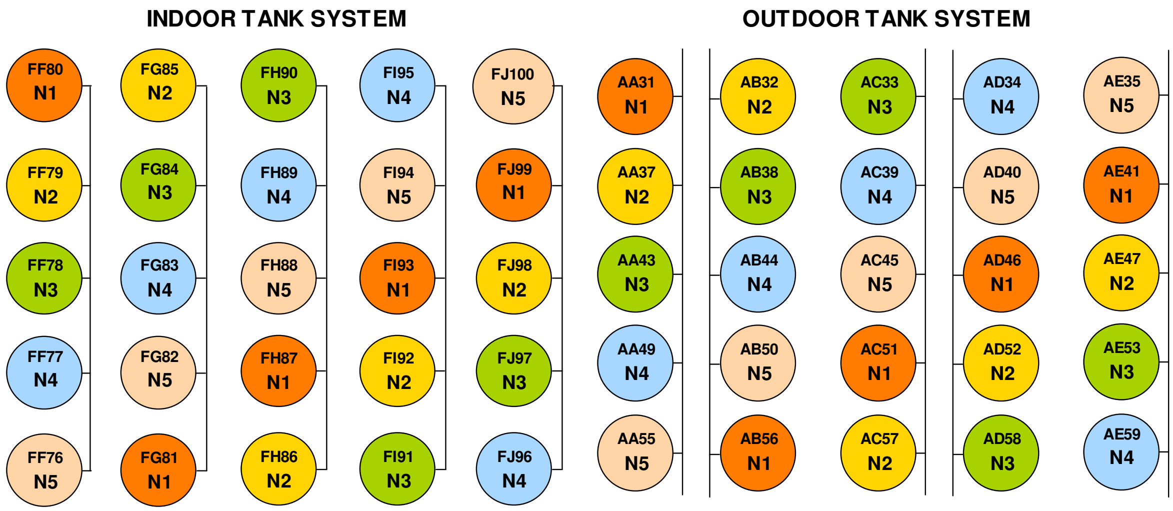

FIGURE 3. Allotment of feeds in the indoor and outdoor rearing tanks followed a random block design. Tanks in the indoor rearing system consisted of 5 individual cells, each composed of five interconnected tanks. The outdoor tanks operated independently of each other.

FIGURE 4.
Shrimp sampling in the outdoor tank system for determination of the population wet body weight during the growth trial.

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3.4 FEED FORMULAS

Initially, a basal diet (**N5**) was formulated to contain levels of fishmeal (18.75%), fish oil (2.00%), soy lecithin (1.50%), and cholesterol (0.15%) to fully meet *L. vannamei* nutritional requirements (Table 1). No addition of Eco Krill meal® or Krill oil was made in the basal diet (Table 1). Remainder formulas (*i.e.*, challenging diets) were formulated to include Eco Krill meal® at 11.00% (**N1**), 5.00% (**N2**) and 1.00% (**N5**) or Krill oil at 2.50% (**N4**) in the basal diet at the cost of fishmeal, fish oil, soy lecithin and cholesterol.

TABLE 1. Formula composition (%) and cost (US$/MT) of diets containing Eco Krill meal® or Krill oil in replacement of fishmeal, fish oil, soy lecithin and cholesterol. **N5** is the control diet.

Ingredients	US$/MT	N1	N2	N3	N4	N5
Wheat flour[1]	$350.00	25.00%	25.00%	25.00%	25.00%	25.00%
Soybean meal[2]	$210.00	34.57%	33.43%	33.93%	30.05%	30.00%
Broken rice[3]	$180.00	11.38%	10.99%	11.58%	13.45%	13.04%
Fishmeal, Anchovy[4]	$1,230.00	**0.00%**	**5.00%**	**10.00%**	**15.00%**	**15.00%**
Fishmeal, Brazilian[5]	$470.00	**0.00%**	**1.25%**	**2.50%**	**3.75%**	**3.75%**
Corn gluten[6]	$520.00	3.00%	3.00%	3.00%	3.00%	3.00%
Krill meal[7]	$1,750.00	**11.00%**	**5.00%**	**1.00%**	**0.00%**	**0.00%**
Meat and bone meal[8]	$140.00	10.00%	10.00%	5.51%	1.70%	2.31%
Fish oil[9]	$815.00	**0.80%**	**2.00%**	**1.58%**	**0.80%**	**2.00%**
Soy lecithin[10]	$800.00	**0.00%**	**0.00%**	**1.50%**	**0.50%**	**1.50%**
Cholesterol[11]	$54,000.00	**0.00%**	**0.08%**	**0.15%**	**0.00%**	**0.15%**
Krill oil[12]	$3,000.00	**0.00%**	**0.00%**	**0.00%**	**2.50%**	**0.00%**
Magnesium Sulfate	$390.00	0.15%	0.15%	0.15%	0.15%	0.15%
Potassium Chloride	$540.00	0.40%	0.40%	0.40%	0.40%	0.40%
Salt	$90.00	1.00%	1.00%	1.00%	1.00%	1.00%
Vitamin-mineral premix[13]	$9,000.00	1.00%	1.00%	1.00%	1.00%	1.00%
Synthetic Binder[14]	$2,000.00	0.50%	0.50%	0.50%	0.50%	0.50%
Bicalcium phosphate	$1,200.00	1.20%	1.20%	1.20%	1.20%	1.20%
US$/MT[15]	---	$527.25	$536.81	$579.07	$598.48	$622.28
Cost reduction (%)	---	18.0%	15.9%	7.5%	4.0%	---

[1]13.04% crude protein (CP); 1.23% crude fat (fat); 0.78% ash; 0.06% crude fiber (CF); 4,306 kcal/kg of gross energy (GE).
[2]50.00% CP; 2.10% fat; 10.12% ash; 7.74% CF; 4,722 kcal/kg GE.
[3]Usina Catende, Catende, Brazil. 8.18% CP; 1.75% fat; 0.88% ash; 0.11% CF; 3,541 kcal/kg GE.
[4]COPEINCA Corporacion Pesquera INCA S.A., Lima, Peru. 68.87% CP; 6.98% fat; 16.28% ash; 0.12% CF; 4,003 kcal/kg GE.
[5]INPEL Indústria de Resíduos de Pescado Ltda., Rio Grande do Sul, Brazil. 56.55% CP; 5.72% fat; 23.33% ash; 0.81% CF; 3,420 kcal/kg GE.
[6]Corn Products Brasil, Protenose®, São Paulo, Brazil. 62.53% CP; 9.51% fat; 4.66 ash; 0.21% CF; 4,990 kcal/kg GE.
[7]Eco Krill meal®, Aker Biomarine ASA, Oslo Norway. 59.00% CP; 25.00% fat; 10.00% ash; 0.00% CF; 2,700 kcal/kg GE.
[8]46.29% CP; 16.46% fat; 31.13% ash; 0.64% CF.
[9]98.00% fat; 8,620 kcal/kg GE.
[10]100.00% fat; 7,590 kcal/kg GE

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[11]Cholesterol XG, Solvay Pharmaceuticals BV/NL. 91% cholesterol.
[12]Aker Biomarine ASA, Oslo Norway. 98.00% fat.
[13]Rovimix Camarao Intensivo. DSM Produtos Nutricionais Brasil Ltda., São Paulo, Brazil. Guarantee levels per kg of product: vitamin A, 1,250,000 IU; vitamin D3, 350,000 IU; vitamin E, 25,000 UI; vitamin K3, 500.0 mg; vitamin B1, 5,000.0 mg; vitamin B2, 4,000.0 mg; vitamin B6; 10.0 mg; nicotinic acid, 15,000.0 mg; pantothenic acid, 10,000.0 mg; biotin, 150.0 mg; folic acid, 1,250.0 mg; vitamin C, 25,000.0 mg; cholin, 50,000.0 mg; inositol, 20,000.0 mg; iron 2,000.0 mg; copper, 3,500.0 mg; chelate copper, 1,500,0 mg; zinc, 10,500.0 mg; chelate zinc, 4,500.0 mg; manganese, 4,000.0 mg; selenium, 15.0 mg; chelate selenium, 15.0 mg; iodine, 150.0 mg; cobalt, 30.0 mg; chromium 80.0 mg; filler, 1,000.0 g.
[14]Pegabind™, Bentoli Agrinutrition, Texas, USA. Synthetic pellet binder composed of urea formaldehyde.
[15]FOB prices at origin.

