UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
DEPARTAMENTO DE PESCA E AQÜICULTURA
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQÜICULTURA

UTILIZAÇÃO DE MELAÇO COMO FONTE DE CARBONO EM CULTIVO DO CAMARÃO BRANCO DO PACÍFICO *Litopenaeus vannamei* (BOONE, 1931) SEM RENOVAÇÃO DE ÁGUA E SOB DIFERENTES RELAÇÕES CARBONO/NITROGÊNIO.

JOÃO PAULO VIANA DE LIMA

Recife, PE
Abril – 2007

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JOÃO PAULO VIANA DE LIMA

Dissertação apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aqüicultura da Universidade Federal Rural de Pernambuco, como parte dos requisitos necessários para a obtenção do grau de Mestre em Recursos Pesqueiros e Aqüicultura.

Orientador: Dr. Eudes de Souza Correia**,** Depto. de Pesca e Aqüicultura, UFRPE.

Recife, PE
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Universidade Federal Rural de Pernambuco
Programa de Pós-Graduação em Recursos Pesqueiros e Aqüicultura

Parecer da comissão examinadora da defesa de dissertação de mestrado de

JOÃO PAULO VIANA DE LIMA

Utilização de melaço como fonte de carbono em cultivo do camarão branco do Pacífico *Litopenaeus vannamei* (Boone, 1931) sem renovação de água e sob diferentes relações carbono/nitrogênio.

Área de concentração: **Aqüicultura**

A comissão examinadora, composta pelos professores abaixo, sob a presidência do primeiro, considera o candidato **João Paulo Viana de Lima** como APROVADO.

Recife, 27 de abril de 2007.

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À **Deus**, pela preciosa vida

OFEREÇO.

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"Toda aventura humana está baseada em sonhos, esperanças e desejos de realizar... e a história recente da indústria brasileira de cultivo de camarão não é senão um desses sonhos num caminho acelerado para transformar-se em realidade".

(Wurmann, 2001)

"A natureza em seus caprichos e mistérios condensa em pequenas coisas o poder de dirigir as grandes; nas sutis, a potência de dominar as mais grosseiras; e nas coisas simples, a capacidade de reger as complexas."

Artur Primavesi

RESUMO

O acúmulo de formas tóxicas de compostos nitrogenados na água é um grande problema para os sistemas aqüícolas. Pesquisas recentes têm demonstrado resultados satisfatórios em termos de produção e eficiência de retenção do nitrogênio, através da adição de fontes de carbono orgânico (açúcar, melaço, etc.). O presente estudo investigou o efeito da adição de melaço em diferentes relações C:N sobre a qualidade da água, atividade microbiana e a produção semi-intensiva do camarão *Litopenaeus vannamei*, em tanques de cultivo experimental sem renovação de água. Foram adotados quatro tratamentos e três réplicas, sendo três com aplicação diária de melaço nas relações C:N 5, 10 e 15:1, e um controle, sem aplicação desta fonte de carbono. Foram utilizados 12 tanques em fibra de vidro (500 L), estocados com 25 camarões.m^{-2} (1,90±0,37 g). A alimentação constou de ração comercial com 35% de proteína bruta e foi ofertada diariamente em bandejas às 8 e 16h. Coletas de água para análise química e de material biológico (fitoplâncton e bactérias) foram realizadas quinzenalmente ao longo do cultivo. As relações 15:1 e 10:1 apresentaram os menores ($P<0,05$) níveis de oxigênio dissolvido (4,64 e 4,76 mg L^{-1}, respectivamente) que está relacionado ao maior aporte de carbono orgânico nestas relações. O melaço reduziu significativamente ($P<0,05$) as concentrações dos compostos nitrogenados, nitrito e nitrato, bem como reduziu as densidades de cianobactérias nos ambientes com relações C:N de 10 e 15:1. Nenhum efeito ($P\geq0,05$) foi observado em relação às bactérias autotróficas, heterotróficas e *Vibrio* spp. O peso final dos camarões (~12,3 g) e o ganho de peso individual (~1,04 g.$semana^{-1}$), nas relações C:N mais altas (10:1 e 15:1), foram superiores ($P<0,05$) aos demais tratamentos. A taxa de crescimento específico foi elevada em todos os tratamentos (2,53 a 2,69 % dia^{-1}), entretanto os indivíduos na relação 10:1 foi superior ($P<0,05$) ao controle. Os valores de produção variaram de 267,4 a 301,0 g m^{-2}, e não foram diferentes estatisticamente ($P\geq0,05$) entre os tratamentos. O melaço pode ser utilizado como fonte de carbono para incrementar a relação C:N, melhorando a qualidade da água e os níveis de produtividade em cultivo semi-intensivo de *L. vannamei* sem renovação de água.

ABSTRACT

The accumulation of toxic nitrogenous compounds in the water is a common problem to aquaculture systems. Recent works have showed good results in terms of production and nitrogen retention efficiency, through the addition of organic carbon source (sugar, molasses, etc). This work investigated the effect of molasses addition in different C:N ratios on the water quality, microbial activity and production, in semi-intensive experimental culture tanks of *Litopenaeus vannamei* with no water exchange. Four treatments and three replicates were adopted, which three with daily molasses addition in 5, 10 and 15:1 C:N ratio and one control with no carbon source addition. Twelve 500 L fiber glass tanks were stocked with 25 shrimps.m^{-2} (1.90±0.37 g). Shrimps were fed with a 35% crude protein commercial diet offered in feeding trays at 08:00 and 16:00. Phytoplankton, bacteria and water samples were collected fortnightly during the culture. The 15:1 and 10:1 C:N ratios showed lower ($P<0.05$) oxygen dissolved levels (4.64 and 4.76 mg L^{-1}, respectively) which is related with the major organic carbon supply in these ratios. Molasses addition resulted in lower ($P<0.05$) nitrogenous compounds levels (nitrite and nitrate), as well reducing in the cianobacteria densities in the C:N 10 and 15:1 treatments. No significant differences ($P\geq0.05$) were found in *Vibrio* spp, autotrophic and heterotrophic bacterial densities. Shrimp final weight (~12.3 g) and weigth gain (~1.04 g.$week^{-1}$) in high C:N ratios (10:1 and 15:1) were higher ($P<0.05$) than in the others treatments. Specific growth rate was high in all treatments (2.53 to 2.69 % day^{-1}), but the 10:1 ratio was higher than the control. Yield values ranged from 267.4 to 301.0 g m^{-2} with no significant difference ($P\geq0.05$) among treatments. This study shows that the molasses can be used as carbon source in order to increase C:N ratio, improving the water quality and the *L. vannamei* semi-intensive culture performance with no water exchange.

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1. INTRODUÇÃO

O cultivo de camarões marinhos em escala comercial no Brasil teve seu início na década de 70, com a introdução da espécie exótica *Marsupenaeus japonicus* e, posteriormente, o cultivo das espécies nativas *Farfantepenaeus brasiliensis*, *Farfantepenaeus subtilis* e *Litopenaeus schmitti*. Porém, apenas na década de 90 com a introdução da espécie exótica *Litopenaeus vannamei* (Boone, 1931), a carcinicultura brasileira começou a ter representatividade na produção mundial de crustáceos.

A adoção do *L. vannamei* como espécie alvo da carcinicultura brasileira foi decorrente do seu alto grau de rusticidade, rentabilidade, crescimento, conversão alimentar e grande aceitação no mercado internacional que, aliados às condições edafo-climáticas das diversas macro-regiões do Brasil e, de forma especial da Região Nordeste, possibilitaram o desenvolvimento do setor (ANDREATTA e BELTRAME, 2004).

Analisando-se a evolução da produção mundial de camarão, envolvendo captura e cultivo, verifica-se que houve um incremento médio anual de 4,71% no volume total de camarão inteiro, passando de 2.983.674 t em 1993 para 4.728.765 t em 2003. Por sua vez, a produção derivada apenas da carcinicultura aumentou de 835.204 t (1993) para 1.703.957 t (2003), correspondendo a uma taxa média anual de 7,39% (ROCHA, 2005).

Desde o início da sua produção comercial em 1996 e até 2003, o cultivo brasileiro de camarão marinho vinha apresentando crescimentos elevados e bastante consistentes em termos de produtividade, produção e volume exportado. No entanto, a partir de 2004, o seu desempenho foi afetado por problemas decorrentes de efeitos combinados do vírus IMNV (Mionecrose Infecciosa) e da ação *antidumping*, frente a um mercado mundial operando com preços baixíssimos e uma taxa de câmbio reduzida (RODRIGUES, 2005). Isto contribuiu para o decréscimo da produção brasileira de camarão que, em 2004, atingiu uma produção de 75.904 t, representando uma redução de 15,84% em relação à produção de 2003 (ROCHA, 2005).

Segundo Madrid (2005), é imperioso que os produtores tenham mudanças de atitudes em relação ao enxugamento dos custos de produção para que se possa recuperar a sustentabilidade econômica. Dentre os custos operacionais, a ração é o item de maior peso, respondendo por 40 a 60% dos gastos com produção na maioria dos empreendimentos (LOVELL, 1989; AKIYAMA et al., 1992; D´ABRAMO e SHEEN, 1996; MADRID, 2005).

A busca pelo incremento da produtividade aquática com o objetivo de minimizar a utilização da ração vem sendo uma preocupação constante da carcinicultura nacional. Uma

das formas de se promover a redução dos custos com ração é a utilização do alimento natural (CORREIA, 1998), que também contribui reduzindo a degradação da qualidade da água (MARTINEZ-CORDOVA et al., 1998).

A capacidade de produção dos viveiros de camarão depende da maximização da produtividade primária e da minimização da perda de nutrientes. A produção de camarões em viveiros pode ser consideravelmente aumentada com a utilização de alimento suplementar e o uso de fertilizantes (BOYD, 1997a; ALONGI et al., 1999).

A prática da adubação ou fertilização tem sido utilizada como uma importante ferramenta no cultivo de organismos aquáticos. Adicionam-se nutrientes à água a fim de estimular a abundância do fitoplâncton e a proliferação do bentos, incrementando a produtividade natural dos viveiros e o crescimento dos camarões (COLMAN e EDWARDS, 1987; SCHROEDER et al., 1990; BOYD e TUCKER, 1998; BOYD, 2001).

Segundo Nunes (2000), a fertilização da água e a implementação de práticas para incrementar a produtividade natural são tão importantes quanto o uso de uma ração nutricionalmente completa e bem balanceada. Diversos autores demonstraram que, mesmo mediante o suprimento alimentar artificial diário, os camarões derivam a maioria do carbono utilizado no crescimento do consumo da biota natural do ambiente de cultivo. Segundo Anderson et al. (1987), o alimento natural pode contribuir com até 77% do carbono empregado em crescimento pelo *L. vannamei*.

Técnicas de cultivo em sistemas fechados, desenvolvidas nos Estados Unidos desde os anos 90, estão sendo bastante difundidas nas fazendas de camarão marinho, a partir da produção de camarões com baixa ou nenhuma troca de água, com o objetivo de garantir maior segurança e diminuição dos efluentes nas fazendas, reduzindo as possibilidades de impacto ambiental (HOPKINS et al., 1993; SANDIFER e HOPKINS, 1996; BROWDY et al., 2001a; BURFORD et al., 2003; WASIELESKY et al., 2006).

As bactérias desempenham um papel importante na dinâmica de nutrientes dos sistemas de produção aqüícola (MONTOYA e VELASCO, 2000). Sistemas de troca zero de água consistem em estimular a formação de uma biota predominantemente aeróbica e heterotrófica a partir da fertilização com fontes ricas em carbono orgânico (açúcar, melaço, etc.) e aeração constante do ambiente de cultivo (WASIELESKY et al., 2006). O melaço pode ser utilizado na preparação dos viveiros de camarão marinho (TALAVERA et al., 1998), atuando como uma fonte alternativa de carbono para a aqüicultura (SCHNEIDER et al., 2006).

