Replacement of Fishmeal By Fish Silage in *Litopenaeus Vannamei* Diets in Biofloc System: Growth Performance and Shrimp Quality

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Abstract

The aim of this study is to evaluate the tilapia silage as alternative protein source in diets for *Litopenaeus vannamei* reared in clear-water and biofloc conditions. The experiment was performed in a "macrocosm-microcosm" device consisting of two individual systems: biofloc and clear-water. The trial used forty 40L rectangular bins in a density of 63 shrimp/m^2. The juveniles were distributed in a bi-factorial completely randomized experimental design with four replicates to each treatment. The treatments were based on the percentage of silage inclusion (control, 1.5, 3.0, 4.5 and 6.0% of inclusion) in biofloc or clear-water based system, totalizing ten treatments. Survival were not affected by both system and diet and stayed above 80% in all treatments. Shrimp mean final weight and SGR was statistically influenced by system ($p<0.01$) but not by the diet, presenting high values in biofloc condition. Shrimp quality was not affected by diet. Replacement of fishmeal by fish silage in *L. vannamei* diets is a good option regarding to shrimp production and quality.

Keywords: silage, nutrition, waste, replacement, Pacific white shrimp

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1. Introduction

In recent years, studies approaching the production of Pacific white shrimp *Litopenaeus vannamei* in biofloc system desired great attention (Avnimelech, 2012). The recent diseases outbreaks and low productivity lead the scientists to search for an alternative system to improve efficiently the aquaculture growth.

Biofloc system, also called as biofloc technology (BFT) has the advantage to allow the production of a great amount of shrimp per area or volume with no water exchange. This provides better biosecurity for the production, especially if the farm is situated in areas with high concentration of aquaculturists using the same water source. BFT has gained popularity because it offers a practical solution to maintain water quality and recycle feed nutrients simultaneously (Xu and Pan, 2012). Other advantage of the biofloc system is the possibility to use alternatives low protein diets and consequently decrease the production costs (Ballester *et al*., 2010; Scopel *et al*., 2011), mainly due to the continuous availability of natural food source in a form of bacteria, protozoa, nematodes, microalgae, rotifers and copepods (Decamp *et al*., 2002; Azim and Little, 2008; Ray *et al*., 2010).

Fishmeal is one of the most expensive and unsustainable ingredient used in aquaculture diets (Naylor *et al*., 2009). Therefore, the replacement or reduction of fishmeal use is of great interest for the aquaculture industry. On the other hand, problems related to the fishmeal replacement by alternative ingredients have been identify including deficiency of some essential amino acids, presence of anti-nutritional factors, palatability and digestibility (Forster *et al*., 2003; Naylor *et al*., 2009). Although problems exist, many cases of success have been reported in *L. vannamei* diets (Davis and Arnold, 2000; Forster *et al*., 2003; Samocha *et al*., 2004; Amaya *et al*., 2007; Cruz-Suarez *et al*., 2007; Hernández *et al*., 2008; Suarez *et al*., 2009; Bauer *et al*., 2012)

Fish silage can be produced using by-products of the fisheries and aquaculture processing residues. Fish silage is an alternative protein source to the fishmeal (Vidotti *et al*., 2003) and possesses a simpler and cheaper production method (Gallardo *et al*., 2012). Furthermore, the use of fish silage as a substitute for protein ingredients, in feed for aquatic organisms, is an alternative to solve sanitary and environmental problems caused by the

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lack of adequate disposition for the waste from the fish industry. Besides, it is also a way of decreasing feeding costs, and, consequently, production costs, since feeding corresponds to about 60% of the overall expenses with production (Arruda *et al*., 2007).

On this context, tilapia, the main species in Brazilian aquaculture, has demonstrated positive results as fish silage incorporated into diets (Carvalho *et al*., 2006; Fernandes *et al*., 2007), possible due to its nutritional quality (Oliveira *et al*., 2006).

The aim of this study is to evaluate the tilapia silage as alternative protein source in diets for *L. vannamei* reared in clear-water and biofloc conditions.

2. Material and Methods

2.1 Experimental design and culture conditions

The study was conducted in the Aquaculture Sector, Department of Animal Sciences, Universidade Federal Rural do Semi-Árido (UFERSA) in Rio Grande do Norte State, Brazil. The Pacific white shrimp *L. vannamei* post-larvae were supplied by a local commercial hatchery.

Before the experiment, shrimps were stocked in a 15m^3 fiberglass circular tank (macrocosm) aiming to an acclimation and prior biofloc formation. Water was vigorously aerated using one air diffuser (composed by ¾" PVC pipe with several 1mm holes) located in the center of the macrocosm tank. In order to maintain biofloc culture medium, shrimp were stocked at a density of 200 shrimp m^{-2} and maintained until the end of the experiment. Shrimp were fed twice a day (08:00am and 6:00pm) with 35% crude protein commercial feed (Aquabalance 35 Presence™) in two feed tray in order to monitor the food consumption. Liquid sugar cane molasses as a carbon source was added daily after the feed addition to maintain a high C:N ratio (20:1) to ensure optimal heterotrophic bacteria growth (Avnimelech, 1999; Crab *et al*., 2009). Vertical substrates were added to provide an additional area of 30% of the tank. Limited water exchange (not exceeding 0.5% daily) was carried out by a central drain to prevent accumulation of sludge throughout the experimental period. Dechlorinated freshwater was added to compensate sludge removal and evaporation losses.