Formulas **N1**, **N2** and **N3** progressively reduced the contribution of fishmeal to total feed protein input in comparison to the basal diet (**N5**) while increasing the participation of Eco Krill meal®. Concomitantly, meat and bone meal was added to meet targeted feed protein levels. As a result, treatment formulas **N1**, **N2** and **N3** contained a lower amount of marine animal protein sources at the cost of increased levels of meat and bone meal and vegetable protein ingredients (Fig. 5). Formulas **N1**, **N2** and **N3** also progressively reduced the inclusion of fish oil, soy lecithin and cholesterol as higher levels of Krill meal was used. In formula **N4**, Krill oil was used in full replacement of cholesterol and partial substitution of fish oil and soy lecithin (Table 1). In this case, all other macro ingredients maintained the same inclusion level as the basal diet (**N5**), except in regards to meat and bone meal which reduced to compensate a higher fat input as a result of Krill oil addition. As fishmeal, fish oil, soy lecithin, and cholesterol were replaced by Eco Krill meal® or Krill oil, a significant formula cost reduction was observed, ranging from 4% to 18%.

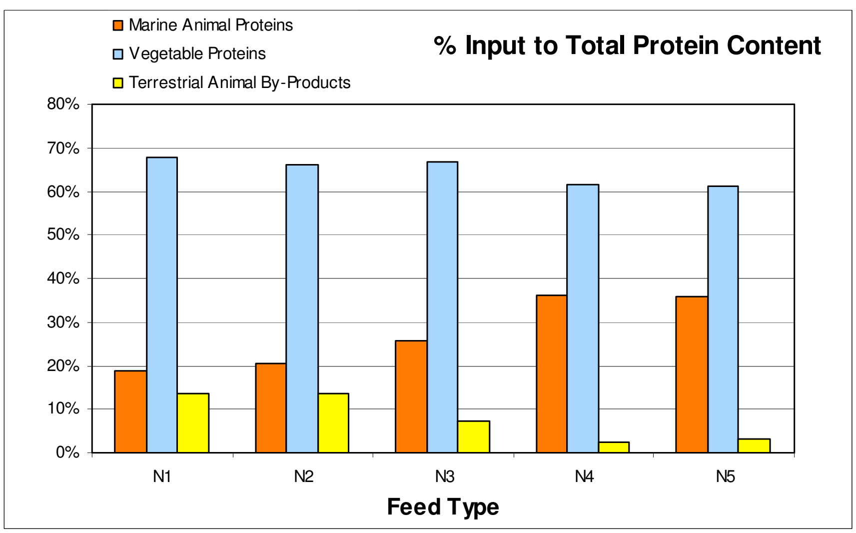

FIGURE 5. Percentage input of major group of macroingredients to total protein content of formulated feeds used in the growth performance trial with *L. vannamei*.

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3.5 FEED MANUFACTURE

Feeds **N1**, **N2**, **N3**, **N4**, and **N5** were designed to be nearly isonitrogenous and isocaloric and result in a similar proximate chemical composition. For formulation, the Solver tool from Microsoft Excel 2003 was used. Diets were manufactured with laboratory-scale feed equipment. Initially all grain ingredients (except wheat flour) were grinded in a coffee-grinder to achieve a particle size lower than 400 microns. Marine animal by-products were sieved through a 225-micron mesh net.

After grinding or sieving, all dry and liquid ingredients were weighed to a 0.01 electronic scale and mechanically mixed during 15 minutes. Boiling water was added to the ingredient mixture at a rate of 2:1 and allowed to mix for 20 minutes until a feed dough was produced. The feed dough was then transferred to a pot for steam-cooking during 40 minutes when the temperature of the feed dough reached 95°C. After steam-cooking, the feed cake was immediately moved to a meat mincer for double extrusion of feed particles to a 2-mm diameter. All feed pellets in a spaghetti-like format were dried in a convection oven at 70°C. Feed was turned over twice every two hours, prior to disintegration of pellets to a 5-mm length with a food chopper. After disintegration of pellets, an additional one-hour of drying was allowed to reach a final feed moisture level of 8-10%. Finished diets were stored in air-tight containers at -20°C until use (Fig. 6)

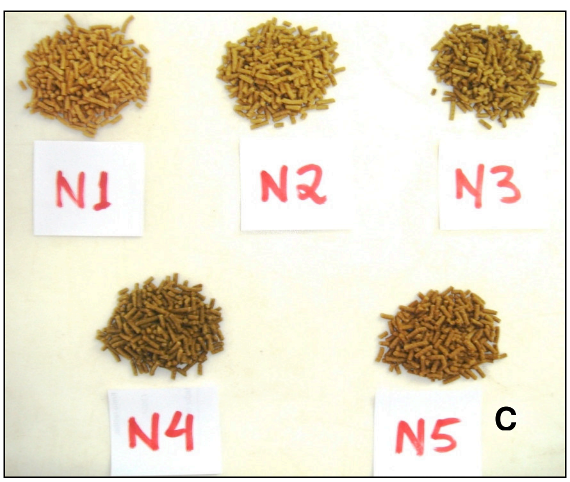

FIGURE 6.
(**A**) major ingredients replaced by Eco Krill meal® and Krill oil (Aker Biomarine ASA) used in a performance trial with *L. vannamei* in both clear and green water culture systems. (**B**) feed strands after oven-drying and prior to disintegration to 5.0 mm pellets. (**C**) lab-manufactured diets used in growth trials with *L. vannamei*. Feeds contained either Eco Krill meal® or Krill oil in replacement for key ingredients in formulation.

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3.6 FEEDING AND MANAGEMENT

Prior to the start of comparisons, shrimp were first reared for 10 days on a commercial extruded diet (Aquaxcel™, Burris Mill Aquaculture, USA). Feed contained 42% crude protein and 9% fat (Aquaxcel™ 4209 - 2.0 mm, Burris Mill Aquaculture, USA). After the acclimation period, shrimp started to be fed on laboratory-manufactured diets. Shrimp were continuously exposed to feed over 24-h periods. To avoid feed loss in tanks and rapid water quality deterioration, all feed was delivered in feeding trays made from a rectangular nylon mesh of 141 mm in diameter surrounded by a PVC circular frame of 150 mm in diameter and 3.5 cm in height (Fig. 7). Trays were installed in the middle of each tank bottom at a density of one unit per tank.

FIGURE 7.
Feeding trays used during the growth trials with *L. vannamei*.

Animals were fed twice daily at 0730 h and 1600 h over the complete rearing period. Meals were delivered on a feed consumption basis, allowing rough changes in feed rations to take place at each feeding time. When necessary, adjustments in meals were carried out in respect to each feeding time (*i.e.*, feed remains at 0700 h to adjust feed rations for 0730 h the next day). A protocol was used to adjust rations 25% above or below original calculated meals (Table 2). On Sundays, shrimp were fed only in the morning with 100% of the daily meal. In this case, uneaten feed were collected the next day after a 24-h immersion period.

TABLE 2. Feed table used during the indoor growth trials with *L. vannamei*.

% Amount of Feed Left in Trays	Feed Ration Adjustment
< 25%	No change in feed ration
≥ 25%	Deliver 75% of original feed ration
No feed remains	Increase 25% of original feed ration

Water quality parameters (*i.e.,* pH, temperature and salinity) were monitored once daily in the morning in each tank. In the indoor system, sand filters only operated overnight from 1700 h to 0700 h in the morning. Whenever filter pressure increased, tank water was back flushed to discharge organic solid material. Back flushing took place within an interval of 7 to 15 days or depending on shrimp stage of development. In the outdoor tank system, 25%

of culture water was discharged on a weekly basis from each tank and replaced by newly pumped brackishwater.