Em pesquisas recentes foi demonstrado que a adição de carboidratos em viveiros extensivos de camarão melhorou a eficiência de retenção dos compostos nitrogenados, tendo efeitos positivos sobre a produção (HARI et al., 2004). Além do controle do nitrogênio, este processo leva à produção de proteínas microbianas que são uma fonte efetiva de proteína para os camarões (AVNIMELECH, 2000; BURFORD et al., 2004), deste modo reduzindo a demanda por proteína no alimento suplementar (AVNIMELECH, 1999).

O melaço é um subproduto do processo de refino do açúcar (NAJAFPOUR e SHAN, 2003) e um dos mais importantes materiais utilizados na produção comercial do etanol, devido ao seu baixo custo e disponibilidade (FAHY et al., 1997). Além de ser mais barato que a glicose, o melaço contém elementos minerais e vitaminas que podem ser usados como potencializadores do crescimento das bactérias (SQUIO e ARAGÃO, 2004).

O melaço possui, geralmente, 17 a 25% de água e um teor de açúcar (sucrose, glucose, frutose) de 45 a 50% (NAJAFPOUR e SHAN, 2003). Estima-se que o teor de carbono no melaço seja 20 a 30%. Samocha et al. (2007), em pesquisas recentes com adição de melaço como fonte de carbono suplementar no cultivo de camarão, informam uma densidade específica de 1,3 e um teor de carbono de 24% (*v*/*v*).

Atualmente, o melaço vem sendo utilizado como promotor de crescimento bacteriano em viveiros de cultivo de camarão no Brasil e no mundo. No entanto, sua eficiência é ainda muito pouco conhecida (WASIELESKY et al., 2006).

2. OBJETIVOS

- **Geral**

  Avaliar a influência do melaço como fonte de carbono orgânico em diferentes relações C:N no cultivo semi-intensivo do *Litopenaeus vannamei* sem renovação de água, visando reduzir o impacto ambiental dos efluentes, favorecendo o desenvolvimento sustentável da carcinicultura.

- **Específicos**

- Analisar a influência da adição de melaço, como fonte de carbono, sobre a qualidade da água de cultivo;
- Verificar e quantificar as possíveis alterações da carga bacteriana e das comunidades fitoplanctônicas;
- Avaliar o consumo de ração, conversão alimentar, relação de eficiência protéica, crescimento e sobrevivência do *L. vannamei* em condições de cultivo experimental, em função da utilização de melaço;
- Estabelecer a relação C:N que proporciona melhor desempenho no cultivo de *L. vannamei* sob condições semi-intensivas.

3. REVISÃO DE LITERATURA

3.1 Alimento Natural

A alimentação é um fator de extrema importância para um sistema de cultivo, pois influencia diretamente na sobrevivência e no crescimento dos organismos aquáticos, bem como a viabilidade econômica do cultivo, visto que pode representar até mais de 60% dos custos de produção.

O alimento natural (especialmente organismos zooplanctônicos e bentônicos) é de suma importância para a nutrição dos camarões cultivados (MARTINEZ-CORDOVA et al., 2003). Segundo Jory *apud* Martinez-Cordova et al. (2003), a utilização de dietas com níveis elevados de proteína é desnecessária quando há uma grande abundância de alimento natural no sistema de cultivo.

As rações são utilizadas em cultivos semi-intensivos e intensivos para aumentar a produção além dos níveis suportados pela produtividade natural do viveiro. No sistema semi-intensivo, a contribuição do alimento natural na alimentação dos camarões é bastante significativa, podendo alcançar até 85% (NUNES et al., 1997). Em viveiros de engorda que operam com produtividades abaixo de 1,0 t/ha/ciclo, as rações satisfazem entre 23% e 47% dos requerimentos nutricionais do *L. vannamei*, sendo o restante suprido pelo alimento natural (ANDERSON et al., 1987). Em sistemas mais intensivos, a contribuição do alimento natural diminui, mas ainda é considerada significativa (> 25%) (NUNES, 2000).

Entre os organismos componentes do alimento natural disponíveis aos animais cultivados em viveiros destacam-se as microalgas, representadas principalmente pelas diatomáceas e clorofíceas; e o zooplâncton, representado pelos rotíferos, cladóceros e copépodos (SILVA, 2004). A comunidade bentônica é representada por organismos microbianos (bactérias e fungos), micro-invertebrados e fitobentos, anelídeos e insetos aquáticos que vivem sob os detritos do fundo do viveiro (CORREIA, 1998).

A intensificação dos cultivos de *L. vannamei* requer o estabelecimento de uma comunidade planctônica bem desenvolvida, uma vez que esta é utilizada pelos camarões como complemento alimentar, fornecendo-lhes importantes compostos nutricionais como ácidos graxos, que são essenciais à sobrevivência e crescimento dos camarões (MAIA et al., 2003). Camarões marinhos em transição da fase de pós-larvas para juvenis podem alimentar-se indiretamente das microalgas aderidas a detritos e diretamente de copépodos, larvas de moluscos e do próprio detrito (ALONSO-RODRIGUEZ e PÁEZ-OSUNA, 2003; MARTINEZ-CORDOVA et al., 2002).

Os microrganismos (plâncton, bactérias, etc.) são de grande importância para os sistemas aqüícolas, particularmente com respeito à produtividade primária, ciclagem dos nutrientes, nutrição dos animais cultivados, qualidade da água, controle de doenças e do impacto dos efluentes ao meio ambiente (MORIARTY, 1997).

Rubright et al. *apud* Moss et al. (1992) sugerem que, em cultivo semi-intensivo, a fauna bêntica é capaz de dar suporte ao crescimento dos camarões nas quatro primeiras semanas de cultivo. A importância da meiofauna para o crescimento dos camarões está no fato destes organismos servirem de elo entre as bactérias e os camarões. Estudos de Hunter et al. *apud* Moss et al. (1992) indicam que o consumo de microalgas pelo *L. vannamei* em viveiros de terra semi-intensivos pode contribuir substancialmente para sua dieta.

Atualmente, a utilização de sistemas sem renovação de água tem despertado o interesse dos pesquisadores quanto às propriedades nutricionais dos flocos bacterianos (agregados microbianos ou bacterianos). Flocos bacterianos são formados durante o ciclo de produção e são constituídos principalmente de bactérias, microalgas, fezes, exoesqueletos, restos de organismos mortos, cianobactérias, protozoários, pequenos metazoários e formas larvais de invertebrados, entre outros (BURFORD et al., 2003; WASIELESKY et al., 2006).

Segundo Burford et al. (2004), mais de 29% do alimento consumido por *L. vannamei* pode ser proveniente do floco bacteriano presente no meio heterotrófico (meio onde predominam organismos heterotróficos, mantidos através do balanço da relação carbono/nitrogênio/fósforo). O filme bacteriano e outros organismos geralmente constituem de 5 a 10% da massa das partículas de detritos (CHAMBERLAIN et al., 2001a) e podem ser promovidas pela adição de silicato e calcário (BROWDY et al., 2001b).

Partículas floculadas possuem elevados níveis de proteínas, aminoácidos e outros elementos alimentares essenciais em níveis satisfatórios (TACON et al., 2002; DECAMP et al., 2003; BURFORD et al., 2004). Contêm também vitaminas e minerais em bons níveis, sendo desnecessária a adição destes fatores de crescimento na ração, reduzindo em 30% os custos destes insumos (CHAMBERLAIN et al., 2001b). Segundo Avnimelech (2006), uma alimentação baseada em microrganismos é de alta qualidade. Entretanto, a utilização da proteína microbiana vai depender da habilidade do animal em capturar a bactéria e de digerir a proteína (KOCHBA et al., 1994).

3.2 Fertilização Inorgânica e Orgânica

A adição de fertilizantes em viveiros de cultivo é uma prática comum na aqüicultura (JANA et al., 2001; BOYD, 2003). Os nutrientes dos fertilizantes são incorporados à biomassa planctônica (algas e zooplâncton) e, através de uma complexa rede de assimilação e reciclagem dos nutrientes, chegam aos organismos cultivados (HANSEN et al., 2003). Esta biomassa é nutricionalmente rica e pode ser utilizada para a alimentação dos organismos cultivados, como também para o estabelecimento da cadeia trófica no ambiente de cultivo (ARANA, 2004).

Segundo Boyd (1997a), mediante o uso apropriado de fertilizantes químicos, a produção da aqüicultura pode ser aumentada de duas a dez vezes acima daquela obtida em viveiros não fertilizados.

Os cinco principais fatores que regulam a produtividade dos viveiros são as disponibilidades de nitrogênio inorgânico solúvel (N), fósforo (P), carbono (C), luminosidade, e temperaturas satisfatórias da água (FOGG, 1975; McCOY, 1983).

Fertilizantes químicos ou inorgânicos são substâncias que contêm, principalmente, nitrogênio, fósforo e potássio, isolados ou em combinação (BOYD, 2001). Estes são classificados pelo conteúdo de nutrientes nas suas fórmulas, sendo expressos em percentagem de peso na forma de nitrogênio (N), óxido de fósforo (P_2O_5) e óxido de potássio (K_2O). O nitrogênio está presente em fertilizantes como nitrito (NO_2^-), nitrato (NO_3^-), amônia (NH_4^+), ou uréia [$(NH_2)_2CO$]; o fósforo como ortofosfato (PO_4^-); e o potássio como íon de potássio (K^+). Os fertilizantes à base de nitratos, mesmo com custos superiores, apresentam vantagens sobre os fertilizantes amoniacais, pois o nitrato não é tóxico e é totalmente oxidado no ambiente de cultivo (BOYD, 1997b; BARBIERI e OSTRENSKY, 2002).

O nitrato também tem mais efeito do que a amônia no desenvolvimento das diatomáceas (BOYD, 2001), que é o grupo de microalgas mais desejado nos cultivos de camarão, além de servirem como bio-indicadores de boa qualidade da água (BRITO et al., 2006). No entanto, se a água contém concentrações de silicato abaixo de 1,0 mg/L de silício (Si), aplicações de silicato de sódio de 50 a 100 kg/ha (~ 0,7 a 1,4 mg Si/L) também podem aumentar a proporção das diatomáceas (BOYD, op. cit.).

Os fertilizantes orgânicos suplementam as fontes de carbono, beneficiando o crescimento de bactérias e organismos bentônicos e também estimulando o crescimento do fitoplâncton (MacLEAN et al., 1994; QIN et al., 1995; CORREIA, 1998; BURFORD et al., 2003). A decomposição destes fertilizantes libera CO_2 (dióxido de carbono) utilizado diretamente na fotossíntese (AVAULT JR., 1996). Fertilizantes orgânicos contêm quase todos

os elementos nutrientes essenciais e enriquecem o conteúdo de matéria orgânica do solo dos viveiros (JANA et al., 2001).

O melaço pode ser utilizado como um fertilizante orgânico no cultivo de camarão, aplicado diretamente no solo dos viveiros ou na coluna d'água. No Panamá, utiliza-se de 12 a 17 galões/ha/semana na preparação dos viveiros e manutenção da produtividade primária ao longo do cultivo (TALAVERA et al., 1998). O carbono aportado pelo melaço é utilizado pelas bactérias e algas na constituição dos tecidos e como fonte de energia, principalmente no processo de fotossíntese.

Certas fazendas de camarão, no Peru, utilizam o melaço com o objetivo de inibir a proliferação de bactérias oportunistas do gênero *Vibrio*, em doses de 5 a 7 galões/ha/semana. Outra utilização seria na preparação do "vomito" (mistura líquida de fertilizantes orgânicos e inorgânicos), tanto para o controle de bactérias como para a proliferação de algas na coluna d'água, melhorando, até certo ponto, a qualidade da água (TALAVERA et al., 1998).