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The experiment was performed in a “macrocosm-microcosm” device (Wasielesky *et al*., 2006; Emerenciano *et al*., 2012a) consisting of two individual systems: biofloc (BS) and clear-water (CWS). The trial was initiated stocking *L. vannamei* juveniles (1.43±0.33g) in forty (20+20) 40L rectangular bins (27x37x54cm) in a density of 63 shrimp m^{-2} (12 juveniles per bin). The juveniles were distributed in a bi-factorial completely randomized experimental design (water type and % of silage inclusion as the main factors) and reared for 45 days. Four replicate tanks were randomly assigned to each treatment. The treatments were based on the percentage of silage inclusion (control, 1.5, 3.0, 4.5 and 6.0% of inclusion) in biofloc or clear-water based system, totalizing ten treatments. The formulation of diets was described below. The juveniles were fed twice a day (08:00am and 06:00pm) using a feed tray to monitor feed consumption. The water was pumped from the macrocosm tank to the experimental units by a submerged pump (¾ HP pumps) and returned by gravity. Water flow in all experimental units was checked two times per day in order to maintain a minimum flow to recirculate the water. The experimental tanks were also siphoned to remove debris once a week.

For the clear-water treatments, the same scheme described above was performed, except by the macrocosmo tank that was not stocked with animals and receive no carbon source in order to maintain the water clear. Aeration were supplied by two 4 HP blowers connected to an emergency diesel electric generator to keep optimum dissolved oxygen levels in both systems.

Temperature, salinity, pH (YSI model ph100) and dissolved oxygen (YSI model 55) concentration were monitored 2 times per day after food time. Settling solids (Imhoff cones) was monitored daily (08:00am). Ammonia (NH_4-N) and nitrite (NO_2-N) were measured once a week (UNESCO, 1983). All shrimps were weighed to the nearest 0.1g at the beginning and the end of experiment. Specific growth rate (%), final weight (g), survival (%) and feed conversion rate (FCR) were measured.

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2.3 Fish silage production

The Nile tilapia silage used in this study was produced in the Laboratory of Seafood Technology and Quality Control (LAPESC/UFERSA) using residues of Nile tilapia processing including head, bones, skin, fins and viscera. The acid silage was produced using the methodology described by Arruda *et al*. (2006) with some modifications: 2% formic acid and 3% phosphoric acid, and 1% ascorbic acid as antifungal. The fish silage was dried in the oven at 60^{o}C by 24 hours (to obtain moisture below 13%), ground in a Rotor Mill (Rotating Knives and Swing Hammer MA900, Marconi Equip. Lab. Ltda, Brazil) and homogenized (10 mesh). Before formulation of the experimental diets, fish silage was neutralized by adding 1.6% calcium hydroxide to raise the silage pH from 2.8 to 7.1. The Nile tilapia silage contained 83.8% dry matter, 33.7% crude protein, 37.4% crude lipid and 21.5% ash on a dry matter basis.

2.4 Diet formulation

Five experimental diets were formulated to be isocaloric and isoproteic and to attend the nutritional requirements of the species (Table 1). Tilapia silage ranged from zero to 6% of the diet in replacement of fishmeal. All diets were processed in the Laboratory of Aquatic Animal Nutrition of the Universidade Federal do Ceará using the method described by Nunes *et al*. (2011). All diets were kept frozen at − 20°C until use.

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Table 1. Ingredient and chemical composition of diets used in the study.

Ingredients	Unit	Dietary Inclusion (%, as is basis)				
		CTL[1]	SIL[2] 1.5%	SIL 3.0%	SIL 4.5%	SIL 6.0%
Soybean meal	%	40.00	40.00	40.00	40.00	40.00
Wheat bran	%	20.00	20.00	20.00	20.00	20.00
Fishmeal 01	%	12.00	12.00	12.00	12.00	12.00
Salmon meal	%	8.00	7.54	6.82	6.10	5.38
Tilapia silage	%	0.00	1.50	3.00	4.50	6.00
Wheat midlings	%	5.24	3.90	3.62	3.33	3.04
Salmon oil	%	3.18	3.48	2.99	2.50	2.01
Powder molasses	%	3.00	3.00	3.00	3.00	3.00
Soy lectin	%	2.81	2.81	2.81	2.81	2.81
Calcium phosphate	%	2.00	2.00	2.00	2.00	2.00
Sardine hydrolisate	%	2.00	2.00	2.00	2.00	2.00
Vitamin and mineral premix DSM[3]	%	1.00	1.00	1.00	1.00	1.00
Aglutinant	%	0.50	0.50	0.50	0.50	0.50
Coline chloride 60%	%	0.22	0.22	0.22	0.22	0.22
Ascorbic acid (Stay C. DSM)	%	0.04	0.04	0.04	0.04	0.04
Etoxiquim 66% (Impextraco)	%	0.01	0.01	0.01	0.01	0.01
Basic Nutrients						
Ash	%	8.33	8.52	8.72	8.91	9.11
Crude Fat	%	9.24	10.00	10.00	10.00	10.00
Crude Fiber	%	3.14	3.02	2.99	2.96	2.93
Crude Protein	%	35.00	35.00	35.00	35.00	35.00
Digestible Energy	kcal/kg	3,036	3,015	2,942	2,868	2,794
Digestible Protein	%	30.83	30.41	29.98	29.56	29.13
Total Energy	kcal/kg	3,746	3,709	3,624	3,538	3,453
Water	%	10.42	10.46	10.59	10.73	10.86