3.7 DETERMINATION OF APPARENT FEED CONSUMPTION

Food conversion ratio (FCR) was determined on the basis of apparent feed consumption. The Apparent Feed Consumption (Cs) measures shrimp feed intake on a dry matter basis. As such, determinations of shrimp feed intake consider dry matter leaching rate for each feed in relation to their immersion period in seawater. The immersion period indicate the time feed was immersed in water prior to collection of uneaten food, if any. In this study, there were three immersion periods, 8.5, 15.0 and 24.0 h that refer to feeding intervals 0730-1600 h, 1600-0700 h and 0700-0700 h the next day, respectively. The latter feeding interval was only adopted on Sunday meals. For each feed and immersion period, feed moisture, its percentage of water absorption (WA_i) and its dry matter leaching rate (DM_i) were calculated. These parameters were determined in triplicate for each feed type.

Feed dry matter leaching rate (DM*i*) and the percentage of feed water absorption (WA*i*) were determined in 500-l tanks containing seawater, but without stocked shrimp and aeration (Fig. 8). Feed was administered in PVC feeding trays measuring 14.3-cm in diameter and boarders with 3.5-cm in height. A total of 3 g of feed was distributed in each feeding tray and collected at the immersion periods evaluated.

FIGURE 8.
For determination of the percentage of feed water absorption and feed leaching rate all feeds were immersed in seawater simultaneously and allowed to drain in feeding trays before any weighing.

The percentage of dry matter leaching (DM*i*) at its respective immersion interval was calculated by the formula: $DMi = [1-(Wdi/Wf)] \times 100$, where: DM*i* = percentage of dry matter leaching at time *i* (%); Wf = dry feed weight before immersion in seawater (g), and; Wd*i* = dry feed weight after immersion in seawater at time *i* (g). Dry feed weight refers to the feed weight after drying at 70°C for 72 h. Water feed absorption (WA*i*) in its respective immersion interval was given as: $WAi = [(Wmi - Wf) + (Wc - Wdi)/Wf] \times 100$, where: WA*i* = percentage of feed water absorption at time *i* (%); Wm*i* = wet feed weight after immersion in seawater at time *i* (g); Wmd*i* = dry weight of wet feed (Wm*i*) at time *i* (g); and, Wc = crude feed weight prior to oven-drying (g). Apparent Feed Consumption (Cs) was determined for each feeding treatment by the equation: $Cs = \sum[(Fc \times DM_i)-(Fm_i \times WA_i)]$, where: Cs = Apparent

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Feed Consumption; Fc = crude weight of feed delivered, and; Fm_i = wet weight of uneaten feed collected from feeding tray at time *i*.

In the present study, for each feed type, observations for WAi were calculated as an average value of all feeding intervals. In the case of DM*i*, values for feeding intervals 8.5 and 14.0 h were combined and the mean calculated.

4 – RESULTS AND DISCUSSION

4.1 WATER QUALITY

Water quality parameters over the growth cycle were found to be within the ideal range for the culture of *L. vannamei*. In the indoor system, no statistically significant differences (ANOVA; $P > 0.05$) were observed among feeding treatments in water salinity (32.4 ± 2.6‰; n = 1,225), pH (7.72 ± 0.23; n = 1,225), and temperature (27.4 ± 0.8°C; n = 1,225). Over the rearing period, there was an increase in water salinity, consistent with the dry season that takes place in the state of Ceará in June through January (Fig. 9). During these months, there is often an increase in water salinity in the Estuary of Pacoti river, the source of water used in shrimp nutrition trials in the lab. Water temperature remained consistent throughout the trial period, while water pH dropped. The reduction in water pH is associated with a higher organic load in culture water due to an increased shrimp biomass and higher daily rations.

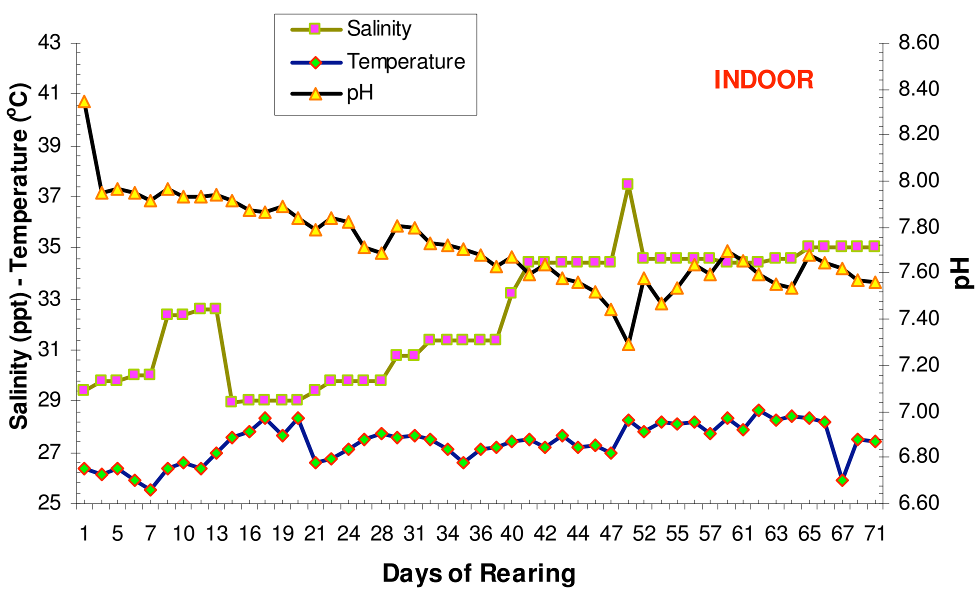

FIGURE 9. Patterns in water quality in 500-l indoor tanks stocked with *L. vannamei* at 57 shrimp/tank (100 animals/m^2). Tanks were used in trials for Aker Biomarine ASA. The system operated under a water recirculation regime. Animals were reared over a 72-day rearing cycle. Data represent means of 25 tanks.

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In the outdoor system, water salinity (36.6 ± 3.5‰; n = 1,150) and temperature (29.6 ± 0.9°C; n = 1,150) showed no significant differences among feeding treatments (ANOVA; $P > 0.05$). However, water pH was statistically higher for **N2** (8.18 ± 0.08; n = 230) and **N3** (8.18 ± 0.09; n = 230), followed by **N1** (8.12 ± 0.17; n = 230) and **N4** (8.11 ± 0.18; n = 230; Turkey's HSD; $P < 0.05$). **N5** displayed the lowest pH among all feeding treatments (8.03 ± 0.23; n = 230). A higher pH in green water tanks is often linked with a greater algae biomass. Higher levels of nitrogen and phosphorous in water can promote phytoplankton blooms and result in an increase in water pH. However, pH was within levels considered adequate for *L. vannamei*. It is unlikely the pH differences among feeding treatments interfered in shrimp growth.

As opposed to the indoor system, drops in water pH were less intense. This was favored by primary productivity and water discharge. Water exchange occurred on a weekly basis to control the build-up of organic matter on the tank bottom. On the other hand, water salinity increased up to 40‰ in comparison to 35‰ in the indoor system. The greater exposure of outdoor tanks to sunlight favored both a greater increase in water salinity and temperature in comparison to the indoor tanks. On average, water temperature in outdoor tanks was 2.2°C higher than indoor tanks. Water temperature in a parameter directly associated with shrimp growth. Water temperature in the range of 27°C and 30°C is found to be the best for the growth of penaeid shrimp. As such, the higher temperature values in the outdoor system may have enhanced shrimp growth performance.