Investigações extensivas têm sido feitas em relação à fertilização de viveiros de água doce, onde as taxas de aplicação usualmente consistem em 2 a 9 kg/ha de P_2O_5 isoladamente ou aplicações desta mesma dosagem de N e P_2O_5 (BOYD, 2001). Para viveiros de água estuarina, as quantidades recomendadas giram em torno de 10 a 20 kg de N e 1 kg de P por hectare, variando conforme a concentração destes na água, entretanto mantendo-se a relação N:P de 20:1 (KUBITZA, 2003; BOYD, 2001).

Cliford (1992) menciona que a manutenção de concentrações de nitrogênio próximas a 1,3 mg/L e fósforo ao redor de 0,15 mg/L favorecem o estabelecimento de populações de algas diatomáceas no fitoplâncton. Segundo Brito et al. (2006), os níveis recomendados de nitrogênio se situam entre 2 e 4 mg/L, enquanto os de fósforo entre 0,2 e 0,4 mg/L, sendo que as relações de N:P devem ser aproximadamente de 20-10:1.

Freqüentemente, observa-se que a aplicação de um mesmo programa de adubação em diferentes fazendas resulta em respostas variáveis quanto à produção e a manutenção do plâncton e dos organismos bentônicos. Isto faz com que as doses adequadas de fertilizantes e a resposta aos programas de adubação sejam específicas para cada propriedade, e até mesmo para cada viveiro dentro da mesma propriedade (BOYD, 1990; KUBITZA, 2003).

Segundo Correia (1998), o efeito da fertilização também pode estar condicionado à acidez, alcalinidade e dureza da água e/ou do solo, que podem ser corrigidas através de calagem, utilizando cal hidratada [$Ca(OH)_2$], calcário calcítico ($CaCO_3$) ou dolomítico [$CaMg(CO_3)_2$], aplicados diretamente no fundo do viveiro ou dissolvidos e espalhados na água, antes da aplicação dos fertilizantes.

3.3 Manejo de Água no Cultivo de Camarão

O cultivo de camarão tornou-se uma importante indústria em áreas tropicais e subtropicais ao redor do mundo (BURFORD et al., 2003), contando, em 2004, com uma produção mundial de 1.908.000 t, o que representou um incremento de 10,7% em relação ao ano anterior (ROCHA, 2005).

Nos últimos anos, apesar dos incrementos de produção, o surgimento de enfermidades tem se tornado um problema para os cultivos de camarão em muitos países no Sul da Ásia, e Américas do Sul e Central. Muitas dessas doenças têm origem viral (BROCK et al., 1997; LIGHTNER, 1999; LIGTHTNER e PANTOJA, 2004; NUNES et al., 2004; GARCIA e OLMOS, 2007) e são exacerbadas pela má qualidade da água de cultivo e pelos elevados níveis de trocas de água (LeMOULLAC, 2000).

O crescimento acelerado da carcinicultura, em conjunto com o surgimento de enfermidades e a descarga direta de efluentes no meio ambiente, têm despertado a preocupação de vários grupos ambientalistas quanto à sustentabilidade ecológica desta atividade (NAYLOR et al., 2000; PÁES-OSUNA, 2001; BURFORD et al. 2003; HARI et al., 2006).

A renovação de água é uma técnica de manejo comum em cultivos de camarão, sendo bastante utilizada para manter níveis adequados de qualidade da água de cultivo (CHIEN, 1992; BURFORD et al., 2003; GÓMEZ-JIMÉNEZ et al., 2005). A troca de água também é utilizada para ajustes de temperatura e salinidade (AVAULT JR., 1996).

Viveiros de cultivo no sistema intensivo adotam taxas de renovação de água de 5 a 30% do volume do viveiro por dia (HOPKINS et al., 1993; MONTOYA et al., 1999; McINTOSH et al., 2001; GÓMEZ-JIMÉNEZ et al., 2005), enquanto que, em viveiros com baixa densidade de estocagem, utilizam-se taxas de 1 a 5% apenas para compensar as perdas por infiltração e evaporação (CHIEN, 1992; AVAULT JR., 1996). HOPKINS et al. (1993) estimam que para produzir 1 kg de camarão são necessárias 39 a 199 t de água.

Segundo Boyd (1997a), rotinas diárias de troca de água são ineficientes e desnecessárias, extrapolando-se os custos com bombeamento de água. Ainda segundo o mesmo autor, a renovação de água nos viveiros deve ser adotada apenas em casos específicos como ajuste da salinidade, remoção de produtos metabólicos tóxicos ou para conter *blooms* de algas. ALONSO-RODRIGUEZ e PÁES-OSUNA (2003) relatam que *blooms* de algas produzem alterações nos níveis de oxigênio dissolvido podendo causar a mortalidade dos camarões.

A liberação de efluentes sem tratamento representa uma perda econômica de nutrientes valiosos, reduzindo a rentabilidade dos cultivos. Um dos maiores desafios encarados pelos produtores de camarão está no desenvolvimento de estratégias que reduzam os resíduos nutrientes dos viveiros (CASILLAS-HERNÁNDEZ et al., 2006).

Os efluentes dos viveiros de camarão contêm partículas mortas e vivas de matéria orgânica, matéria orgânica dissolvida, amônia, nitrito, nitrato, fosfato, partículas sólidas suspensas e outras substâncias consideradas como potenciais poluentes (HARGREAVES, 1998; PÁES-OSUNA, 2001).

Estratégias de manejo para minimizar o aporte de nutrientes devem incluir melhorias na formulação das rações e manejo alimentar dos organismos cultivados, redução das densidades de estocagem, redução ou eliminação das trocas de água, juntamente com o aperfeiçoamento dos projetos e manejo dos sistemas de tratamento de efluentes (NUNES e PARSONS, 1998; BURFORD et al., 2001; PÁES-OSUNA, 2001; JACKSON et al., 2003).

Tentativas para melhorar a sustentabilidade e a biosseguridade na aqüicultura têm sido adotadas desde os anos 90, como a utilização de sistemas de recirculação – *Recirculating Aquaculture Systems* (RAS) com baixa renovação de água, aproveitando os efluentes após decantação e oxidação biológica da matéria orgânica (HOROWITZ e HOROWITZ, 2000; VELASCO e LAWRENCE, 2001; GROSS et al., 2003; HOLL et al., 2006; MICHAUD et al., 2006; SCHNEIDER et al., 2006); e sistemas de cultivo sem renovação de água – *Zero Water Exchange Systems* (HOPKINS et al., 1993; SANDIFER e HOPKINS, 1996; AVNIMELECH, 1998; McINTOSH, 1999; BROWDY et al., 2001a; CHAMBERLAIN et al., 2001a; PÁES-OSUNA, 2001; BOYD e CLAY, 2002; TACON et al., 2002; DECAMP et al., 2003; GÓMEZ-JIMÉNEZ et al., 2005; EBELING et al., 2006).

As vantagens desses sistemas são a redução da demanda por água, redução da emissão de efluentes e do impacto ao meio ambiente, controle da qualidade da água, aumento da conversão alimentar, e controle dos níveis de nitrogênio inorgânico com produção de proteína microbiana, dobrando a utilização da ração (AVNIMELECH, 1998; WASIELESKY et al., 2006). Além disso, reduzem os riscos de introdução e disseminação de enfermidades, fornecendo os benefícios nutricionais da produtividade natural do viveiro (McINTOSH et al., 2000; BRATVOLD e BROWDY, 2001; BURFORD et al., 2003; GÓMEZ-JIMÉNEZ et al., 2005; WASIELESKY et al., 2006).

Sistemas sem renovação de água demandam altos níveis de aeração e mistura da água, necessários para conter a crescente demanda por oxigênio, resultado da intensa atividade bacteriana (ERLER et al., 2005). A intensificação da densidade de estocagem tornou-se

requisito básico para a viabilidade econômica deste tipo de cultivo (McNEIL, 2000; WASIELESKY et al., 2006).

Atualmente, sistemas sem renovação de água trabalham com densidades de estocagem acima de 60 pós-larvas/m³, com alguns empreendimentos chegando a utilizar 500 pós-larvas/m³. Cultivos intensivos de camarão são definidos por produções de 0,5 a 1,0 kg/m³ (5 a 10 t/ha), super-intensivo de 1 a 5 kg/m³ (10 a 50 t/ha) e hiper-intensivo com produções acima de 5 kg/m³ (McNEIL, 2000). Hopkins et al. (1995) e Velasco et al. (1998) relataram boa sobrevivência e crescimento em cultivo de camarão marinho em alta densidade e sem renovação de água.

Em Belize Aquaculture Ltda (BAL), na América Central, é utilizado com sucesso o sistema de produção sem renovação de água visando à redução dos efluentes, incremento da biossegurança e aumento das produções (McINTOSH, 1999; McNEIL, 2000; McINTOSH, 2001; ERLER et al., 2005). BAL desenvolveu uma abordagem integrada para o cultivo de camarão, utilizando estoques de pós-larvas selecionadas, ração com baixo nível protéico (~ 20%), elevadas densidades de estocagem (~120 animais/m²) em viveiros revestidos com lona plástica e sob constante aeração, sistema de recirculação e tratamento completo da água após a despesca. Estas técnicas de manejo resultaram em níveis de produção em torno de 15 t/ha/ciclo (McINTOSH, 1999; BOYD e CLAY, 2002; BURFORD et al., 2003).

Nos últimos anos vêm-se desenvolvendo pesquisas em cultivos intensivos que combinam o tratamento de água com a reciclagem de alimento artificial não consumido, utilizando-se viveiros de suspensão ativa – *Active Suspension Ponds* (ASP) (AVNIMELECH et al., 1994; CHAMBERLAIN e HOPKINS, 1994; AVNIMELECH, 2003; BURFORD et al., 2003; AVNIMELECH, 2006) ou sistemas de cultivo sem renovação de água através de uma biota predominantemente aeróbica e heterotrófica – *Zero Exchange, Aerobic, Heterotrophic Culture Systems* (ZEAH) (McINTOSH, 1999; McNEIL, 2000; CHAMBERLAIN et al., 2001c; McGRAW, 2002; ERLER et al., 2005; WASIELESKY et al., 2006). Estes sistemas têm em comum a predominância de bactérias aeróbicas heterotróficas que colonizam partículas de resíduos orgânicos e absorvem o nitrogênio, fósforo e outros nutrientes da água (CHAMBERLAIN et al., 2001a).

3.4 Os Microrganismos e a Qualidade da Água

A qualidade da água e o controle de enfermidades são interdependentes e ligados às atividades microbianas dos sistemas aqüícolas (ABRAHAM et al., 2004). Processos microbianos afetam os fatores de qualidade da água como oxigênio dissolvido, amônia (NH_3), nitrito (NO_2^-) e sulfeto (MORIARTY, 1997).

Em alguns estudos, a qualidade da água nos cultivos de camarão tem sido melhorada com a aplicação de produtos probióticos (GATESOUPE, 1999; DEVARAJA et al., 2002), especialmente *Bacillus* spp. A razão é que bactérias gram-positivas (*Bacillus* spp.) são geralmente mais eficientes na conversão da matéria orgânica que bactérias gram-negativas (VERSCHUERE et al., 2000). O uso de *Vibrio* spp. como probiótico, ainda é controverso porque dentro deste gênero existem espécies que estão associadas às enfermidades ocorridas na carcinicultura (MORIARTY, 1997, 1998).

Os víbrios são bactérias de grande importância para a carcinicultura e fisiologicamente estão presentes no trato digestivo dos camarões, entretanto, quando em desequilíbrio podem causar enfermidades com elevados índices de mortalidade (MENDES et al., 2005).

Enfermidades causadas por *Vibrio* spp. provavelmente ocorrem quando os animais cultivados estão estressados por densidades de estocagem elevadas, baixos níveis de oxigênio, concentrações elevadas de amônia e sulfito ou por uma alimentação insatisfatória (MORIARTY, 1997). Segundo Mendes et al. (2005), em camarões marinhos cultivados no Brasil, foram identificadas pelo menos oito espécies que podem causar infecções entéricas, sistêmicas ou externas, tais como *Vibrio anguillarium*, *V. alginolyticus*, *V. parahaemolyticus*, *V. splendidus*, *V. cholerae*, *V. damsela*, *V. harveyi*, *V. vulnificus*.