[1] CLT= control; [2] SIL= fish silage; [3] Vitamin A 1.250.000 UI; Vitamin D3 350.000 UI; Vitamin E 25.000 UI; Vitamin K3 500 mg; Vitamin B1 5.000 mg; Vitamin B2 4.000 mg; Vitamin B6 10,0 mg; Nicotinic acid 15.000 mg; Pantothenic acid 10.000 mg; Biotin 150 mg; Folic acid 1.250 mg; Vitamin C 25.000 mg; Choline 50.000 mg; Inositol 20.000,0 mg; Iron 2.000 mg; Copper 3.500 mg; Chelated Cooper 1.500 mg; Zinc 10.500 mg; Chelated Zinc 4.500 mg; Manganese 4.000 mg; Selenium 15, mg; Chelated Selenium 15 mg; Iodine 150 mg; Cobalt 30 mg.

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2.4 Effect of growing systems and diets on shrimp production

The shrimp were counted and weighed in the end of the experimental trial in order to verify the influence of growing systems and diets on shrimp performance. Specific growth rate (SGR), mean final weight, survival and food conversion ratio (FCR) were evaluated.

2.4 Effect of growing systems on shrimp quality

To verify the influence of growing systems and fish silage replacement on the shrimp quality and shelf life, the Quality Index Method (QIM) was used. The aspects analyzed were based on QIM schemes developed by Otwell and Marshall (1986), Oliveira *et al*. (2009) and Oliveira (2013). Three assessors were selected among the staff of the LAPESC, and trained according to international standards (ISO 8586, 1993), including detection and recognition of tastes and odors, use of measurement scales, and in the development and use of descriptors. Changes that were occurring during the storage of raw shrimp (15 days storage in flake ice at 1±0.2°C) were described day-by-day according to standardized methodology published by Martinsdóttir *et al*. (2001; 2002). The quality parameters observed in the samples were odor; presence of melanosis; texture; head adherence; shell adherence; and overall appearance. The amount to a total value called Quality Index (QI) ranged in this experiment from 0 to 36. Sensory analysis took place shortly after samples were taken for microbiological and physico-chemical analyses (each 72 hour). They were performed mostly on the same hours on the determined dates, in an adequate environment inside the laboratory, and panelists did not discuss samples amongst each other. All observations of the shrimp were conducted under standardized conditions following the general guidance for the design of test room and testing conditions described in ISO 8589 (2007).

Nitrogen of total volatile bases (TVB-N), trimethylamine (TMA-N) and pH analyses were performed in triplicate at each 72 hours and were made using official methodology (BRASIL, 1981). Microbiological analyses (total mesophilic and psychrotrophic count)

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were performed according to the Manual of Brazilian Official Analytical Methods (BRAZIL, 2003).

2.5 Statistical Analysis

After check for homoscedasticity and normality, shrimp performance data were analyzed using a two-way ANOVA and Tukey's test to compare the means with α fixed in 0.05 using R (version 3.0.2). Survival data in percentage was transformed using the arcsine transformation in order to normalize the data before the analysis, however the original means and standard deviation are presented.

For the shrimp quality data, the linear equation (for QIM scheme), which was best fit and the correlation coefficient (r) between the QI and the storage time in ice, were calculated using the software SigmaPlot for Windows V. 10 (Systat Software, Inc.). Calibration models were calculated using the average QI of three samples evaluated per storage day. All regressions were calculated using XLSTAT Trial Version 2014.2.02 (Addinsoft 1995-2014).

3. Results

Water quality parameters stayed in the usual ranges for *L. vannamei* with temperature ranging from 24 to 32 ºC, pH 6.7 to 9.7, salinity 4 to 5 and dissolved oxygen always kept > 3.7 mg/l. Ammonia (<0.52mg/l) and nitrite (<0.25mg/l) stayed in safe concentration to the animals during all the experiment. Settling solids were maintained between 10 and 15 ml/l.

Regarding to growth performance (Table 2), survival were not affected by both system and diet and stayed above 80% in all treatments. Shrimp mean final weight and SGR was statistically influenced by system ($p<0.01$) but not by the diet, presenting high values in biofloc condition.

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Table 2. Performance of *L. vannamei* fed increasing percentages of tilapia waste silage in clear-water and biofloc systems during 45-d.

Diet (% of tilapia silage)	SGR (%/day)	Mean final weight (g)	Survival (%)	FCR
0	1.86 ±0.20	6.51 ±0.76	85.29 ±17.18	1.59 ±0.37
1.5	1.90 ±0.14	6.62 ±0.60	90.15 ±6.27	1.56 ±0.10
3.0	1.93 ±0.18	6.79 ±0.80	92.93 ±9.74	1.38 ±0.29
4.5	1.83 ±0.23	6.43 ±0.94	94.32 ±4.30	1.61 ±0.15
6.0	2.07 ±0.11	7.46 ±0.56	88.88 ±13.94	1.35 ±0.23
System				
Clear Water	1.82 ±0.20b	6.35 ±0.54b	87.50 ±12.12	1.65 ±0.23b
Biofloc	2.01 ±0.12a	7.17 ±0.79a	94.23 ±6.25	1.35 ±0.18a

Values are means (±standard error) of three tanks; Different letters in columns denote significant differences between experimental systems with $\alpha = 0.05$ level by Tukey's HSD multiple range test; SGR, specific growth rate; FCR, food conversion ratio.