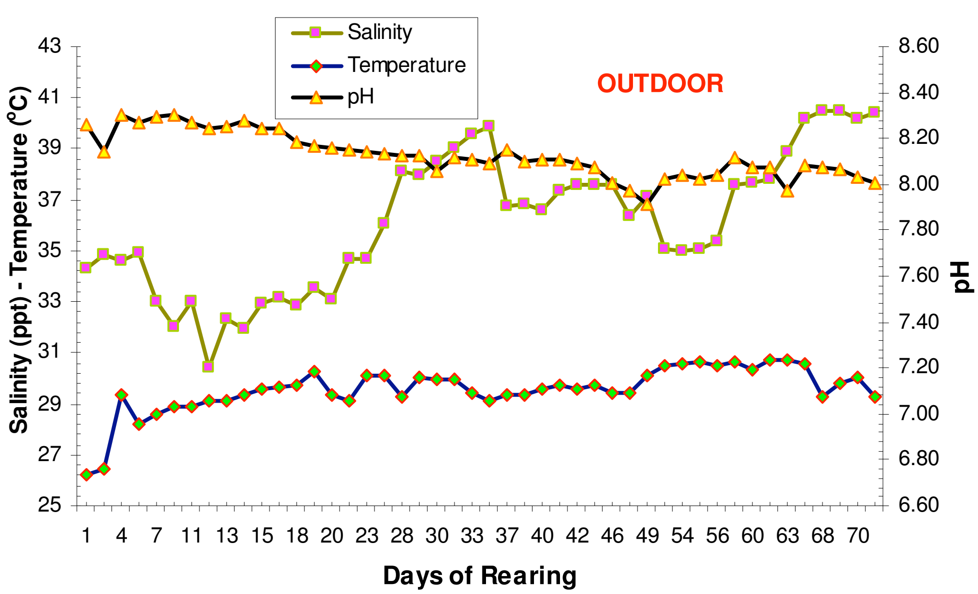

FIGURE 10. Patterns in water quality in 1,000-l indoor tanks stocked with *L. vannamei* at 61 shrimp/tank (60 animals/m^2). Tanks were used in trials for Aker Biomarine ASA. The system operated under a water recirculation regime. Animals were reared over a 72-day rearing cycle. Data represent means of 25 tanks.

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4.2 FEED PHYSICAL CHARACTERISTICS

Lab manufactured diets displayed different characteristics in regards to their physical condition. Both the feed moisture and the percentage of water absorption (WA) varied significantly among diets (ANOVA; $P < 0.05$; Table 3). Feed moisture content differed between diets **N4** and **N5**; the latter showed the lowest moisture content (Turkey's HSD; $P < 0.05$). The variation in feed moisture observed among feeds was probably due to variations in feed composition. Feed **N4**, for instance, received the highest amount of liquid ingredients (3.80%) in comparison to other formulas (less than 3.0%), although it did not vary from **N5** (3.50%). Another possibility for the variation in moisture level is the feed manufacturing process. The addition of water during ingredient mixture follows precise measurement, but conditioning of the feed dough may vary in terms of cooking temperature and amount of steam added.

In regards to WA (water absorption), differences were only observed between diets **N2** and **N5** (Turkey's HSD; $P < 0.05$). There was a significant and inverse correlation between feed moisture content and WA (Pearson correlation; $r = -0.644$; $P < 0.01$). However, variations in moisture content did reflect in feed dry matter leaching rate (DM). DM was consistent among feeds for the **8.5 + 15.0 h** immersion period (ANOVA; $P > 0.005$), achieving an average of 16.9 ± 2.3%. For the **24 h** immersion period, DM was statistically higher for **N5**, followed by **N1** and **N4**, and finally **N2** and **N3** (Turkey's HSD; $P < 0.05$).

DM is an important factor in shrimp feeds as it reflects the ability of feed pellets to maintain their physical integrity in water. As such, the greater the DM the less physical stability pellets will have in water. It is suggested that commercial shrimp feeds must remain at least 80% intact in water for a period of 4 h. However, feeds must not exceed 95% in physical stability in order to avoid negatively influencing shrimp food consumption. Shrimp feeds slowly in the tank bottom and requires handling feed pellets prior to ingestion. While high feed water stability levels can make nibbling of feeds more difficult for shrimp, low water stability can lead to a rapid water absorption by feed pellets. This can compromise their physical integrity and lead to leaching of nutrients in water. In a work conducted under indoor conditions with 11 commercial diets manufactured in Brazil, DM for a 2.5-h immersion period reached 14.2 ± 8.8% (Nunes, unpublished). Although, this value is lower than the one attained in the present work, feed immersion period used in the present work was much higher than the one used with the commercial diets.

TABLE 3. Feed physical characteristics measured through immersion in seawater in 500 l tanks. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**).

Feed Codes	Moisture Content	% of Water Absorption (WA)	Dry Matter Leaching Rate (DM)	
			8.5 + 15.0 h	24.0 h
N1	8.78 ± 0.38% ab	116.7 ± 13.2% a	15.7 ± 1.0%	63.8 ± 0.6% a
N2	10.22 ± 0.19% ab	94.3 ± 14.0% b	16.7 ± 0.9%	60.7 ± 0.4% b
N3	10.67 ± 0.33% ab	96.5 ± 12.2% ab	17.2 ± 3.4%	60.0 ± 0.1% b
N4	11.11 ± 1.26% a	106.2 ± 19.1% ab	17.8 ± 3.1%	62.7 ± 0.8% a
N5	8.22 ± 1.84% b	117.4 ± 21.7% a	17.3 ± 2.3%	66.0 ± 0.8% c
ANOVA *P*	< 0.05	< 0.05	NS	< 0.05

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4.3 SHRIMP PERFORMANCE

4.3.1 SHRIMP WEEKLY GROWTH

Shrimp grew continuously over the culture period. In the indoor tank system, weekly growth rates tended to increase as the culture period progressed (Fig. 11). On average, shrimp grew at a rate of 1.00 ± 0.01 g/week in indoor tanks. This rate is considered exceptional, particularly as shrimp were farmed under a high stocking density (100 shrimp/m^2) and in the absence of naturally occurring food sources.

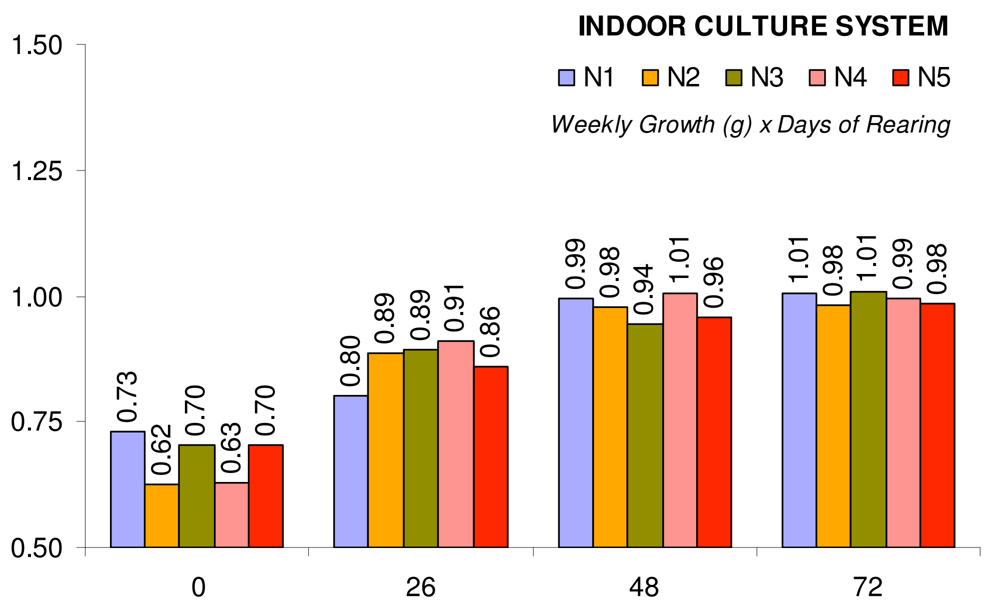

FIGURE 11. Weekly growth rates (g) of *L. vannamei* over a 72-day rearing period in indoor tanks of 500 l. Each bar represents the mean value for 50 animals collected from five different tanks. On day 72, bars indicate the mean value for all animals collected at harvest. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**).