As bactérias desempenham importante papel nos sistemas de produção aqüícola (MORIARTY, 1997; BROWDY et al., 2001a), tornando-se imprescindível uma compreensão adequada das diferenças entre bactérias autotróficas e heterotróficas para o desenho e manejo de muitas operações aqüícolas (McGRAW, 2002). As bactérias heterotróficas obtêm carbono e energia para crescimento a partir de compostos orgânicos que existem na natureza, ao contrário das autotróficas que obtêm energia a partir da luz (fotoautotróficas) e a partir da oxidação de compostos inorgânicos, tais como a amônia (quimioautotróficos).

O ecossistema microbiano em ambientes aqüícolas é geralmente dominado por organismos heterotróficos competindo por substratos orgânicos como fontes de carbono e energia (VERSCHUERE et al., 2000). No entanto, sistemas de cultivo autotróficos e heterotróficos são complementares. As bactérias heterotróficas produzem dióxido de carbono (CO_2) como produto final do seu metabolismo, o qual provê uma fonte de carbono para as

autotróficas que, ao crescer, produzem biomassa que será consumida eventualmente pelas heterotróficas (MORIARTY, 1997; McGRAW, 2002).

O manejo da qualidade da água é uma importante ferramenta para o sucesso dos sistemas de cultivo, pois tem influência direta na reprodução, crescimento e sobrevivência dos organismos aquáticos, especialmente em sistemas semi-intensivos e intensivos (CHIEN, 1992). As águas e efluentes de viveiros de camarão geralmente são ricos em sólidos suspensos, matéria orgânica e outros nutrientes, e a concentração destes elementos está estritamente ligada ao manejo adotado e ao sistema de cultivo (ALONSO-RODRIGUEZ e PAEZ-OSUNA, 2003).

Segundo Nunes e Parsons (1998), em viveiros de camarão de água estuarina, somente uma porção da matéria orgânica e dos nutrientes da ração aportada ao sistema (10 a 15% do carbono orgânico e 20 a 70% do nitrogênio e fósforo) é convertida em biomassa pelos camarões e removida durante a despesca. Em sistemas convencionais, apenas 20 a 30% do carbono, nitrogênio e fósforo, adicionados com a ração, são assimilados pelos camarões (CHAMBERLAIN et al., 2001a; JACKSON et al., 2003; THAKUR e LIN, 2003; AVNIMELECH, 2006).

A baixa assimilação dos nutrientes pode ser causada por uma inadequada formulação da ração, excessos de alimentação, baixa qualidade dos ingredientes ou pouca estabilidade da ração (BURFORD e WILLIAMS, 2001).

O alimento não consumido, as fezes e outros resíduos excretados, como a amônia, tornam-se disponíveis favorecendo o rápido crescimento do fitoplâncton e dos organismos heterotróficos (NUNES e PARSONS, 1998; TOOKWINAS e SONGSANGJINDA, 1999). A mineralização da matéria orgânica acumulada, em condições anaeróbicas, também leva à formação de produtos metabólicos tóxicos como a amônia e o nitrito, deteriorando a qualidade da água no ambiente de cultivo (AVNIMELECH e RITVO, 2003).

Um dos maiores problemas de qualidade da água em sistemas aqüícolas intensivos é o acúmulo de formas tóxicas de nitrogênio inorgânico na água (AVNIMELECH, 1999). Animais aquáticos, assim como peixes e camarões, excretam amônia, que pode se acumular no viveiro. Mesmo em baixas concentrações, a amônia e o nitrito (NH_3 e NO_2^-) são altamente tóxicos para os camarões e, portanto, devem ser removidos do sistema (CHIEN, 1992; BOYD e TUCKER, 1998; GROSS et al., 2003).

Vários processos microbianos podem ser utilizados para reduzir os níveis de amônia nos ambientes de cultivo. Estes processos incluem a nitrificação, denitrificação, mineralização, fotossíntese e o crescimento de bactérias heterotróficas (BRUNE et al., 2003).

Os sistemas de cultivo tradicionais estão baseados na biossíntese das algas (sistema fotoautotrófico) para remover a maior parte do nitrogênio inorgânico (HOPKINS et al., 1996; AVNIMELECH et al., 1994; EBELING et al., 2006). A grande desvantagem deste sistema é a variação diurna de oxigênio dissolvido, pH e nitrogênio amoniacal e, a longo prazo, as constantes mortes e as mudanças nas densidades das algas (BURFORD et al., 2003).

Segundo Schroeder (1978), a produtividade das algas também é limitada pela intensidade de energia solar que incide na superfície dos viveiros e pelas concentrações de nitrogênio e fósforo. Populações de algas em viveiros sem manejo, normalmente fixam entre 2 e 3 g de carbono/m²/dia, enquanto que, em viveiros com elevada taxa de mistura, fixam de 10 a 12 g de carbono/m²/dia (BRUNE et al, 2003).

Os fungos, todos que são aeróbios, também são considerados eficientes em converter matéria orgânica em material celular, mas geralmente preferem condições mais ácidas que as encontradas nos viveiros (SCHROEDER, 1978).

Os microrganismos nitrificantes são responsáveis pela oxidação da amônia para nitrito e, subsequentemente, para nitrato (VERSCHUERE et al., 2000). Estes são principalmente autótrofos obrigatórios, que consomem dióxido de carbono como fonte primária de carbono, e aeróbios obrigatórios, pois requerem oxigênio para crescer (HAGOPIAN e RILEY, 1998).

A conversão biológica da amônia em nitrito é desenvolvida por bactérias que oxidam a amônia – *Ammonia Oxidizing Bacteria* (AOB), que incluem bactérias do gênero *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, *e Nitrosovibrio*; já a subseqüente oxidação, do nitrito em nitrato, é realizada por bactérias que oxidam o nitrito – *Nitrite Oxidizing Bacteria* (NOB), que são do gênero *Nitrobacter*, *Nitrococcus*, *Nitrospira e Nitrospina* (EBELING et al., 2006). Quanto ao nitrato, este pode ser convertido em gás nitrogênio através da ação de bactérias denitrificadoras e volatilizado para a atmosfera (BOYD e QUEIROZ, 2001). Segundo BOYD (2001), a denitrificação representa a forma de maior perda de nitrogênio dos viveiros.

Os principais fatores que influenciam na taxa de nitrificação são as concentrações de amônia e nitrito, a relação carbono/nitrogênio, o oxigênio dissolvido, o pH, a temperatura e a alcalinidade (EBELING et al., 2006). Ao contrário das algas, populações microbianas são mais estáveis e independem de condições luminosas (SCHROEDER, 1978; AVNIMELECH, 2006).

Estudos realizados em viveiros de camarão têm demonstrado resultados satisfatórios em termos de produção e eficiência de retenção do nitrogênio, através da adição de fontes de carbono orgânico e manutenção de um sistema constante de aeração e mistura, para estimular

o desenvolvimento de bactérias heterotróficas (AVNIMELECH, 1999; McINTOSH, 1999; HARI et al., 2004; ERLER et al., 2005).

Sistemas heterotróficos reduzem o risco de introdução e disseminação de doenças, inibem o crescimento de *Vibrio* spp. e outros grupos de bactérias potencialmente patogênicas, além de complementar a produtividade natural presente nos viveiros (McINTOSH et al. 2000; BROWDY et al., 2001a; MOSS et al., 2001; WASIELESKY et al., 2006).

A habilidade para o controle das concentrações de nitrogênio está na manipulação da relação entre a quantidade de carbono orgânico e nitrogênio inorgânico (C:N), e tem sido utilizada com freqüência para indicar a qualidade dos substratos orgânicos de viveiros de aqüicultura (AVNIMELECH, 1999). A importância da relação C:N do viveiro se deve ao fato da deficiência de qualquer nutriente exigido pelas bactérias heterotróficas poder limitar a taxa de decomposição da matéria orgânica e, com isso, o desenvolvimento e a formação dos flocos bacterianos.

Para aperfeiçoar a produção e, conseqüentemente, a retenção dos nutrientes na biomassa bacteriana, Burford et al. (2003) informam que a relação C:N deve situar-se acima de 10:1. Schneider et al. (2005) sugerem que a relação C:N requerida no substrato é de aproximadamente 15 g C/g N. Segundo Wasielesky et al. (2006), a relação C:N ideal para formação do floco microbiano, com predomínio de bactérias heterotróficas, deve situar-se entre 14 e 30:1. No entanto, misturas balanceadas de carbono e nitrogênio numa relação de 20:1 são, aparentemente, mais facilmente assimiladas (CHAMBERLAIN et al., 2001a).

Goldman et al. *apud* Jana et al. (2001) mostraram que a eficiência de crescimento das bactérias diminui com o incremento da relação C:N e C:P no substrato. Um crescimento balanceado de bactérias requer substratos com carbono, nitrogênio e fósforo em uma relação atômica de 106:12:1, embora algumas bactérias tenham capacidade de variar estes requerimentos (JANA et al., 2001).

A relação C:N na água está vinculada à disponibilidade e competição por carbono orgânico e amônia. Para uma alta relação C:N, bactérias heterotróficas competem com as autotróficas por oxigênio dissolvido e espaço. Quando há uma baixa relação C:N, as bactérias autotróficas são privilegiadas (MICHAUD et al., 2006). Portanto, informações sobre uma ótima relação C:N e N:P são pré-requisitos para se entender as atividades microbianas e para o desenvolvimento de um protocolo racional de fertilização de ambientes para cultivo de organismos aquáticos (JANA et al., 2001).

4. ARTIGO CIENTÍFICO

Parte dos resultados obtidos durante o trabalho experimental dessa dissertação é apresentada no artigo intitulado **"Molasses utilization in *Litopenaeus vannamei* culture with different carbon/nitrogen ratios"** (manuscrito), que se encontra anexado.

MANUSCRITO

"MOLASSES UTILIZATION IN *Litopenaeus vannamei* CULTURE WITH DIFFERENT CARBON/NITROGEN RATIOS"

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Molasses utilization in *Litopenaeus vannamei* culture with different carbon/nitrogen ratios

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Abstract

This study investigated the effect of molasses addition in different C:N ratios on the water quality, microbial activity and *Litopenaeus vannamei* semi-intensive production, in experimental culture tanks with no water exchange. Four treatments and three replicates were adopted, where three treatments with daily molasses addition in 10, 20 and 30:1 C:N ratios, and one control with no carbon source addition. Twelve 500 L fiber glass tanks stocked with 25 shrimps.m^{-2} (1.90±0.37 g) were used. Commercial shrimp pelleted ration (35% crude protein) was offered in feeding trays at 08:00 and 16:00. Phytoplankton, bacteria and water samples were collected fortnightly during the culture. The C:N ratios 30 and 20:1 showed lower ($P<0.05$) oxygen dissolved levels that is related with the major organic carbon supply in these ratios. Molasses addition resulted in lower ($P<0.05$) nitrogenous compounds levels, as nitrite and nitrate, as well in reduced cyanobacteria densities in 20 and 30:1 C:N ratios. No significant differences ($P\geq0.05$) were found in *Vibrio* spp, autotrophic and heterotrophic bacterial densities. Shrimp final weight and weight gain in high C:N ratios (20 and 30:1) were higher ($P<0.05$) than the others treatments. Specific growth rate was high in all treatments (2.53 to 2.69 % day^{-1}), and the 20:1 ratio was higher than the control. Yield values ranged from 267.4 to 301.0 g m^{-2} with no significant difference ($P\geq0.05$) among the treatments. This study showed that the molasses can be used as carbon source in order to increase C:N ratio, improving the water quality and the *L. vannamei* culture performance with no water exchange.