During ice storage, the intensity of the sensory attributes changed in all shrimp farmed in biofloc systems (BS) and clear-water systems (CWS), showing gradual and consistent changes for all sensory parameters evaluated, reaching a score of 30 demerit points at 15th day of storage. The QIM scheme developed for shrimp farmed in BS and CWS stored in flake ice for 15 days is shown in Figure 1.

As can be seen in Figure 1, the sensorial attributes analyzed presented increased scores over time. These were added up to become the Quality Index (QI), a tool used to measure the shelf life of the group samples. The QI was calculated for each storage day of sampling and showed a linear relationship with storage time. High correlation (for all groups) between the total QI score (sum of all attributes) for each storage day and days in flake ice was found (Figure 1, Table 3) indicating loss of freshness during the days of storage. Its evolution could be expressed by the equations (linear regression model).

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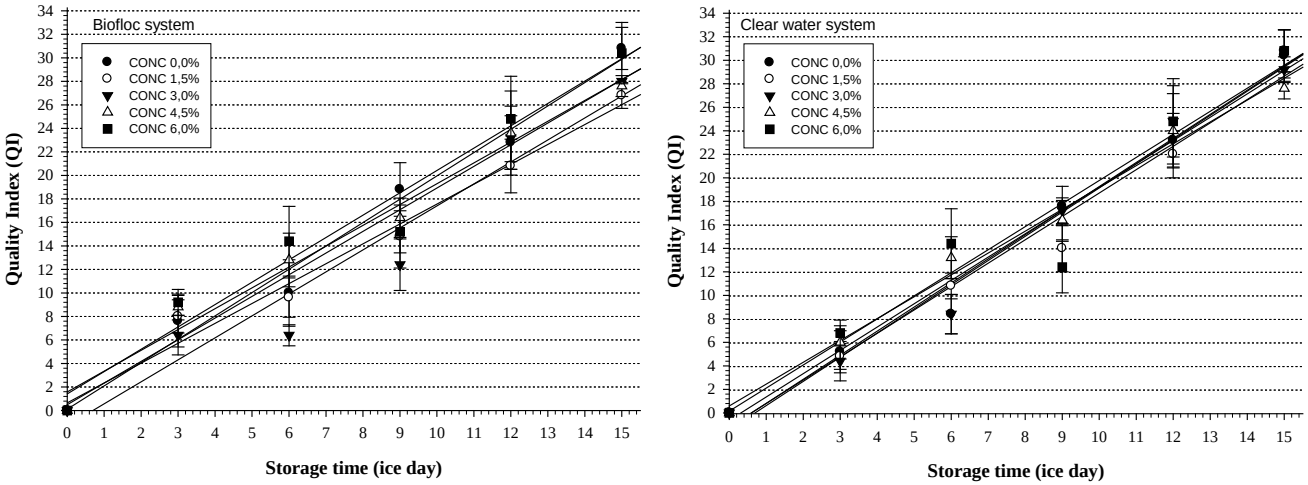

Figure 1. Results of the Quality Index Method (QIM) for shrimp grown in biofloc system, and in clear water system during storage on ice (0±1 °C).

Although mean QI scores of CWS group were lower throughout the experiment, there was no difference between BS group on the following days ($p<0.05$). Linear regressions were plotted to predict the shelf life of each sample group. Considering the maximum QI score for acceptable shrimp to be 65% of the total, i.e., 23.4 demerit points, it can be stated that the average shelf life of BS and CWS (Figure 1, Table 3) samples is, respectively, 12.18±0.16 and 13.19±0.86 days.

According to Figure 1 and Table 3, there are no significant differences among groups (i.e., percentage of fish silage replacement) on shrimp shelf lives in CWS and BS. These results showed that attributes gradually deteriorated with time as it is assumed in the Quality Index Method that the scores for all quality parameters increase with storage time. End of shelf life is usually determined when spoilage related sensory attributes become evident and most panelists detect them. The quality rejection of white shrimp (without additives, i.e. sodium metabisulphite) has reached the average limit of acceptability at 12 days of storage of shrimp farmed in CWS, and 13 days for BS (Table 3), according to the external attributes of sensory evaluation used and the maximum acceptable IQ (65% of total demerit points, i.e., 23.4).

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Table 3. Results of linear regression and shelf life of the samples of white shrimp (*L. vannamei*) grown in clear water system and in biofloc system during storage (0 ± 1°C).

Clear Water System			
	Linear regression model	**r^2**	**Shelf life (days)**
Control	y = 2,0495 x - 1,2381	0,9856	12,02
1,5%	y = 1,9886 x - 1,1809	0,9782	12,36
3,0%	y = 2,0571 x - 1,4286	0,9839	12,06
4,5%	y = 1,8590 x + 0,5905	0,9903	12,27
6,0%	y = 1,9619 x + 0,1524	0,9399	11,85
Biofloc System			
	Linear regression model	**r^2**	**Shelf life (days)**
Control	y = 1,9848 x + 0,1143	0,9853	11,73
1,5%	y = 1,6914 x + 0,6476	0,9808	13,45
3,0%	y = 1,8705 x - 1,2952	0,9418	13,20
4,5%	y = 1,7714 x - 1,5810	0,9828	14,10
6,0%	y = 1,9010 x + 1,4095	0,9654	12,04

y = maximum acceptable IQ (65% of total demerit points, i.e., 23.4); x = ice days; r^2 = coefficient of regression.

The pH analysis during storage, all samples began with values of 6.8 in both BS and CWS groups. On the 9th day, there was a significant increase and values rose to 7.7 and 7.3, respectively; and from this moment forward pH stabilized in BS and increased and increased in small proportion, presented values of 7.9 and 7.8, respectively, until the end of the experiment (Figure 2).

