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Análise de minerais por Ativação Neutrônica Instrumental (INAA) e Espectrometria de Massa (ICP-MS) em cultivo de camarões marinhos (*L. vannamei*) em bioflocos

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Tese apresentada ao Programa de Pós-graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais como requisito para Obtenção do Grau de Doutor em Zootecnia.

Área de concentração: Produção de animais aquáticos

Prof. Orientador: Kleber Campos Miranda Filho

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“There are many hypotheses in science which are wrong. That’s perfectly all right: it’s the aperture to finding out what’s right. Science is a self-correcting process.”

Carl Sagan

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RESUMO

De acordo com National Research Council (NRC), alguns microminerais (Cu, Zn, Mn e Se) são considerados essenciais ao desenvolvimento da espécie *Litopenaeus vannamei*. No entanto, pouco se sabe sobre o acúmulo desses elementos em sistemas de aquicultura que empregam pouca ou nenhuma troca de água como o sistema Biofloc Technology (BFT). O objetivo deste trabalho foi analisar a concentração de microminerais necessários e tóxicos presentes no alimento, biofloco e nos camarões criados em sistema superintensivo (400 camarões/m^2), distantes da costa, sem renovação de água e em baixa salinidade. Na primeira fase da pesquisa (capítulo 2), foi realizada a análise multielementar dos insumos utilizados na carcinicultura utilizando a Análise de Ativação Neutrônica Instrumental (INAA), baseada no método *k0*, utilizando reator nuclear como técnica referencial, com suporte de analises das amostras em em outra técnica: Espectrometria de Massa com Plasma acoplado indutivamente (ICP-MS); como foco ao aporte de informações adicionais em minerais considerados essenciais. Após o cultivo, observou-se um acúmulo superior de elementos no cefalotórax do camarão quando comparado ao resto do corpo. O Fator de Transferência Trófica (FTT) foi> 1 para os microminerais necessários Cu e Se via alimentação para amostras de animais inteiros. O biofloco concentrou todos os elementos requeridos pela espécie, presentes na ração comercial. Na segunda fase (capítulo 3), tratou-se da descrição da gama variada de elementos, classificados como potencialmente tóxicos ao sistema de produção. Concentrações de elementos lantanídeos e actinídeos em flocos microbianos também foram observadas, elementos como bromo (Br) e césio (Cs) obtiveram FTT da ração utilizada para o animal> 1. Dos 28 elementos presentes nos insumos, 23 concentraram no sistema de produção via sólidos suspensos e 16 elementos foram incorporados na composição do tecido animal. Tanto insumos usados como os animais produzidos em um ciclo em BFT estavam dentro dos limites exigidos pela legislação brasileira. Especial atenção deve ser dada a pesquisa sobre sistemas fechados de aquicultura, pois há uma variedade de elementos que podem acumular ao longo dos ciclos de produção, e seus efeitos sobre a saúde e nutrição dos camarões e seres humanos são pouco conhecidos.

Palavras-chave: BFT; carcinicultura; microminerais; nutrição; sanidade

ABSTRACT

According to the National Research Council (NRC) some microminerals (Cu, Zn, Mn and Se) are considered required for development of *Litopenaeus vannamei*. However, little is known about the fate of these elements in aquaculture systems that employ low or no water exchange such as the Biofloc Technology system (BFT). The objective of this study was to analyze the concentration of microminerals required and toxics present in feed, biofloc and in shrimp raised in superintensive system (400 shrimps/m^2), distant from the coast with no water renewal in low salinity. In the first stage of the research (chapter 2), a multielementary analysis of aquacultural inputs used in shrimp farming was performed using the Instrumental Neutron Activation Analysis (INAA), based on the *k0* method using nuclear reactor as referencial technique. Same samples were also analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with focus for all minerals cited as essencial. We observed a greater accumulation of elements in the shrimp cephalothorax when compared with the rest of the body. Trophic transfer factor (TTF) was > 1 for essentials Cu and Se, via feed for whole animal samples. In relation to commercial feed, the biofloc concentrated all the required elements. In the second phase (chapter 3), was observed a greater accumulation of several elements, classified as potentially toxic to production system were identified. Concentrations of lanthanides and actinides elements in biofloc were also observed, elements such as bromine (Br) and cesium (Cs) obtained TTF of the aquafeed used for the animal > 1. Of the 28 elements present in the inputs, 23 were concentrated in the production system via suspended solids and 16 elements were incorporated into the animal tissue composition. Both supplies and animals were within the limits required by Brazilian legislation. Special attention should be given to research into closed aquaculture systems, as there are a variety of elements that can accumulate along the production cycles and their effects on heath and nutrition of cultured animals and human beings are little known.

Key-words: BFT; heath; microminerals; nutrition; shrimp farming

INTRODUÇÃO

O setor aquícola tem se desenvolvido para atender a demanda de alimentos como consequência do declínio da estagnação das fontes extrativistas (FAO, 2018). O aumento da demanda por pescado é um dos fatores que tem exigido a intensificação da maioria dos sistemas aquícolas (Coloso, 2015). Este crescente segmento da produção global de alimentos aumentou rapidamente nas últimas cinco décadas, com uma taxa média anual global de crescimento acima de 6,0% (FAO, 2018).

A produção de alimentos e rações para organismos aquáticos, de acordo com a FAO (2015) é uma das indústrias de mais rápida expansão do mundo, com taxas de crescimento superiores a 30% ao ano, com crescimento três vezes mais rápido em comparação com a produção para animais terrestres.