Keywords: molasses, C:N ratio, shrimp culture, heterotrophic bacteria, water quality, *Litopenaeus vannamei.*

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1 Introduction

Shrimp farming is an important industry in tropical and subtropical areas around the world. The accelerated expansion of this activity, diseases outbreaks in addition to the direct discharge of waste nutrients from shrimp farms into adjacent waters have raised global concerns of environmental groups concerning the sustainability of shrimp farming (Naylor et al., 2000; Burford et al., 2003; Hari et al., 2006).

Discharges of untreated pond effluents represents an economic loss of costly nutrients, thereby reducing farm profitability (Smith et al., 2002). The development of managing strategies to reduce nutrient wastes in ponds appears as a key point toward the success of the activity (Jackson et al., 2003; Casillas-Hernández et al., 2006).

In conventional culture systems, only about 20 to 30% of the carbon, nitrogen and phosphorus in feeds are assimilated by shrimp (Chamberlain et al., 2001; Jackson et al., 2003; Thakur e Lin, 2003; Avnimelech, 2006). The remainder is dispersed in the pond as uneaten food, shrimp faeces or others excreted metabolic residues (Nunes e Parsons, 1998; Tookwinas e Songsangjinda, 1999).

The shrimp farm effluents contain living and dead particulate organic matter, dissolved organic matter, ammonia, nitrite, nitrate, phosphate, suspended soil particles and other substances that can be considered potential pollutants (Hargreaves, 1998; Páes-Osuna, 2001).

One of the major quality problems in intensive aquaculture systems is the accumulation of toxic inorganic nitrogen species in the water (Avnimelech, 1999). Aquatic animals, such as fish and shrimp, excrete ammonia, which may accumulate in the pond. Even in low levels, ammonia and nitrite (NH_3 and NO_2^-) are highly toxic for shrimps and therefore should be removed from the water (Chien, 1992; Boyd e Tucker, 1998; Gross et al., 2003).

The microbial community plays an important role in the nutrient dynamics of aquaculture systems production (Moriarty, 1997; Montoya e Velasco, 2000; Browdy et al., 2001; McGraw, 2002). Nitrification, denitrification, photosynthesis, mineralization or heterotrophic bacterial re-growth can be used to reduce ammonia levels in the conventional ponds (Brune et al., 2003).

Recent studies in shrimp ponds have demonstrated satisfactory results in terms of production and efficiency nitrogen retention, through the addition of organic carbon sources (sugar, molasses, etc.) and maintenance of a constant system of mixing and aeration, for stimulate the development of heterotrophic bacteria (Avnimelech, 1999; McIntosh, 1999; Hari et al., 2004; Erler et al., 2005).

Heterotrophic systems reduce the risk of introduction and spread of diseases, inhibiting the growth of potentially pathogenic bacteria, as *Vibrio* spp, besides complementing the natural productivity within ponds (McIntosh et al. 2000; Browdy et al., 2001; Moss et al., 2001; Wasielesky et al., 2006).

Molasses is a by-product of the sugar refinery process (Najafpour and Shan, 2003) and can be used in marine shrimp pond preparation (Talavera et al., 1998), acting as an alternative carbon source for aquaculture (Schneider et al., 2006; Samocha et al., 2007). It also contains miner al elements and vitamins that can be used to improve bacterial growth (Squio and Aragão, 2004).

The ability to control inorganic nitrogen concentrations through the manipulation of the relationship between organic carbon and inorganic nitrogen (C:N) and it has been used frequently to indicate the quality of the organic substrate in ponds (Avnimelech, 1999). The importance of pond C:N ratio is due to the fact that the deficiency of any nutrient demanded by heterotrophic bacteria can limit the decomposition rate of the organic matter, the development and the formation of bacterial floc that are used as food by the shrimps.

To improve the flocs production, and consequently, the retention of the nutrients in the bacterial biomass, Burford et al. (2003) inform that the C:N ratio should be located above 10:1. Schneider et al. (2005) suggest that the C:N ratio requested in the substrate is of approximately 15 g C/g N. Wasielesky et al. (2006) affirm that the ideal C:N ratio for formation of the microbial flocs, with prevalence of heterotrophic bacteria, is between 14 and 30:1. However, balanced mixtures of carbon and nitrogen are more easily assimilated in 20:1 ratio (Chamberlain et al., 2001).

In the present study the effects of molasses in different C:N ratios on the water quality, microbial activity and production of the Pacific white shrimp, *Litopenaeus vannamei* (Boone, 1931), in experimental culture tanks with no water exchange were investigated..

2 Materials and methods

2.1 Site and experimental conditions

The experimental culture was carried out at Aquacultural Station of Universidade Federal Rural de Pernambuco, Recife, PE, during 70 days using twelve 0.5m³ fiber glass circular tanks, supplied with continuous aeration. Bottom tanks were recovered by a 5cm estuarine sediment layer previously treated with lime (100 g m^{-2}) and the tanks were filled

with 400L salt water (30‰). Weekly water replacements were used to compensate evaporation losses.

Tanks were fertilized only before shrimp stocking, using monoammonium phosphate – MAP (11% N and 44% P_2O_5), calcium nitrate (15% N) and sodium silicate (30% SiO_2), to reach concentrations of 3-4 mg L^{-1} nitrogen, 0.15-0.20 mg L^{-1} phosphorous and 1-2 mg L^{-1} silicium.

An entirely randomized design was adopted, consisting in molasses addition to reach C:N ratios of 10, 20 and 30:1 (RM_{10}, RM_{20} and RM_{30}, respectively), and a control treatment (CTL) without molasses addition. All treatments were done in triplicate.

2.2 Molasses addition

The amount of molasses added to the culture tanks was calculated basing on carbon:nitrogen ratios (*C/N*) established, in the feed nitrogen quantity converted into ammonia (ΔN) and in the molasses carbon content (*%C*), according to Eq. 1:

$$\Delta_{Molasses} = [\Delta N \text{ x } (C/N)] \text{ x } \%C^{-1} \qquad (1)$$

It can be assumed that the ammonia flux into water, directly by excretion or indirectly by microbial degradation of the organic N residues, is roughly 50% of the feed nitrogen flux (Avnimelech, 1999):

$$\Delta N = Q_{Feed} \text{ x } \%N_{Feed} \text{ x } \%N_{Excretion} \qquad (2)$$

where, Q_{Feed} is the daily feed quantity supplied and $\%N_{Feed}$ is the feed nitrogen input (*%Crude Protein* x 6.25^{-1}).

The molasses quantity to be added in each experimental unit to attend the required C:N ratios in treatments was calculated using Eqs. (1) e (2):

$$\Delta_{Molasses} = [(Q_{Feed} \text{ x } \%N_{Feed} \text{ x } \%N_{Excretion}) \text{ x } (C/N)] \text{ x } \%C^{-1} \qquad (3)$$

Molasses used contained 25% of carbon in relation to raw material. Thus, assuming 35% protein feed pellets (5.6% N) and that 50% of the feed nitrogen are excreted ($\%N_{Excretion}$), we get:

$$\Delta_{Molasses} = [(Q_{Feed} \text{ x } 0.056 \text{ x } 0.5) \text{ x } (C/N)] \text{ x } 0.25^{-1} = Q_{Feed} \text{ x } 0.112 \text{ x } (C/N) \qquad (4)$$

The described equations were adapted from the studies accomplished by Avnimelech (1999), Hari et al. (2004, 2006) and Ebeling et al. (2006). Molasses was added at noon daily to the cultivation tanks, diluted in water and spread in the experimental units.

2.3 Animals, feed management and production evaluation

Twenty *L. vannamei* shrimps (1.90±0.37 g) were randomly stocked per tank (~25 shrimps•m^{-2}) and submitted to fortnightly measurements. Shrimps were fed a commercial diet (Camaronina35™, 35%-crude protein, Agribrands Purina do Brasil), offered *ad libitum* in feeding trays twice a day (8 and 16 hrs.). The uneaten feed was daily collected and stored under refrigeration to posterior dry weight quantifying. Shrimp production performance was evaluated through final weight (W*f*), weight gain (W*G*), survival (*S%*), final biomass (B*f*), biomass gain (B*G*), consumed feed (C_{feed}), specific growth rate (*SGR*), feed conversion ratio (*FCR*), protein efficiency ratio (*PER*) and shrimp yield (*Y*).

2.4 Water quality analysis

Water temperature, dissolved oxygen (YSI Incorporation, YSI–550A oxymeter) and pH (Homis, 1002PH digital pHmeter) were daily measured (7 and 16 hrs), while Secchi transparence and salinity (Atago, S–10E refractometer) were weekly measured. Fortnightly water samples were collected to nitrite (Bendochneider and Robinson (1952) *apud* Golterman et al., 1978), nitrate (Mackereth et al., 1978), total ammonia (Koroleff, 1976), alkalinity (Felfödy et al., 1987), α-chlorophyll (Nusch, 1988), silicate (Golterman et al., 1978), total phosphorous, inorganic phosphate, total suspended solids (TSS) and chemical oxygen demand (COD) determinations, according to APHA (1995). At the ending of experiment, it was also determinate organic matter level in sediment (EMBRAPA, 1997) and 5-days biochemical oxygen demand ($cBOD_5$) (APHA, 1995).

2.5 Bacteriological and phytoplankton analysis

Water samples to phytoplankton analysis were taken every two weeks. Two-litters samples were filtered on plankton net (25μm) and concentrated in 100 mL, to which was added 4% of 1%-borax neutralized formaldehyde for organisms preservation. The phytoplankton qualitative and quantitative (cells.mL^{-1}) analysis were done through Newell and Newell (1963) direct counting method, using 1-mL of sub samples and optical microscope.

Fortnightly water samples were taken for autotrophic, heterotrophic and *Vibrio* spp. population density (CFU mL^{-1}) evaluation. Autotrophic and heterotrophic bacteria were counted by depth and surface sowing techniques (respectively), according to Oliveira (2003). *Vibrio* were analyzed according to Silva (1997) and identified according to FDA (1998).

2.6 Statistical analysis of results

A one-way analysis of variance (ANOVA) was used to evaluate the effects of molasses addition in different C:N ratios, complemented by Duncan's test at 5% probability level. Survival and phytoplankton and bacteria population density data were *arcsen* $x^{0.5}$ and *log x* transformed, respectively. When variance heterogeneity persisted, the Friedman non-parametric analysis of variance was applied.

The statistical analysis agrees with Zar (1996) and Mendes (1999). Calculation was helped by *STATISTICA* v. 6.0 and *SysEAPRO* v. 1.0.

3 Results and Discussion

3.1 Water Quality

Water quality and sediment data are synthesized in Table 1. It was not observed significant difference ($P \geq 0.05$) on water temperature, salinity and pH among treatments.

During the experimental period, the mean water temperature was 28.3±1.45 °C, ranging from 25.2 to 31.8°C. The temperature amplitude observed was near to ideal for *L. vannamei* of 22 – 32°C (Pillay, 1990) or 26 – 33°C (Nunes, 2002).

The minimal (24‰) and maximum (35‰) salinities indicates a slight high variation. However, the mean salinity (28.3±2.52‰) was adequate for culture of this species. Being a euryhaline species, *L. vannamei* support a high salinity variation (0 to 50‰), but the ideal salinity for culture is 15 – 25‰ (Arana, 2004; Li et al., 2007). According to LeMoullac (2000), the salinity has relatively little effect on metabolic rate of euryhaline shrimps, what indicate a low energy requirement to osmotic regulation. In low salinities, *L. vannamei* is more sensitive to ammonium (Lin and Chen, 2001) and utilize protein as amino acids source to keep osmotic pressure and growth (Rosas et al., 2001).

The mean pH was 8.1±0.15 among treatments, ranging of 7.14 to 8.73. The ideal pH for shrimp culture varies from 6 to 9 (Boyd, 2001) or 7.5 to 8.5 (Chien, 1992). pH values below 7 damages *L. vannamei* growth in heterotrophic system (Wasielesky et al., 2006) and above 9 induces water quality alterations, increasing alkalinity and ammonium toxicity (Avault Jr., 1996).