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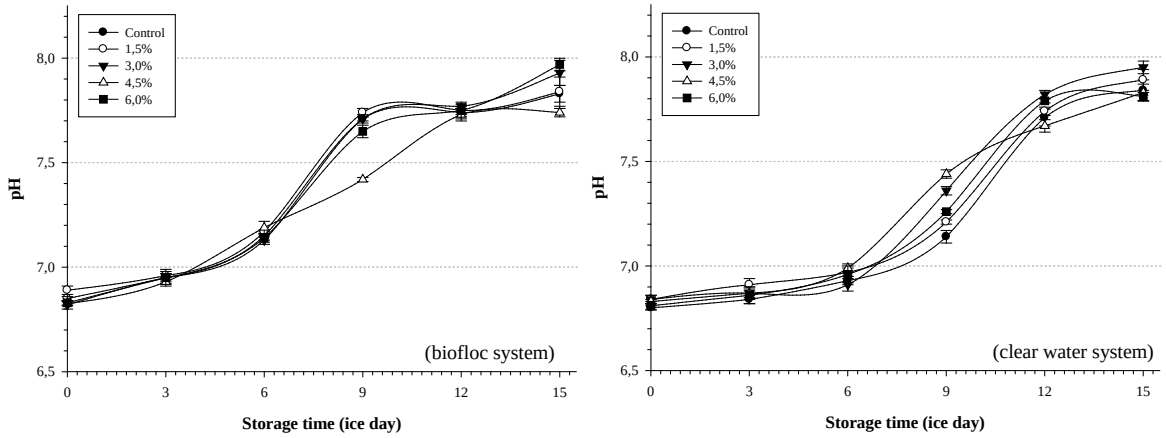

Figure 2. pH results in shrimp grown in biofloc system and clear water system during storage (0 ± 1 °C).

The microbiological results for total mesophilic and psychrotrophic count in shrimp stored on ice (0°C) for 15 days are shown in Figure 3. The results showed a decrease of mesophilic bacterial count from the 1st day (probably due to initial chilling) and the recovery of bacterial flora, remaining stable and remained above the limit of 4 log CFU/g until the end of the experiment. Instead, it was found that from the 1st day of storage, the psychrotrophic bacteria increased almost linearly during storage, reaching values above the limit of 5 log CFU/g, demonstrating its influence on the shelf life of shrimp. Considering both systems of farming (Biofloc and Clear water), the results were more stable in CWS (Figure 3). There was no difference among the groups (replacement of fish silage) in CWS along the storage time, but in BS the results showed more unstable.

de Azevedo, F. 2015. Replacement of Fishmeal By Fish Silage in *Litopenaeus Vannamei* Diets in Biofloc System: Growth Performance and Shrimp Quality. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Rivas Vega, M. y Miranda Baeza, A. (Eds), Nutrición Acuícola: Investigación y Desarrollo, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, ISBN 978-607-27-0593-7, pp. 234-258.