Contrary to the indoor tanks, shrimp in the outdoor system reduced their growth rates as the culture period progressed (Fig. 12). In the start of the growth study, shrimp had reached 1.13 ± 0.09 g/week and ended with 1.04 ± 0.03 g/week at harvest. The depression in growth rates could have been the result of several factors, including: (1) body weight; shrimp as other animals reduce their metabolic rate and tissue synthesis as larger body weight is achieved; (2) water quality; deterioration of water quality parameters, especially the build-up of nitrogenous compounds in the rearing system, could have occurred leading to less favorable growth conditions; and, (3) stocked biomass; shrimp grew more than normally expected, coupled with high survival, could have led to exceeding the critical stocked biomass of the rearing system.

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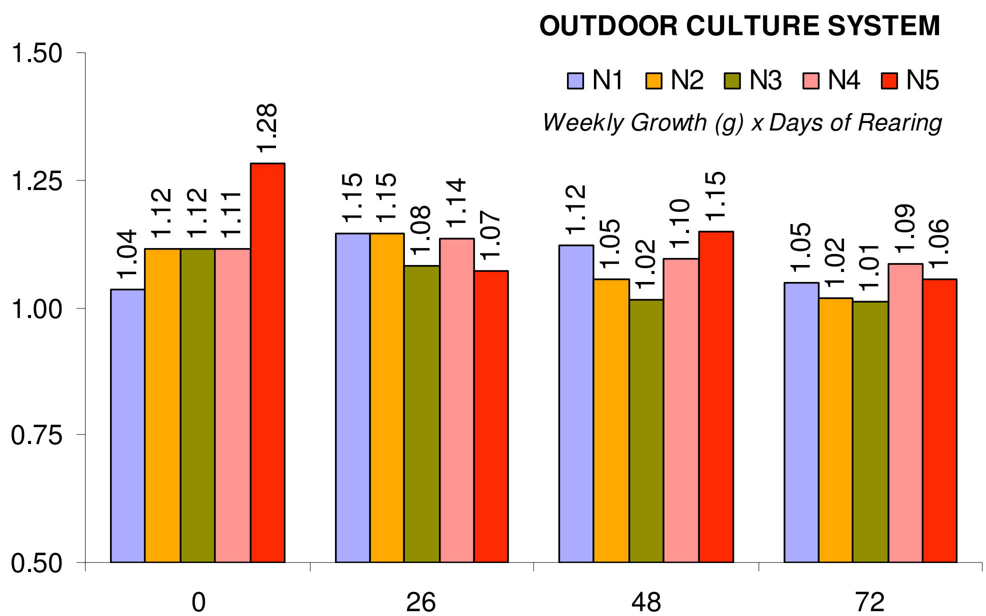

FIGURE 12. Weekly growth rates (g) of *L. vannamei* over a 72-day rearing period in outdoor tanks of 1,000 l. Each bar represents the mean value for 50 animals collected from five different tanks. On day 72, bars indicate the mean value for all animals collected at harvest. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**).

A much higher weekly growth rate was observed in the outdoor tanks system compared to the indoor system. Under outdoor conditions, shrimp consistently kept individual growth rates above 1.00 g as opposed to the indoor tanks which remained with rates below 1.00 g. These differences are due to the lower shrimp stocking density and the continuous availability of natural food in outdoor tanks. Temperature was another critical factor that favored higher growth rates in the outdoor system. On average, differences in water temperature from the indoor to the outdoor system reached 2.2 g. As tanks under the indoor system are sheltered, there is little exposure to sunlight, maintaining water temperature levels below those observed in the outdoor system.

4.3.2 GAINS IN SHRIMP WET BODY WEIGHT

In the indoor tank system, there were no statistical differences in shrimp wet body weight among feeding treatments (Table 4; ANOVA; $P > 0.05$). Gains in wet body weight were very consistent. These results indicate that replacement of Anchovy fishmeal, fish oil, soy lecithin and cholesterol by Krill meal or Krill oil had no detriment in *L. vannamei* growth. Even at higher replacement values (**N1,** no fishmeal, soy lecithin or cholesterol and Krill meal at 11%), shrimp displayed a normal growth and slightly higher than the basal diet.

Animals in the indoor system were farmed under a challenging condition, with the lack of natural food and under a high stocking density. As such, data indicates that Krill meal and Krill oil are able to fully replace fishmeal, soy lecithin or cholesterol and meet *L. vannamei*

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nutritional requirements. Since there is significant formula cost savings when the basal diet (**N5**) is compared to treatment diets (**N1**, **N2**, **N3** and **N4**) this has a very positive economical outcome to commercial feed formulation.

TABLE 4. *L. vannamei* wet body weight fed diets containing either Krill meal or Krill oil under a clear water system stocked at 100 shrimp/m^2. Values presented as mean ± standard deviation obtained from five rearing tanks. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**); and, basal diet with 18.75% Anchovy fishmeal (**N5**).

Diets	Days of Rearing/Shrimp Wet Body Weight (g)			
	0	26	48	72
N1	2.97 ± 0.77	5.95 ± 1.45	9.79 ± 1.52	13.32 ± 2.13
N2	2.82 ± 0.61	6.12 ± 0.86	9.53 ± 1.19	12.93 ± 1.80
N3	2.93 ± 0.66	6.25 ± 1.04	9.40 ± 2.04	13.32 ± 1.86
N4	2.83 ± 0.73	6.21 ± 1.70	9.72 ± 1.43	13.06 ± 2.18
N5	2.94 ± 0.67	6.12 ± 1.26	9.51 ± 1.21	13.05 ± 2.10
***P* value**	NS[1]	NS	NS	NS

[1]Not statistically different according to One-Way ANOVA

Comparatively, shrimp under the outdoor system achieved a higher final body weight compared to those reared under the indoor system (Table 5). These differences were expected as animals from the outdoor system were larger at the end of the acclimation period compared to shrimp in the indoor system. In addition, rearing conditions in the outdoor system were more favorable to growth than those in indoor tanks. Along the rearing period, differences in shrimp body weight started to be clearer on day 48 of culture.

TABLE 5. *L. vannamei* wet body weight fed diets containing either Krill meal or Krill oil under a green water system stocked at 60 shrimp/m^2. Values presented as mean ± standard deviation obtained from five rearing tanks. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**); and, basal diet with 18.75% Anchovy fishmeal (**N5**). Common letters in columns denote non-significant differences between feeding treatments according to Turkey's HSD Multiple Range Test at the $\alpha = 0.05$ level.

Diets	Days of Rearing/Shrimp Wet Body Weight (g)			
	0	26	48	72
N1	3.41 ± 0.80 a	7.67 ± 1.15 a	11.10 ± 1.73 ab	14.19 ± 2.38 ab
N2	3.52 ± 0.72 a	7.78 ± 1.28 a	10.75 ± 2.02 ab	14.00 ± 2.48 a
N3	3.53 ± 0.70 a	7.55 ± 0.82 a	10.50 ± 1.39 a	13.94 ± 2.12 a
N4	3.52 ± 0.79 a	7.74 ± 1.03 a	11.04 ± 1.27 ab	14.69 ± 2.11 b
N5	3.76 ± 0.71 a	7.75 ± 1.05 a	11.63 ± 1.71 b	14.62 ± 2.22 b
***P* value**	NS[1]	NS	< 0.05	< 0.05

[1]Not statistically different according to One-Way ANOVA

After 48 days of rearing, shrimp fed **N3** were statistically smaller than those fed the basal diet (**N5**; Turkey's HSD; $P < 0.05$). At harvest, shrimp fed **N4** and **N5** achieved larger body weights compared to those fed **N1** and **N2** (Turkey's HSD; $P < 0.05$). On the other hand, no differences were found between **N4**, **N5** and **N1**.