The reduced pH fluctuation can be attributed to adequate alkalinity levels (>100 mg $CaCO_3$ L^{-1}), what means an excellent capacity of acid-basic equilibrium in the culture water.

According to Wasielesky et al. (2006), alkalinity helps to maintain pH, besides be an important calcium source for shrimp ecdysis. In aquaculture, the alkalinity should not be inferior to 20 mg $CaCO_3$ L^{-1} due to phosphorous insolubility (Wurts, 2002); should be between 75 and 150 mg $CaCO_3$ L^{-1} for shrimps (Boyd, 2001), and between 100 and 140 mg $CaCO_3$ L^{-1} for *L. vannamei*, specifically (Clifford, 1994). The alkalinity levels were slightly increased throughout culture (Figure 1A), and significantly (P<0.05) affected by molasses addition on C:N ratios used (Table 1).

The concentration of total suspended solids (TSS) (0.128 to 0.152 g L^{-1}) varied similarly among treatments throughout culture (Figure 1B), and it was not influenced (P≥0.05) by molasses addition. However, the concentration of α-chlorophyll were strongly influenced (P<0.05) by molasses addition, responding inversely to C:N ratios, with lower concentrations in RM_{30} (0.162 mg L^{-1}) and RM_{20} (0.286 mg L^{-1}) (Figure 1C). Probably the heterotrophic tendency observed in treatments RM_{20} and RM_{30} should have inhibit the autotrophic development and consequently reduced the α-chlorophyll levels. According to Boyd (2001), productive ponds frequently show α-chlorophyll concentrations from 0.05 to 0.2 mg L^{-1}. Clifford (1994) reported that adequate α-chlorophyll and TSS levels to *L. vannamei* ranging from 0.05 to 0.075 mg L^{-1} and 0.05 to 0.15 g L^{-1}, respectively. Erler et al. (2005) and Hari et al. (2006) did not observe statistical difference in α-chlorophyll due to organic carbon sources addition. The mean concentration of α-chlorophyll in tanks (Table 1) was superior to that obtained by Matias et al. (2002) (0.09 to 0.12 mg.L^{-1}) and compatible to Burford et al. (2003) (0.13 to 0.44 mg.L^{-1}).

Based on Secchi disk data, it was observed statistical difference (P<0.05) among treatments related to water visibility. The mean visibility varied from 13.27 to 16.03 cm, with lower values in CTL and RM_{20}, when related to RM_{30}. Correlating visibility with α-chlorophyll and TSS concentrations, it was observed that visibility values were mainly elapsed to α-chlorophyll concentration, due to equality in TSS among treatments.

The mean concentrations of dissolved oxygen (OD) were maintained above 4.0 mg L^{-1} what, according to Chien (1992) and Boyd (1997), could be considerate adequate to shrimp culture. The reduced amplitude (~3.6 mg O_2 L^{-1}) observed in experimental tanks results of constant aeration system, which differ values for production ponds reported by Tookwinas and Songsangjinda (1999) (~10 mg O_2 L^{-1}) and Matias et al. (2002) (~9 mg O_2 L^{-1}). However, it was statistical difference (P<0.05) among treatments, where RM_{30} and RM_{20} showed the

lowest OD levels (4.64 and 4.76 mg L^{-1}, respectively), what could be related to bigger organic carbon supply in these treatments.

Table 1. Physicochemical water quality variables and organic matter on sediment in Litopenaeus vannamei experimental tanks, over a 70-day semi-intensive culture (~25 shrimps m^{-2}) with daily sugar cane molasses addition in different carbon:nitrogen ratios [C/N] and no water exchange.

Parameter	Treatment*			
	CTL	**RM_{10}**	**RM_{20}**	**RM_{30}**
Temperature (°C)[A]	28.4[a] ± 1.43	28.0[a] ± 1.39	28.5[a] ± 1.48	28.4[a] ± 1.48
pH[A]	8.2[a] ± 0.19	8.1[a] ± 0.14	8.0[a] ± 0.14	8.0[a] ± 0.14
Dissolved Oxygen (mg L^{-1})[A]	4.97[a] ± 0.78	4.99[a] ± 0.81	4.76[b] ± 0.8	4.64[b] ± 0.81
Salinity (‰)[B]	27.9[a] ± 2.62	28.4[a] ± 2.43	29.2[a] ± 2.38	27.9[a] ± 2.66
Secchi Disk Visibility (cm)[B]	13.27[b] ± 3.93	14.57[ab] ± 4.55	13.37[b] ± 3.54	16.03[a] ± 3.65
Total Ammonia Nitrogen – TAN (mg L^{-1})[C]	0.102[a] ± 0.18	0.250[a] ± 0.27	0.152[a] ± 0.15	0.289[a] ± 0.23
Nitrite-N (mg L^{-1})[C]	0.037[a]±0.035	0.035[a]±0.034	0.001[b]±0.002	0.007[b]±0.019
Nitrate-N (mg L^{-1})[C]	0.884[a] ± 0.66	0.456[b] ± 0.55	0.048[c] ± 0.10	0.180[bc] ± 0.42
Total phosphorus (mg L^{-1})[C]	0.454[a] ± 0.17	0.474[a] ± 0.17	0.397[ab] ± 0.11	0.335[b] ± 0.13
Inorganic phosphate (mg L^{-1})[C]	0.006[a]±0.006	0.007[a]±0.006	0.002[b]±0.002	0.004[ab]±0.003
Alkalinity (mg $CaCO_3$ L^{-1})[C]	143.4[b] ± 21.5	163.1[ab] ± 31.3	157.7[ab] ± 31.3	178.9[a] ± 42.1
Chlorophyll-a (mg L^{-1})[C]	0.335[a] ± 0.23	0.318[a] ± 0.24	0.286[ab] ± 0.26	0.162[b] ± 0.13
Silicate (mg L^{-1})[C]	0.334[a] ± 0.25	0.343[a] ± 0.29	0.265[a] ± 0.15	0.315[a] ± 0.24
Total Suspended Solids – TSS (g L^{-1})[C]	0.146[a]±0.067	0.128[a]±0.055	0.143[a]±0.064	0.152[a]±0.086
COD (mg O_2 L^{-1})[C]	1,123[a] ± 333.8	1,200[a] ± 257.5	1,177[a] ± 267.3	1,175[a] ± 344.0
$cBOD_5$ (mg O_2 L^{-1})[D]	132.7[b] ± 118.7	167.3[ab] ± 44.1	282.0[a] ± 10.4	221.3[ab] ± 53.7
Organic Matter (sediment) (g kg^{-1})[D]	4.77[a] ± 2.0	5.06[a] ± 0.95	4.54[a] ± 1.15	7.01[a] ± 0.98

*Values are given as averages ± standard deviation. Different superscript letters ([a,b,c]) in the same line denote significant difference ($P<0.05$) between the treatments by Duncan's test. [A,B,C]Daily, Weekly and Fortnightly measured parameter, respectively. [D]Measured parameters at the end of the culture. $cDBO_5$ – 5-day Carbonaceous Biochemical Oxygen Demand and COD – Chemical Oxygen Demand CTL (control): with no molasses addition; RM_{10}, RM_{20} and RM_{30}: C/N ratios in 10, 20 and 30:1, respectively.

The molasses addition in different C:N ratios significantly interfered ($P<0.05$) on 5-days biochemical oxygen demand ($cBOD_5$), ranging from 132.6 to 282 mg O_2 L^{-1} among

treatments. The highest $cBOD_5$ levels were observed in 20:1 and 30:1 C:N ratios, what corroborate with OD concentrations verified in these treatments. It was observed a small elevation on Chemical Oxygen Demand (COD) throughout culture in all treatments (Figure 1D). The mean COD was 1,168.7±298.0 mg O_2 L^{-1}, without statistical difference ($P \geq 0.05$) among treatments. The $cBOD_5$ and COD values registered during experimental period were superior to that reported by Hari et al. (2004): 1.6 to 2.0 mg O_2 L^{-1} and 384.5 to 386.0 mg O_2 L^{-1}, respectively. Matias et al. (2002), using probiotics and molasses in *Penaeus monodon* culture, reported 16.54 to 22.3 mg O_2 L^{-1} for $cBOD_5$ and 852.2 to 870.3 mg O_2 L^{-1} for COD, and Samocha et al. (2007), rearing *L. vannamei* using different molasses quantities and 30%-CP feed, did not observe significant difference in $cBOD_5$ (7.4 to 9.7 mg O_2 L^{-1}) and COD (1,359 to 1,495 mg O_2 L^{-1}).

The molasses addition reduced ($P<0.05$) nitrite and nitrate concentrations throughout culture (Figures 1E and 1F). The molasses acted in opposite way to C:N ratio; for higher ratios (RM_{20} and RM_{30}) it was observed lower concentrations of these nitrogen compounds. The mean nitrite and nitrate concentrations ranging from 0.007 to 0.037 mg L^{-1} and 0.05 to 0.88 mg L^{-1}, respectively. The total ammonia nitrogen – TAN (NH_3 + NH_4^+) was in undetectable levels until 28th culture day and it varied in a so similar way among treatments (Figure 1G). Analyzing mean TAN concentrations, it was not observed significant difference ($P \geq 0.05$) due to molasses addition in different C:N ratios. However, at the end of culture, RM_{20} showed the lowest TAN concentration (0.01 mg L^{-1}), which statistically differ ($P<0.05$) of RM_{10} (0.52 mg L^{-1}). Barbieri and Ostrensky (2002) recommend nitrite concentration below of 0.5 mg L^{-1}, nitrate between 0.4 and 0.8 mg L^{-1} and TAN between 0.1 and 1.0 mg L^{-1}. Chien (1992) indicate maximum of 0.1 and 1.0 mg L^{-1} for unionized ammonia (NH_3) and nitrite, respectively.

The evolution of others chemical water quality parameters (total phosphorous, inorganic phosphate and silicate) is showed in Figures 1H, 1I and 1J. Total phosphorous (0.335 to 0.474 mg L^{-1}) and inorganic phosphate (0.002 to 0.007 mg L^{-1}) statistically differed ($P<0.05$) among treatments, with lowest concentrations in 20:1 and 30:1 C:N ratios. The molasses addition in different C:N ratios did not interfere ($P \geq 0.05$) in silicate levels (0.314±0.23 mg L^{-1}). The sediment organic matter ranged from 4.54 to 7.01 g kg^{-1}. The RM_{30} treatment showed the highest organic matter level, but without significant difference ($P \geq 0.05$) among treatments.