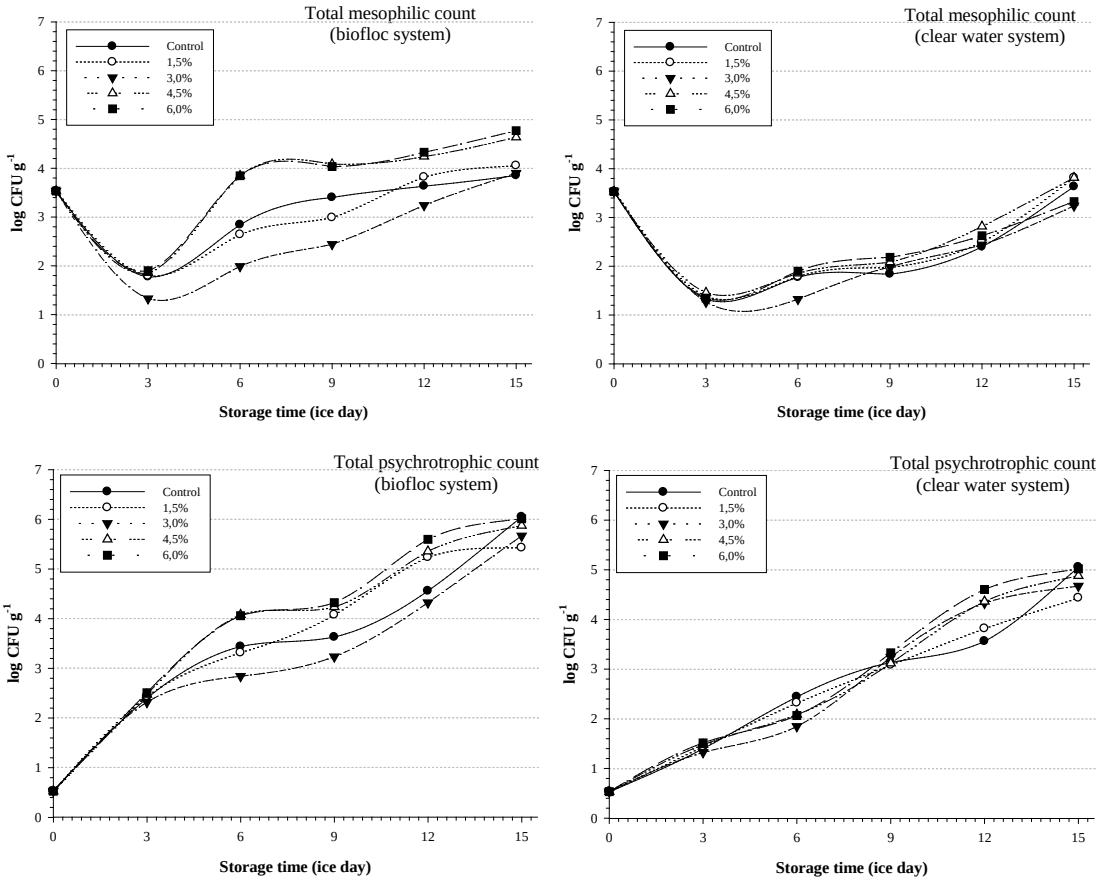

Figure 3. Mesophilic and Psychrotrophic bacteria counts in shrimp grown in biofloc system and clear water system during storage (0 ± 1 °C).

TVB-N values for BS showed an increasing trend for all samples (Figure 4) throughout the entire storage period (1.34 - 1st day), with higher values (18.56 mg N/100g on 15th day of storage), but all values were within the limit set by internationally (30 mg N/100g). TVB-N values for CWS showed the same tendency for all samples (Figure 4) throughout the entire storage period (1.34 - 1st day), but at the end of experiments (on 15th day of storage) showed higher values (20.16 mg N/100g), but all values were within the limit set by internationally (30 mg N/100g). These results could probably due to the log phase in bacterial growth (Figure 3).

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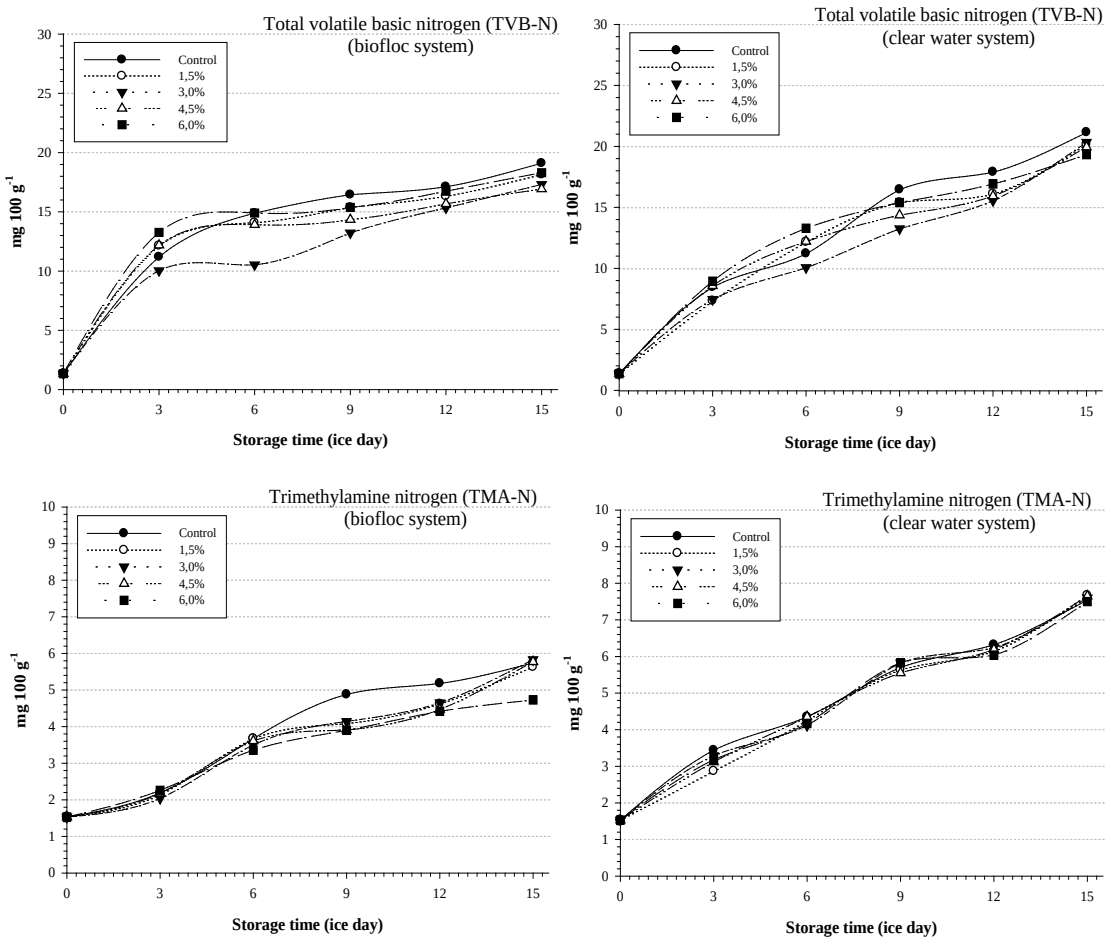

Figure 4. Total volatile bases nitrogen (TVB-N) and Trimethylamine nitrogen (TMA-N) in shrimp grown in biofloc system and clear water system during storage (0 ± 1 °C).

4. Discussion

The water quality parameters remained within the recommended range for *L. vannamei* culture (VanWyk and Scarpa, 1999), including settling solids maintained below to 15 ml/l (Taw, 2010).