The inclusion of Krill oil in diet **N4** enhanced *L. vannamei* growth. In diet **N4**, as Krill oil was added at the cost of cholesterol, and partially, of fish oil and soy lecithin, there was no impact on fishmeal inclusion levels. As such, this diet was probably superior in terms of essential fatty acids (EFA) and phospholipids when compared to **N5** and other diets. Although shrimp fed diets **N2** and **N3** achieved a lower body weight at harvest compared to the basal diet, there was still a significant savings in formula cost which compensates these differences in weight gain. The inconsistencies in shrimp wet body gain in relation to diet type found between the two culture systems is probably a result of the different survival rates achieved between the two systems.

4.3.3 FINAL SHRIMP SURVIVAL

Final shrimp survival in both the indoor and outdoor culture systems can be considered excellent. For both systems, there were no statistically significant differences in final survival among feeding treatments (ANOVA; $P > 0.05$; Figs. 13 and 14). However, differences were observed for the same feed used under the indoor versus outdoor condition. On average, a lower survival rate was observed for the indoor system (mean of 81.9%) compared to the outdoor (mean of 91.4%). As under the outdoor system, culture conditions impose less stress to farmed shrimp, a higher survival rate is often achieved.

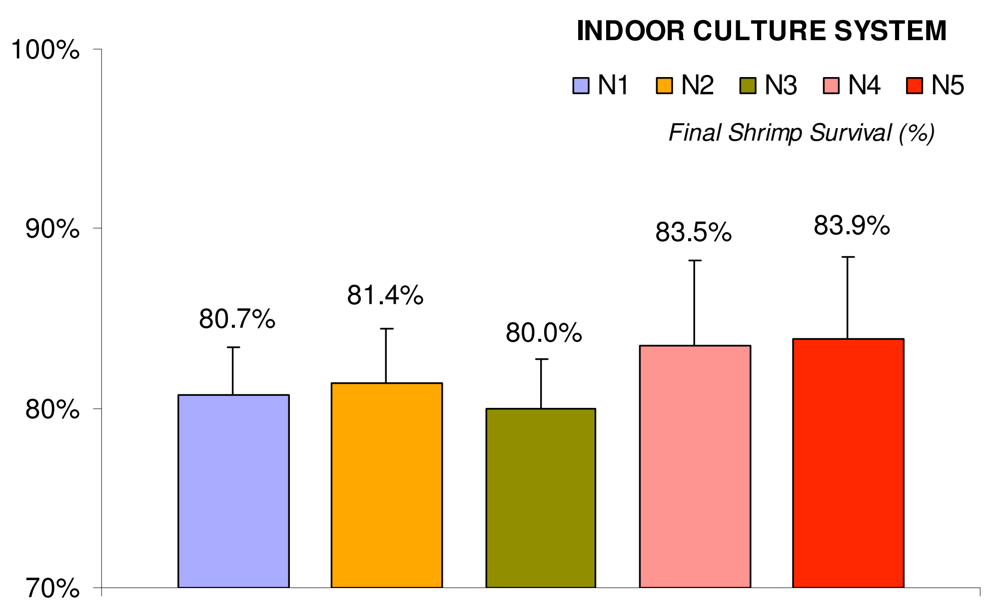

FIGURE 13. Final survival of *L. vannamei* farmed for 72 days under a clear water culture system under 100 shrimp/m^2. Data presented as mean ± standard error of five different tanks. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**).

On the other hand, it is expected that under more challenging culture conditions, nutrients to overcome stress are more important than those used for growth. As such, under the indoor culture conditions, while statistically significant differences were not observed, survival rate was marginally higher for shrimp fed **N4** and **N5** compared to other feeds. It is clear that these two feeds provided a greater protection for shrimp compared to other diets. It is interesting to note that a similar result was not observed when Krill meal was included at higher (**N1**, 11%) or lower level (**N3**, 1%). Perhaps this effect was not related to Krill meal or fishmeal inclusion themselves, but to a reduction in other ingredients, known to supply key nutrients to shrimp. This includes fish oil, soy lecithin and cholesterol which provide essential dietary nutrients such as fatty acids, phospholipids and sterols. Shrimp are not able to synthesize *de novo* sterols and phospholipids. Cholesterol is an essential sterol involved in the molting process in shrimp. Phospholipids are important in cholesterol transport, facilitate the storage of lipids in the hepatopancreas, an important energy reserve during the molting process and are an important component of cell membranes.

Under the outdoor system, final shrimp survival increased for all feeds (Fig. 14) when compared to the indoor system. The increase was 6.4, 12.4, 18.0, 11.9 and 9.5% for feeds **N1**, **N2**, **N3**, **N4** and **N5**, respectively. Although all survival rates achieved in the outdoor condition could be considered exceptional under a commercial setting, these results suggest that diet **N1** probably lacked one or more key nutrients in relation to other diets. In outdoor tanks, nutrient deficiencies can be offset by the consumption of natural food sources other that the artificial feed itself.

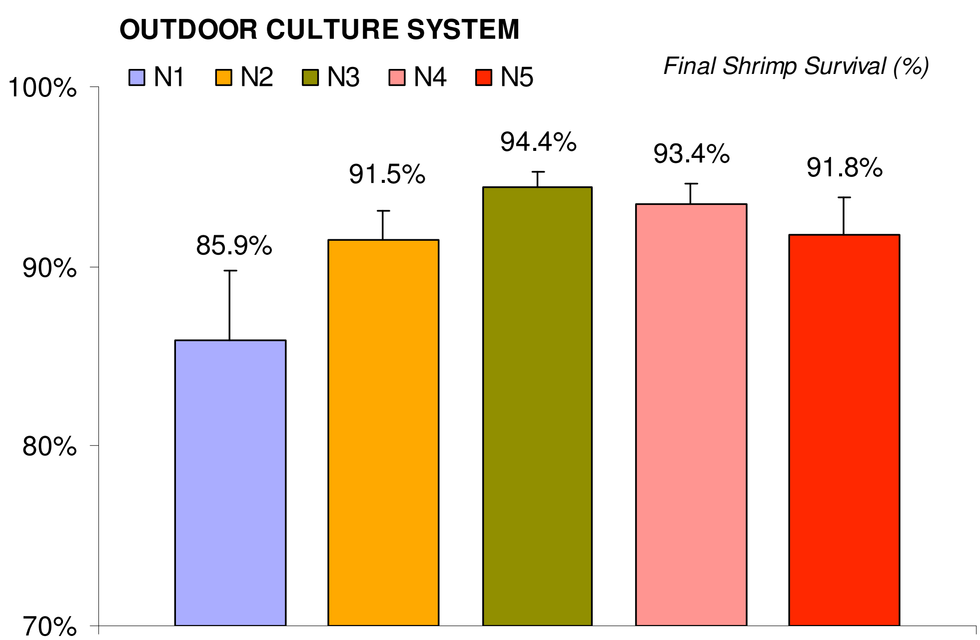

FIGURE 14. Final survival of *L. vannamei* farmed for 72 days under a green water culture system under 60 shrimp/m^2. Data presented as mean ± standard error of five different tanks. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**).

4.3.4 FOOD CONVERSION RATIO (FCR)

Shrimp reared in indoor tanks achieved a mean FCR of 2.18 ± 0.28 (Fig. 15). Although no statistically significant differences were observed in FCR among feeding treatments (ANOVA; $P > 0.05$), shrimp fed **N4** achieved a FCR 8.4% lower than the average obtained in all other treatments.