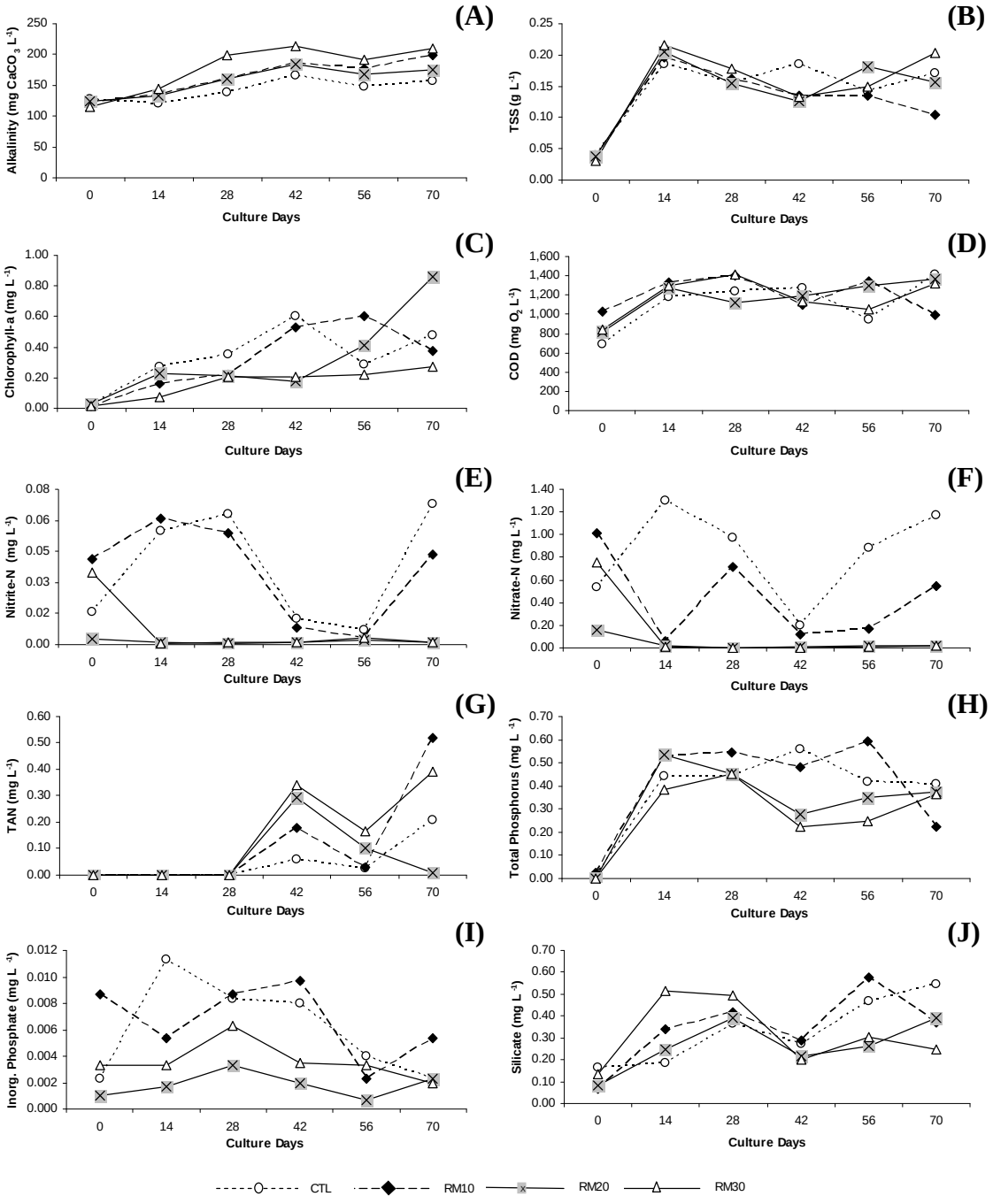

Figure 1. The effects of sugar cane molasses addition in different C/N ratios on the water quality parameters alkalinity (A), total suspended solids – TSS (B), chlorophyll-a (C), chemical oxygen demand – COD (D), nitrite–N (E), nitrate–N (F), total ammonia nitrogen – TAN (G), total phosphorus (H), inorganic phosphate (I) and silicate (J), over a 70-day *Litopenaeus vannamei* semi-intensive culture (~25 shrimps m^{-2}) in experimental tanks with no water exchange. CTL (control): with no molasses addition; RM_{10}, RM_{20} and RM_{30}: C/N ratios in 10, 20 and 30:1, respectively.

3.2 Phytoplankton and bacteria

The mean phytoplankton density in CTL, RM_{10}, RM_{20} and RM_{30} treatments was 1,109, 1,036, 330 and 239 cell.mL^{-1}, respectively, without significant difference ($P \geq 0.05$) among them. In general, the phytoplankton was represented by Bacillariophyceae (diatoms), Chlorophyceae (chlorophyceans), Cyanophyceae (cyanobacteria) and Dinophyceae (dinoflagellates) (Table 2 and Figure 2).

Bacillariophyceae class predominated ($P<0.05$) in higher C:N ratios (RM_{20} and RM_{30}), corresponding to 79 and 75%, respectively, of phytoplanktonic organisms. For lower C:N ratios (RM_{10} and CTL), the predominant organisms ($P<0.05$) were Cyanophyceae, with 81 and 80%, respectively. Chlorophyceae (~7.5%) and Dinophyceae (<1.0%) classes had few representation in all treatments.

Table 2. Phytoplankton and bacterial densities in *Litopenaeus vannamei* experimental culture tanks with daily sugar cane molasses addition in different C/N ratios and no water exchange.

Organism	**Treatments***			
	CTL	**RM_{10}**	**RM_{20}**	**RM_{30}**
Phytoplankton (cells mL^{-1})				
Diatoms	177.0^{a} ± 325.5 (6.75–1,343)	128.5^{a} ± 249.7 (4.75–1,067)	260.3^{a} ± 325.0 (0.0–1,117)	180.4^{a} ± 187.1 (6.50–603.5)
Chlorophyceans	38.15^{a} ± 71.6 (0.50–301.8)	63.0^{a} ± 133.8 (0.0–575.8)	28.22^{a} ± 43.9 (0.0–183.3)	28.17^{a} ± 34.6 (0.0–108.8)
Cyanobacteria	892.8^{a} ± 1,302.3 (0.50–4,089)	841.6^{a} ± 1,681.6 (1.50–6,556)	40.7^{b} ± 112.7 (0.0–475.0)	30.7^{b} ± 65.6 (0.75–252.0)
Dinoflagellates	0.85^{a} ± 3.18 (0.00–13.5)	2.68^{a} ± 10.26 (0.00–43.75)	0.47^{a} ± 0.94 (0.0–3.0)	0.14^{a} ± 0.27 (0.00–0.75)
Bacteria (CFU)				
Vibrio spp. (x 10^{2} mL^{-1})	1.51^{a} ± 3.14 (0.0–11.0)	1.47^{a} ± 3.96 (0.0–16.0)	1.09^{a} ± 2.86 (0.0–12.0)	1.13^{a} ± 2.05 (0.0–7.1)
HET (x 10^{5} mL^{-1})	174.9^{a} ± 434.8 (0.003–1,800)	440.6^{a} ± 858.7 (0.003–2,900)	70,3^{a} ± 125.5 (0.001–400.0)	170.9^{a} ± 371.1 (0.003–1,500)
AUTO (x 10^{4} mL^{-1})	2.08^{a} ± 3.93 (0.0–12.0)	8.76^{a} ± 28.07 (0.0–120.0)	4.15^{a} ± 5.15 (0,0–16.0)	1.60^{a} ± 2.37 (0.0–8.5)

*Values are given as averages ± standard deviation, minimum and maximum in parenthesis. Different superscript letters (^{a,b,c}) in the same line denote significant difference (P<0.05) between the treatments by Duncan's test. CFU – Colony Forming Units. AUTO – Autotrophic Bacteria and HET – Heterotrophic Bacteria. CTL (control): with no molasses addition; RM_{10}, RM_{20} and RM_{30}: C/N ratios in 10, 20 and 30:1, respectively.

The molasses addition significantly interfered (P<0.05) in cyanobacteria density during culture (Figure 2C), ranging from 30.7 to 892.8 cell.mL^{-1}. The lower densities (30.7 and 40.7 cell.mL^{-1}) were observed in 30:1 and 20:1 C:N ratios, respectively. Diatoms (128.5 to 260.3 cell.mL^{-1}), chlorophyceans (28.2 to 63.0 cell.mL^{-1}) and dinoflagellates (0.14 to 2.68 cell.mL^{-1}) did not show statistical difference (P≥0.05) among treatments. Nunes (2001) recommends minimal densities of 50,000 cell.mL^{-1} and 20,000 cell.mL^{-1} for diatoms and chlorophyceans (respectively) and maximum of 40,000 cell.mL^{-1} for cyanobacteria.

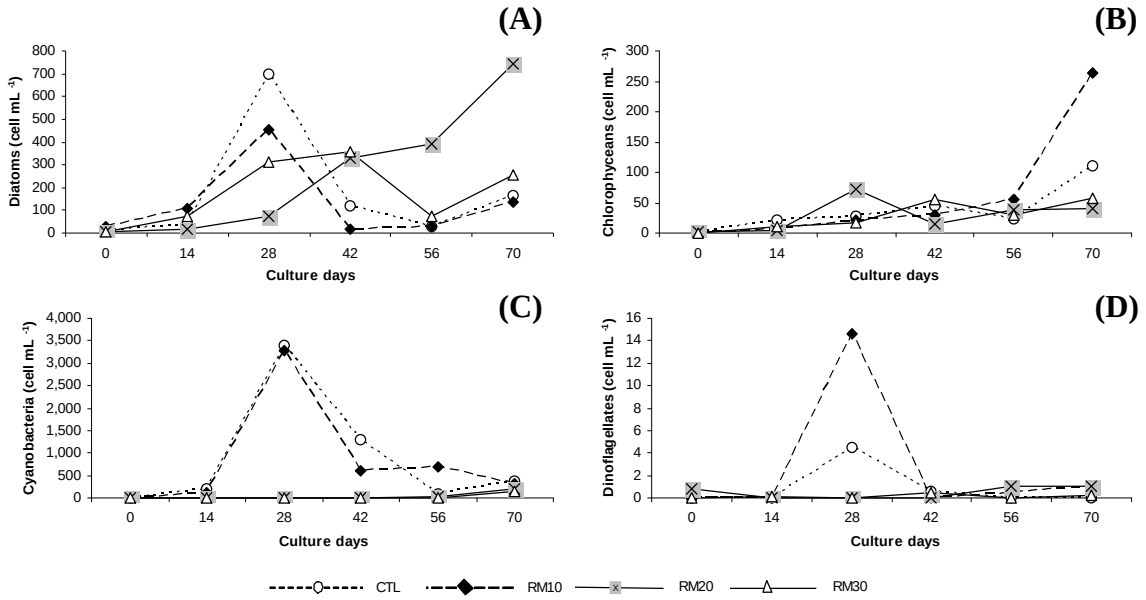

Figure 2. The effect of sugar cane molasses addition in different C/N ratios on the phytoplankton communities Diatoms (A), Chlorophyceans (B) Cyanobacteria (C) and Dinoflagellates (D), over a 70-day *Litopenaeus vannamei* semi-intensive culture (~25 shrimps m^{-2}) in experimental tanks with no water exchange. CTL (control): with no molasses addition; RM_{10}, RM_{20} and RM_{30}: C/N ratios in 10, 20 and 30:1, respectively.

The RM_{10} and CTL treatments had similar phytoplanktonic development during culture period, showing, at 28th culture day, high diatoms, cyanobacteria and dinoflagellates densities (blooms) (Figures 2A, 2C and 2D). These blooms could be related to a larger nutrient supply (Figure 1), deriving from enhance in feed offered in this period. Although diatoms and chlorophyceans had showed distinct development in RM_{20} and RM_{30} (Figures 2A and 2B), these treatments presented similarity relative to cyanobacteria and dinoflagellates, inhibiting development of high densities of these organisms (28th culture day) (Figures 2C and 2D).

Organic carbon addition, through molasses, in tanks did not demonstrate significant effect (P≥0.05) on autotrophic, heterotrophic and *Vibrio* spp. bacteria. The mean population

density of autotrophic bacteria ranging from 1.60 to 8.76 CFU x 10^4 mL^{-1}, while the heterotrophic one ranging from 70.3 to 440.6 CFU x 10^5 mL^{-1} (Table 2). The heterotrophic bacteria density was superior (P<0.05) than autotrophic one. According to Ebeling et al. (2006), heterotrophic bacteria use organic carbon sources more efficiently and grow up fivefold faster than autotrophic bacteria.

The heterotrophic and autotrophic bacteria development happened in an analogous way among treatments, showing slow growth until 42nd day, with maximum densities below to 25.0 CFU x 10^5 mL^{-1} and 5.0 CFU x 10^4 mL^{-1}, respectively (Figures 3A and 3B). Concomitantly, TAN concentrations were in non-detectable levels (Figure 1G). The reduction on TAN and nitrite levels, observed at 56th culture day, is directly related to significant enhance in bacterial densities in this period (Figure 1 and Figure 3). The TAN was mobilized to new bacterial cells synthesis (Hari et al., 2004) and the nitrite has harmful action on bacteria.