In our experimental conditions, both biofloc and clear-water systems fish silage could be included at the highest level (6.0%) without losses in growth and survival. On the other hand, in biofloc condition shrimp got the best performance as compared to clear-water,

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probably due to the continuous availability of natural food source in a form of bacteria, microalgae, protozoa, nematodes, copepods and rotifers (Decamp *et al*., 2002; Azim & Little, 2008; Ballester *et al*., 2010; Ray *et al*., 2010). These microorganisms are a rich source of lipids (Maicá *et al*., 2012), vitamins and essential aminoacids (Ju *et al*., 2008), as well as highly diverse "native protein". The concept of "native protein" is related to protein source without previous treatment mainly including live food (Emerenciano *et al*., 2012b). Bacteria protein-source plays an important role in the equilibrium and re-ingestion of particulate organic matter and faeces (coprophagia) left by shrimp results in a form of constant food supply. The colonization of shrimp gut by bacteria had been shown positive effects such as improvement of shrimp digestive enzymes activity (Xu *et al*., 2012) and increasing the availability of extracellular enzymes (Xu & Pan, 2012) acting as "natural probiotic" (De Schryver *et al*., 2012).

Many studies have been done in the past decades with penaeid shrimp diets replacing fishmeal by alternative protein sources such as vegetable grains and terrestrial animal industry by-products. Problems related to the fishmeal replacement by alternative sources include palatability, digestibility, deficiency of essential amino acids, and presence of anti-nutritional factors (Forster *et al*., 2003; Naylor *et al*., 2009).

Although problems exist, many cases of success have been reported. Forster *et al*. (2003) and Suarez *et al*. (2009) recommended levels until 75% and 80% of fishmeal replacement using cattle by-product and a mixture of canola and soya, respectively. Amaya *et al*. (2007) and Hernández *et al*. (2008) concluded that is possible to replace 16% and 35% of fishmeal by poultry and swine by-product, respectively, without shrimp performance losses. Samocha *et al*. (2004) and Cruz-Suarez *et al*. (2007) achieved success replacing 100% and 80% of fishmeal by soya meal and poultry by-product in *L. vannamei* diets. Paripatananont *et al*. (2001) achieved 50% of replacement using soya protein concentrate in *Penaeus monodon* diets. Recently, Bauer *et al*. (2012) suggested that a mixture of soy protein concentrate and microbial floc meal can be utilized as a substitute for fishmeal in diets for *L. vannamei* juveniles. These studies have been carried out in clear-water condition and few efforts have been done to investigate alternative sources in biofloc conditions. Scopel *et al*. (2011) evaluated the replacement of fishmeal (0, 12.5 and 21.0%)

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by a combination of soya and animal terrestrial by-products. The authors found that 12.5% of replacement did not affect shrimp growth, resulting in growth rates of 0.7 g/week similar to those found in our study in clear-water condition, but less than 0.9 g/week observed in biofloc.

No literature was found related to the use of fish waste silage in *L. vannamei* diets under biofloc condition. In a recent study using clear-water, Gallardo *et al*. (2012) evaluated in *L. vannamei* juveniles feeds containing fish waste silage, fish waste silage with soybean meal or fish waste meal as protein source. The authors reported that shrimp fed with diets containing fish waste silage with soybean meal gained 0.7 g/week higher than those fed with fish waste silage or fish waste meal (0.3 g/week). These values are lower than observed in our study in biofloc conditions (0.9 g/week). Additionally, in our study values of FCR were 1.3 and 1.6 for biofloc and clear-water, respectively, higher than 2.8 and 2.5 observed by Ray *et al*. (2010) and Xu *et al*. (2012) using soy protein-based diets and low protein content diets, respectively, both in biofloc conditions for *L. vannamei.*

In contrast with our work, Costa *et al*. (2009) did an interesting study evaluating shrimp silage in juvenile tilapia (*Oreochromis niloticus*) diets. The authors concluded that it is possible to include 2.75% of shrimp silage, reducing the diets costs in 3.3%, without losses in fish performance. In a similar work, Cavalheiro *et al*. (2007) tested shrimp head silage, which contained approximately 40% protein, as a substitute for fishmeal in tilapia diets at 0%, 33.3%, 66.6% and 100% dietary levels. The results indicate that the shrimp silage could replace fishmeal at 100% level with economic advantages and without sacrificing the quality of the feed.

It is important to note that our experiment was carried out in euryhaline conditions, typically observed in Brazilian Semi-Arid region. The possibility of producing a marine shrimp in the continent is very interesting, as inland cultures demonstrate higher economic viability than the coastal cultures, mainly due to the high cost of land and the rigorous environmental protection legislation of these regions. As *L. vannamei* presents a salinity tolerance ranging from 0.5 to 40 g/l, together with good sensorial qualities and growth performance using alternatives protein source diets makes such species attractive for new investments in many regions around the world.

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The QIM is useful essentially because it evaluates sensory parameters and attributes that change most significantly in each species during degradation (Erkan & Özden 2006; Huidobro *et al*., 2000). According to quality experiments (Figure 1, Table 3) indicate that loss of freshness during the days of storage was verified. The loss of freshness evolution could be expressed by the equations (linear regression model) and these results are similar to studies of QIM for shrimp *Pandalus borealis* (Martinsdóttir *et al*., 2001) and for white shrimp *L. vannamei* (Oliveira *et al*., 2009; Oliveira, 2013).