As shrimp fed **N4** attained a larger body weight, it would be normal to find a higher FCR for this treatment. Animals tend to convert food less efficient as they grow older and larger due to a decrease in protein synthesis. However, total apparent feed consumption (Cs) for **N4** was the lowest among all other treatments (mean of 902 g compared to 977 g for all other treatments). It may be possible that **N4** contained a more balanced energy:protein ratio compared to other diets. Shrimp feeds can contain between 3,100 to 4,000 kcal/kg of gross energy content. Optimal ranges for energy:protein reported in the literature for *Penaeus mondon* and *L. vannamei* are 8,000 and 12,000 kcal/kg of protein, respectively. As for the other feeding treatments, differences in FCR were negligible.

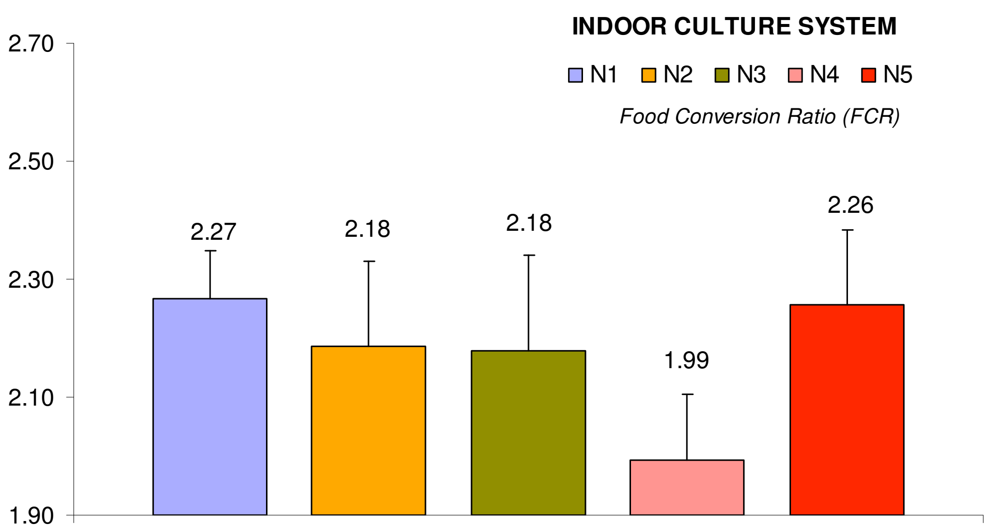

FIGURE 15. Food conversion ratio (FCR) of *L. vannamei* fed diets containing either Krill meal or Krill oil in an indoor rearing system. Data presented as mean ± standard error of five different tanks. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**).

In the outdoor system, FCR for **N4** remained low, but higher than **N3** (Fig. 16). The low FCR for shrimp fed **N3** was driven by a lower shrimp biomass achieved at harvest when compared to **N4** (13.94 g versus 14.69 g). The high FCR achieved in **N1** also suggests that shrimp mortality may have taken place over the final stages of the growth cycle. Overall, although differences in FCR were not statistically significant (ANOVA; $P > 0.05$), there could be substantial feed savings with the inclusion of Krill oil in *L. vannamei* diets.

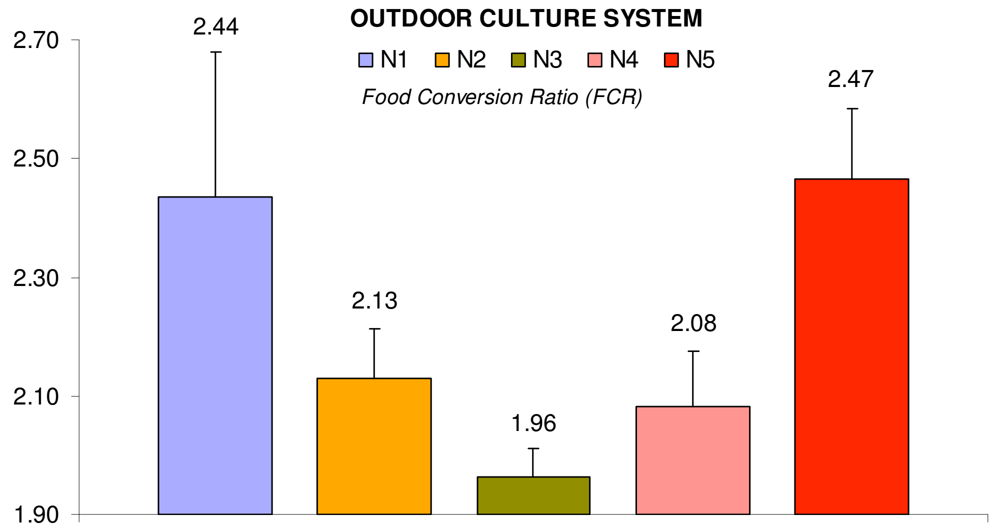

FIGURE 16. Food conversion ratio (FCR) of *L. vannamei* fed diets containing either Krill meal or Krill oil in an outdoor rearing system. Data presented as mean ± standard error of five different tanks. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**).

4.3.5 SHRIMP YIELD

Under culture conditions, shrimp final yield is one of the best parameters to evaluate feed performance as it collectively considers final shrimp survival and final body weight. Due to the more intensive rearing conditions, the highest shrimp yield was achieved under the indoor system (Fig. 17). Yields were higher for **N4** and **N5**, and maintained comparable among **N1**, **N2** and **N3**. This implies that when shrimp is fed on diets containing Krill oil higher productivities can be achieved at a lower cost compared to a basal diet with regular levels of fish oil, soy lecithin and cholesterol (**N5**). In indoor tanks, when shrimp yield for **N4** is compared against the average obtained for **N1**, **N2** and **N3**, there is a 4% difference. When diets containing Krill meal (**N1**, **N2** and **N3**) are contrasted against the basal diet (**N5**) there is a 3.6% reduction in shrimp yield. However, this reduction is compensated by a significant formula cost savings (from 7.5% to 18.0%). From the economical point of view, it appears more advantageous to apply Krill meal at 11% than at lower inclusion levels due to the higher formula costs it can deliver.

On average, shrimp yield in outdoor tanks was 38% lower than the one obtained under indoor conditions. This obviously reflects the initial stocking densities adopted which were lower for the outdoor tanks. Although differences were not significant for shrimp yield among feeding treatments (ANOVA; $P > 0.05$), there was a clear advantage for animals fed **N4** (9.3% higher yield on average). On the other hand, shrimp yield for **N5** and **N3** were quite similar, as opposed to the results obtained under the indoor system. The lowest yield was achieved with **N1**, below 7.8% when compared to other feeds containing Krill

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meal (**N2** and **N3**). This lower yield achieved for **N1** was a reflection of the lower shrimp survival achieved under the outdoor tanks.