According to Mendes et al. (2005), *Vibrio* are the most important bacteria in shrimp culture and physiologically are presented in shrimp midgut; however, when in imbalance, could cause diseases with high mortality. The *Vibrio* mean density ranged from 1.09±2.86 to 1.51±3.14 CFU x 10^2 mL^{-1} and, even without statistical difference (P≥0.05) among treatments, the lower values were observed in higher C:N ratios (RM_{20} and RM_{30}) (Table 2). Along culture, it was identified six *Vibrio* specie: *Vibrio carchariae, V. proteolyticus, V. fluvialis, V. alginolyticus, V. metchnikovii* and *V. nereis*.

The *Vibrio* development was so irregular in CTL, RM_{10} and RM_{20}, showing two density peaks along culture (14th and 42nd days) (Figure 3C). It demonstrates an apparent correlation with phytoplankton development (Figure 2), once algal bloom exactly occurred between *Vibrio* density peaks (28th day).

The process of community succession occurs over time within shrimp ponds in response to the increasing organic load and maturity of the ecosystem (Chamberlain et al., 2001). Analyzing density data (Table 2) and phytoplanktonic and bacterial communities evolution along culture (Figures 2 and 3), can be observed that, at the end of culture, the environment tended to favour heterotrophic bacteria. However, in the experimental tanks was not observed a real heterotrophic system. According to Burford et al. (2003), to characterize a heterotrophic system, the carbon supply should exceed primary production.

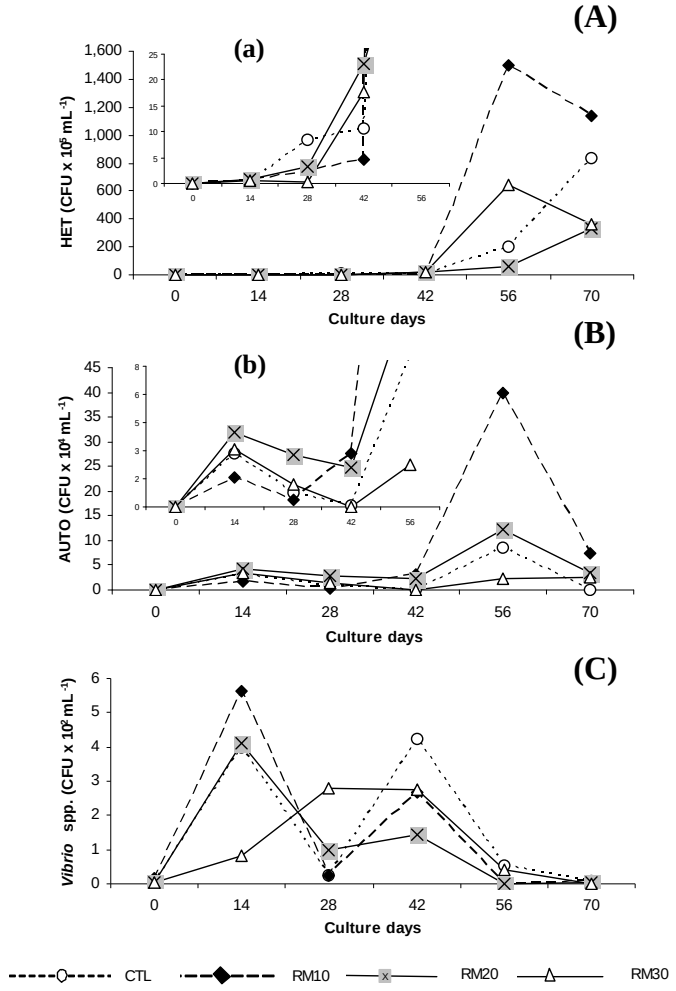

Figure 3. The effect of sugar cane molasses addition in different C/N ratios on the population density (CFU – Colony Forming Units per mL) of HET – heterotrophic (A), and AUTO – autotrophic bacteria (B) and *Vibrio* spp. (C), over a 70-day *Litopenaeus vannamei* semi-intensive culture (~25 shrimps m^{-2}) in experimental tanks with no water exchange. CTL (control): with no molasses addition; RM_{10}, RM_{20} and RM_{30}: C/N ratios in 10, 20 and 30:1, respectively. Details in *a* and *b*.

3.3 Production evaluation

The parameters used on productive performance evaluation of shrimps with molasses addition are summarized on Table 3. Shrimp survival was high for all treatments (91.7 to 100%), without statistical difference ($P \geq 0.05$) among them.

Shrimps showed continuous growth during 70-days culture, developing so similar way, varying from 0.96 to 1.04 g per week among treatments. However, about specific growth rate (SGR), this equality was not observed ($P<0.05$). The treatments with molasses addition (RM_{10}, RM_{20} and RM_{30}) showed SGR (2.63, 2.69 and 2.65 % day^{-1}, respectively),

and RM_{20} was higher (P<0.05) than CTL (2.53 % day^{-1}). Hari et al. (2006) observed higher shrimps SGR and weight gain in extensive ponds with carbohydrate addition.

Shrimps final weight was significantly higher (P<0.05) in high C:N ratios (20:1 and 30:1) treatments, resulting in different (P<0.05) weight gain in these treatments during the culture. Final mean weights of CTL, RM_{10}, RM_{20} and RM_{30} treatments were 11.56, 11.66, 12.25 and 12.27 g, respectively. Relating to final biomass and biomass gain, it was not observed significant difference (P≥0.05) among treatments. The shrimp biomass ranged from 213.9 to 240.8 g, with mean gain of 189.7±16.2 g related to initial biomass (Table 3).

Table 3. Yield performance of Pacific white shrimp *Litopenaeus vannamei* over a 70-day semi-intensive culture (~25 shrimps m^{-2}) in zero water exchange experimental tanks with daily sugar cane molasses addition in different carbon: nitrogen ratios [C/N].

Yield variables	**Treatment***			
	CTL	**RM_{10}**	**RM_{20}**	**RM_{30}**
Initial weight [W*i*] (g)	$1.97^{a} \pm 0.36$	$1.85^{a} \pm 0.38$	$1.86^{a} \pm 0.35$	$1.92^{a} \pm 0.38$
Final weight [W*f*] (g)	$11.56^{b} \pm 1.50$	$11.66^{ab} \pm 1.48$	$12.25^{a} \pm 1.49$	$12.27^{a} \pm 1.61$
Weight gain [W*G*] (g)	$9.59^{b} \pm 0.14$	$9.82^{ab} \pm 0.57$	$10.39^{a} \pm 0.22$	$10.35^{a} \pm 0.23$
Survival (*S%*)	$100.0^{a} \pm 0.0$	$91.7^{a} \pm 7.6$	$98.3^{a} \pm 2.9$	$91.7^{a} \pm 7.6$
Final biomass [B*f*] (g)	$231.2^{a} \pm 3.2$	$213.9^{a} \pm 22.8$	$240.8^{a} \pm 5.9$	$224.7^{a} \pm 15.1$
Biomass gain [B*G*] (g)	$191.7^{a} \pm 2.8$	$177.0^{a} \pm 25.1$	$203.6^{a} \pm 5.7$	$186.4^{a} \pm 14.9$
SGR (% dia^{-1})	$2.53^{b} \pm 0.06$	$2.63^{ab} \pm 0.13$	$2.69^{a} \pm 0.02$	$2.65^{ab} \pm 0.03$
FCR	$1.35^{a} \pm 0.12$	$1.45^{a} \pm 0.10$	$1.41^{a} \pm 0.07$	$1.51^{a} \pm 0.14$
PER	$1.90^{a} \pm 0.16$	$1.76^{a} \pm 0.12$	$1.81^{a} \pm 0.09$	$1.70^{a} \pm 0.16$
Shrimp yield [Y] (g m^{-2})	$289.0^{a} \pm 4.0$	$267.4^{a} \pm 28.5$	$301.0^{a} \pm 7.3$	$280.9^{a} \pm 18.9$

*Values are given as averages ± standard deviation. Different superscript letters in the same line denote significant difference (P<0.05) between the treatments by Duncan's Test. W*G* = W*f* – W*i*; *S%*= 100 x (N x n^{-1}), *n* – initial shrimp number per tank and N – final shrimp number per tank; B*G* = B*f* – (W*i* x *n*); SGR = 100 x (*ln* W*f* – *ln* W*i*) x T^{-1}, SGR – Specific Growth Rate and T – culture period at days (70 days); FCR = C_{feed} x BG^{-1}, FCR – Feed Conversion Ratio; PER = B*G* x (C_{feed} x *CP*)$^{-1}$ and [C_{feed}] – Consumed Feed (dry matter), PER – Protein Efficiency Ratio and *CP* – crude protein in dry matter feed, Y = B*f* x A^{-1}, A – Bottom area of culture tank (0.8 m^2). CTL (control): with no molasses addition; RM_{10}, RM_{20} and RM_{30}: C/N ratios in 10, 20 and 30:1, respectively.

The mean FCR was 1.35, 1.45, 1.41 and 1.51 for CTL, RM_{10}, RM_{20} and RM_{30} respectively, without statistic differences (P≥0.05) among them. According to Barbieri and Ostrensky (2002), FCR values between 0.9 and 1.5 are satisfactory, and could vary in

function of the stocking density. Boyd (1997) reports that shrimps farms generally obtain FCR between 2.0 and 2.4. The PER ranged from 1.70 to 1.90 and it was not significantly influenced ($P \geq 0.05$) by molasses addition.

In this work, shrimp yield values did not show significant difference ($P \geq 0.05$), ranging from 267.4 to 301.0 g m^{-2} among treatments. Hari et al. (2006), testing carbohydrate addition (tapioca flour) in extensive *Penaeus monodon* culture, obtained production of 160 g m^{-2}, FCR of 1.1 and PER of 3.6. Martinez-Cordova et al. (2003) obtained shrimp yield ranging from 219.17 to 261.50 g m^{-2}, in 112-days period, stocking density of 16.6 shrimps m^{-2} and without aeration. McIntosh et al. (2001), using aeration and stocking density of 40 shrimps m^{-2}, obtained values of 441 and 540 g m^{-2} in a 94-days period.

Erler et al. (2005) demonstrated that carbon addition in molasses way could improve growth and FCR of *P. monodon* in no water exchange culture system. Samocha et al. (2007), rearing *L. vannamei* in limited water exchange tanks, did not observe significant effect on water quality and shrimp performance, when fed low protein diet and different molasses addition levels.

4 Conclusion

This study demonstrated that sugar cane molasses can be used as a carbon source to adjust carbon:nitrogen ratios in shrimp culture with no water exchange. The C:N ratios in 20 and 30:1 showed a good efficiency in the water quality control, reducing the nitrogen compounds levels (ammonia and nitrite) and inhibiting the development of undesirables microorganisms in the culture, besides to improve *Litopenaeus vannamei* culture performance. However production systems based on organic carbon source addition demand a higher dissolved oxygen quantity and consequently requiring an adequate aeration system.

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5. CONCLUSÕES

O melaço de cana-de-açúcar pode ser utilizado como fonte alternativa de carbono para ajustar as relações Carbono:Nitrogênio no cultivo de camarão sem renovação de água. As relações C:N 20 e 30:1 proporcionaram um melhor desempenho no cultivo do camarão marinho *Litopenaeus vannamei*, demonstrando ser efetivas no controle da qualidade da água, reduzindo os níveis dos compostos nitrogenados (amônia e nitrito) e inibindo o desenvolvimento de microorganismos indesejáveis ao cultivo. No entanto, sistemas aqüícolas baseados no aporte de fontes orgânicas de carbono demandam uma maior quantidade de oxigênio dissolvido e, portanto, necessitam de um sistema de aeração bastante eficiente.

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