Considering the maximum QI score for acceptable shrimp to be 65% of the total, i.e., 23.4 demerit points, it can be stated that the average shelf life of BS and CWS (Figure 1, Table 1) samples is, respectively, 12.18±0.16 and 13.19±0.86 days. Similarly, Oliveira *et al*. (2009) found 60% to be the maximum acceptable QI score for whole white shrimp (*L. vannamei*), and their samples reached this score in 12 days of iced storage. On the other hand, Norway lobsters stored in flake ice, even though treated with sodium metabisulphite, were only sensory acceptable for 5 days (Aubourg *et al*., 2007) while deep-water pink shrimp on ice was unacceptable after 7 days (Gonçalves *et al*., 2003).

In this study, the pH was above of the permitted pH by the Brazilian law (Brazil, 1997), which establishes pH values for seafood meat suitable for consumption ranging from 5.8 to 6.4. However, Ogawa *et al*. (1970) considered lobster tails good for consumption at pH 7.0 and Ogawa *et al*. (1975) observed that pH of frozen tail lobsters did not demonstrating a great variation, showing maximum values of 6.9 during one month of storage. Vieira *et al*. (1990) observed increasing trend of pH (6.4 to 7.6) for *P. argus* during the storage period.

The pH variation during storage (Figure 2) happens due to the basic compounds, such as TVB-N and TMA, formed from microbial activity, and to the fact that crustaceans have higher content of non-protein nitrogenous compounds, which facilitates the rise in pH values (Huss, 1995; López-Caballero *et al*., 2007). Similar results were reported in deep-water pink shrimp (*P. longirostris*) packaged in different modified atmospheres (Gonçalves *et al*., 2003), Norway lobster (*N. norvegicus*) stored in either flake or slurry ice and treated with sodium metabisulphite (Aubourg *et al*., 2007) peeled shrimp (P. serratus) treated with thymol essential oil (Mastromatteo *et al*., 2010) and especially in white shrimp (L.

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vannamei) treated with catechin (Nirmal and Benjakul, 2009). Kirschnik & Viegas (2004) had pH values ranging from 6.62 to 7.44 for *Macrobrachium rosenbergii* stored on ice for 10 days. Reilly *et al*. (1985) had a pH (7.1 to 8.1) for *P. monodon* stored on ice for 17 days and attributed the increase to the high levels of volatile nitrogen compounds produced by tissue and microbial enzymes, as well as Shamshad *et al*. (1990), who obtained initial pH values of 7.05 rising to 8.25 after 16 days of storage on ice, higher values than those obtained in the present study, and they found that at pH greater than 7.6, the prawns *Penaeus merguiensis* were classified as unacceptable or putrid.

Brazil's official analytical methods (Brazil, 2003) employed in microbiological analyses states plate counts must only be done when there are at least 20 and at most 200 CFU/plate, therefore the maximum countable value is 5.4 log CFU/g.

According to Cyprian *et al*. (2008) the low total counts reported at the beginning of storage time were due to the flesh of newly caught fish being sterile, since the immune system of the fish prevents the bacteria from growing. However, when the seafood dies, the immune system collapses, and consequently during storage, bacteria invade the flesh (Sveinsdóttir *et al*., 2002). At the end of shelf life estimated to be about 12 days the bacterial (psychrotrophic) count reach values near to log 4 CFU/g and remain increase up to the 15^{th} day of storage.

The present results of TVB-N and TMA-N is agree with results found by Vieira *et al*. (1990) after the first week of storage values tended to increase. TVB-N values vary according to the methodology used, the seafood species and the deterioration stage. This can be seen in Moura *et al*. (2003) experiments, which showed TVB-N values between 27.6 and 73.0 mg N/100g, much higher than recommended for freshness and consumption values. Cheuk *et al*. (1979) studying the pink shrimp (*Penaeus duorarum*) and brown shrimp (*Penaeus aztecus*) observed that the onset of deterioration coincided with the values of TVB-N reaching the limit of 30 mg N/100g, which occurred, respectively, 16 and 19 days of storage in ice. Angel *et al*. (1981) studying the Giant Malaysian Prawn (*M. rosembergii*) cooled on ice, detected slow increases on TVB-N, exceeding the value of 30 mg N/100g on 14 days of storage. Basavakumar *et al*. (1998), in experiments with tiger shrimp (*Penaeus monodon*), observed signs of deterioration at 11 days on ice (32.2 mg

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N/100g). Kirschnik & Viegas (2004), studying with *M. rosembergii* stored on ice for 10 days, found, respectively, initial and final values of TVB-N 18.65 to 26.00 and from 10.23 to 27.10 mg N/100g.

Considering the species studied by the cited authors were different from that used in this study, it was not possible to draw a comparison among them. However, in all reports, there was an increase of TVB-N with storage time. It has been suggested that the TVB-N value is affected by species, season, harvesting area, age and sex of fish. According to Mitsubayashi *et al*. (2004) and Siripatrawan *et al*. (2009) TMA-N is produced by decomposition of trimethylamine N-oxide (TMAO) by microorganisms. Volatile compounds such as ammonia, dimethylamine (DMA) and TMA are products of microbial degradation and are collectively regarded as TVB-N. Although TVB-N values in all samples increased throughout the storage period, the TMA-N increased in small proportion, probably due to the fact that lobster has very low values of TMAO.

As mentioned by Barbosa & Vaz-Pires (2004), QIM is a method that implies the transformation of scientific knowledge of the products in a consumer friendly solution that can be used by the seafood retailer and the consumer in common, which is both rare and desirable.

5. Conclusion

Replacement of fishmeal by fish silage in *L. vannamei* diets is a good option regarding to shrimp production and quality.

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