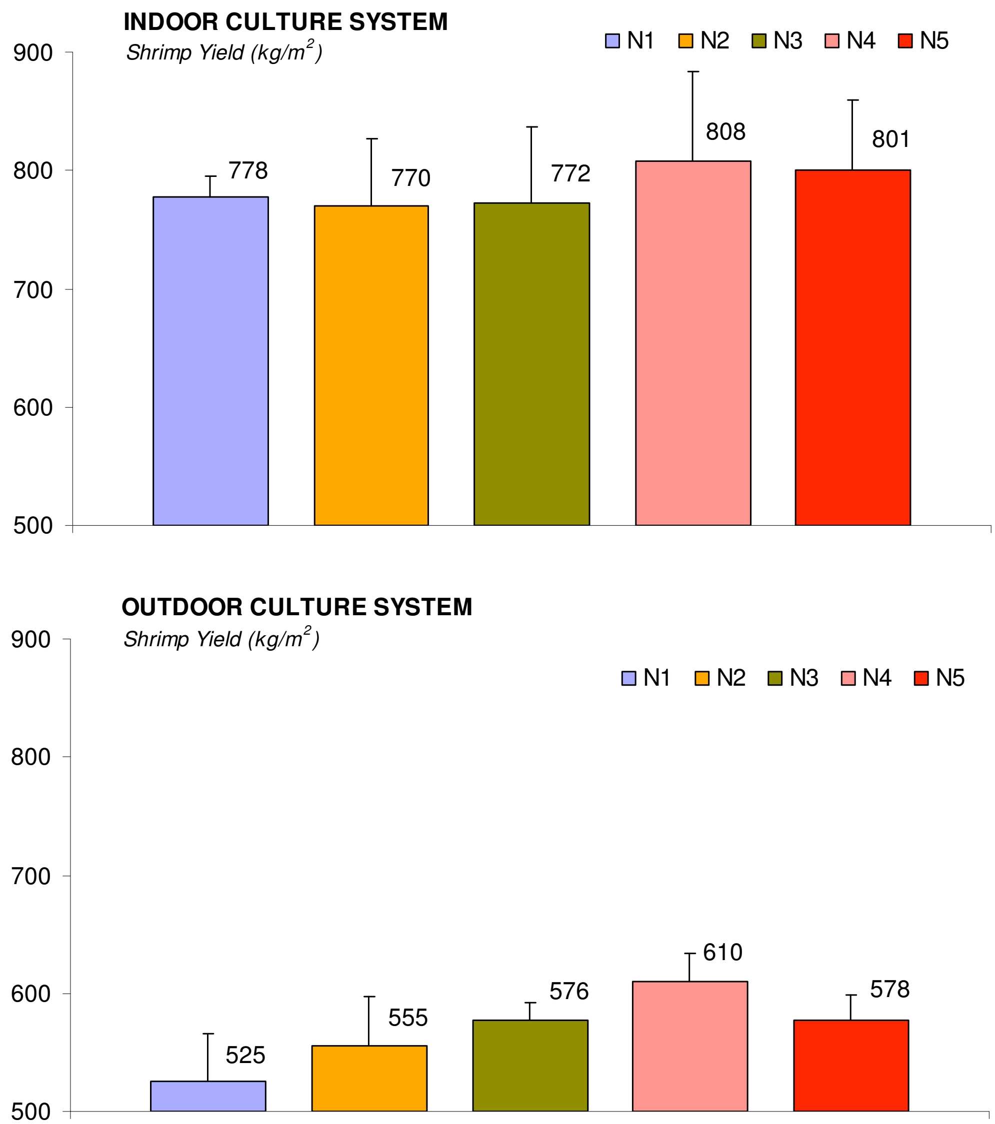

FIGURE 17. Yield (g/m^2) for *L. vannamei* farmed over a 72-day period under a clear and green water system. Data presented as mean ± standard error of five different tanks. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**).

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4.4 ECONOMICAL PERFORMANCE

The economical performance presented here (Table 6) is based on the assumption that shrimp prices are the same for all animals harvested, regardless of their size grade. Production costs only considers those costs related to feed itself. Therefore, this scenario also assumes that other operational costs such as labor, power and seed (post-larvae) are equal for all feeding treatments.

The evaluation shows a different result depending on the feed type and system used. Under the more intensive conditions (indoor system), feed **N4** remained the most attractive in terms of gross profit. The high survival rates, increased body weight, but particularly the high yield at harvest greatly reflected in this outcome. The low FCR did not appear to impact the economical result for feed **N4** as much as other performance parameters. Even though feed **N4** was not the less expensive one, it remained the most competitive. On the other hand, the reasonable results achieved in terms of gross profit for **N1** and **N2** was mainly driven by the lower formula costs coupled with moderate yields. All formulas containing Krill meal were more cost competitive and advantageous than the basal diet (**N5**), including the one with the most modest results (**N3**).

TABLE 6. Economical performance of diets for the rearing *L. vannamei* containing Krill meal or Krill oil in partial or full replacement of Anchovy fishmeal, fish oil, soy lecithin and cholesterol. Diets refer to feeds with 11% Eco Krill meal® (**N1**), 5.00% Eco Krill meal® (**N2**), 1.00% Eco Krill meal® (**N3**), 2.50% Krill oil (**N4**), and basal diet with 18.75% Anchovy fishmeal (**N5**). Price for white shrimp head-on 80:100 grade at US$ 2.50/kg. Values in US$.

Feed	Formula Cost (US$/kg)	Feed Cost (US$/kg)[1]	Shrimp Yield (kg/m^2)	Revenue (US$)[2]	Production Cost (US$/kg)[3]	Gross Profit (US$/kg)[4]
Indoor Tank Conditions - Intensive Culture at 100 shrimp/m^2 under clear water						
N1	$0,43	$0,97	0,78	$1,94	$0,75	**$1,19**
N2	$0,44	$0,96	0,77	$1,93	$0,74	**$1,19**
N3	$0,48	$1,05	0,77	$1,93	$0,81	**$1,12**
N4	$0,50	$1,00	0,81	$2,02	$0,81	**$1,21**
N5	$0,53	$1,19	0,80	$2,00	$0,95	**$1,05**
Outdoor Tank Conditions - Less intensive Culture at 60 shrimp/m^2 under green water						
N1	$0,43	$1,04	0,52	$1,31	$0,55	**$0,77**
N2	$0,44	$0,93	0,56	$1,39	$0,52	**$0,87**
N3	$0,48	$0,95	0,58	$1,44	$0,54	**$0,90**
N4	$0,50	$1,04	0,61	$1,53	$0,64	**$0,89**
N5	$0,53	$1,30	0,58	$1,44	$0,75	**$0,69**

[1] FCR *Formula Cost
[2] Yield * Shrimp Price
[3] Formula cost * Shrimp Yield
[4] Revenue - Cost of Production

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Under the outdoor conditions, there was a shift in gross profit results, particularly in regards to feed **N3**. As **N3** formula was less expensive than **N4**, it became the most advantageous among all diets. Under less intensive shrimp culture systems, benefits of nutritionally enhanced diets can often be obscured due to the presence of naturally occurring food sources and less challenging culture conditions. A factor to consider is that the outdoor system does not entirely reflect a commercial semi-intensive condition where shrimp stocking densities do not surpass 30 animals/m^2. Under these conditions, benefits of high performance diets, such as **N4**, on shrimp growth and economical returns would probably be difficult to discriminate, except when challenging conditions emerged (*e.g.*, post-lavae transfer and acclimation, disease outbreaks, high water salinity, low concentration in dissolved oxygen). This remains to be better investigated.

N2 also delivered a reasonable result in terms of gross profit, close to **N3** and **N4**. On the other hand, there was a substantial decrease when results for **N1** are evaluated. The lower shrimp survival for **N1** affected gross revenue. However, all treatment diets (**N1**, **N2**, **N3** and **N4**) remained more competitive than the basal diet (**N5**).

5 – REMARKS AND CONCLUSIONS

Results from the present study conducted with juveniles of the white shrimp, *L. vannamei*, farmed in clear and green water under laboratory conditions indicated:

(1) Feed Characteristics: addition of Krill meal up to 11% inclusion and Krill oil at 2.5% had no influence on feed water stability measured in this study as dry matter feed leaching rate.
(2) Culture System: the type of culture system (indoor versus outdoor) and management (green versus clear water; high versus low stocking density) adopted had a significant influence on shrimp performance. Higher growth and survival rates were found in outdoor tanks compared to indoor tanks, favored by increased water temperature, availability of natural food and lower stocking densities.
(3) Ingredient Replacements:
    a. Krill Oil: supplementation of Krill oil at 2.5% at the cost of cholesterol, and partially, of fish oil and soy lecithin in a diet containing 18.75% Anchovy fishmeal significantly enhanced *L. vannamei* growth, survival and FCR. Under intensive culture conditions, it resulted in the highest economical performance among all tested diets.
    b. Krill Meal: Krill meal was able to fully replace Anchovy fishmeal and soy lecithin at no significant cost to shrimp performance. The use of Krill meal at all inclusion levels adopted (1%, 5% and 11%) led to a higher economical return when compared to a diet containing 18.5% Anchovy fishmeal, 2.0% fish oil, 1.5% soy lecithin and 0.15% cholesterol.