Na carcinicultura mundial, a produção do camarão marinho *Litopenaeus vannamei* cresceu em volume de 8.000 toneladas em 1980 para 3.668.681 toneladas em 2014, representando 80% da produção mundial de camarões de cultivo (4.580.768 t.), e 46,5% de todo camarão ofertado no mundo. Este aumento na produção desta espécie se deve em grande parte as características zootécnicas da espécie, avanços nas técnicas de cultivo e à intensificação da produção (ABCC, 2016; FAO, 2016).

Segundo Moss (2002), novas tendências de cultivo foram desenvolvidas, com a redução do tamanho dos tanques, redução ou nenhuma troca de água, produções em estufas, sistemas de recirculação, e sistemas cada vez mais fechados, com redução na utilização da água.

Com a evolução dos sistemas tradicionais de produção, entrou em destaque o Sistema de Bioflocos ou “Biofloc Technology System” (BFT), que é caracterizado como um sistema de cultivo utilizando altas densidades, com pouca troca de água, resultando em um aumento da produtividade e redução da emissão de efluentes para o meio ambiente (Burford et al., 2003; Emerenciano, et al., 2013; Samocha et al., 2017)

Emerenciano et al. (2013) relatam que quando a água é reutilizada, alguns riscos, como a introdução de patógenos, o escape de espécies exóticas e a descarga de águas residuais (poluição) são reduzidos e até eliminados. Contudo, segundo Samocha et al. (2017), alguns microminerais podem acumular na água, biofloco e nos animais se a água do cultivo for reusada por alguns ciclos.

O objetivo desse trabalho foi caracterizar os microminerais que podem ser acumulados e influenciar a produção dos comarões, a saúde animal e humana, quando criados em sistema de bioflocos sem renovação de água.

CAPÍTULO I - REVISÃO DE LITERATURA

1. Sistema de Bioflocos (BFT)

O BFT tem como característica a formação de agregados microbianos, chamados de bioflocos, compostos por bactérias, algas, protozoários e zooplânctons (Avnimelech, 2015), e vem sendo explorado em pesquisas quanto sua qualidade nutricional, quando formado dentro do sistema de cultivo (*in situ*) por adição de carbono, e quando produzido em reatores (*ex situ*), com o uso e reuso de efluentes de cultivos de diferentes espécies, para produção de inóculos ou produção de farinhas de biofloco, usadas em dietas de animais aquáticos (Emerenciano et al., 2013; Samocha et al., 2017).

A capacidade de reciclar a matéria orgânica acumulada ocorre com a formação de uma comunidade bacteriana capaz de degradar os resíduos existentes, além de converter os compostos nitrogenados em biomassa microbiana (Samocha et al., 2007).

Este sistema é considerado sustentável por reciclar continuamente os nutrientes, baseado no crescimento de micro-organismos com duas funções: (1) manter a qualidade da água, pela absorção de nitrogênio gerando proteína microbiana disponível no sistema; e (2) nutricional, na melhora do índice de conversão alimentar e redução de custos com ração (Emerenciano et al., 2013).

Assim, o cenário futuro tende a redução da troca de água dos sistemas produtivos, evoluindo para zero de troca e descarga no meio ambiente. No entanto, em sistemas produtivos aquícolas com reuso de água, algumas substâncias como os microminerais podem acumular na água e gerar danos aos animais (Martins et al., 2009).

As diferentes fontes de carbono e insumos usados (cada uma com sua composição individual), salinidades diferentes dos sistemas já pesquisados, e até a fonte de água, influenciam na dinâmica do sistema e na possível toxicidade ao animal por meio de alguns elementos presentes no sistema. Segundo Leffer e Brunsson (2014), a dinâmica natural do ecossistema bacteriano faz com que o BFT seja susceptível a diversas mudanças bruscas que impactam na estabilidade, reprodutibilidade e replicabilidade das avaliações de campo feitas em alguns testes.

Contudo, ao contrário dos animais terrestres que se limitam principalmente a uma fonte dietética de minerais, os animais aquáticos podem utilizar, até certo ponto, microminerais dissolvidos na água para atender aos seus requisitos fisiológicos (NRC, 1993; Davis e Gatlin, 1996; NRC, 2011). Sendo este um fator complicador para pesquisas nutricionais com esses elementos, que já estão presentes em pequenas concentrações, tendo ainda diferentes vias de absorção nem sempre contabilizadas nas pesquisas publicadas.

2. Minerais

Os minerais são elementos químicos sólidos ou cristalinos que devem ser suplementados na dieta do animal, pois não são sintetizados por eles e nem podem ser originados de reações químicas metabólicas. Eles estão presentes em todos os tecidos de animais e plantas, e de modo geral, estão envolvidos em quase todas as vias metabólicas dos organismos, tendo funções importantes no ciclo de vida, entre outras funções fisiológicas vitais à manutenção animal e ao aumento da produtividade animal (NRC, 1993; Underwood e Suttle, 1999).

Segundo Scott et al. (1982), 23 minerais são considerados necessários em uma ou mais espécies animais, e divididos em dois grupos: os macrominerais que estão presentes em maior quantidade no organismo animal (cálcio, cloro, magnésio, fósforo, potássio, sódio e enxofre) e os microminerais presentes em menor quantidade no organismo do animal (alumínio, arsênio, cobalto, cromo, cobre, flúor, iodo, ferro, manganês, molibdênio, níquel, selênio, silício, estanho, vanádio, zinco e outros).

Os macrominerais estão envolvidos, em sua grande maioria, em funções estruturais ou fisiológicas. Já os microminerais possuem funções metabólicas menos estudadas incluindo a resposta imune, reprodução e crescimento, sendo muitas vezes catalisadores ou constituintes dos sistemas enzimáticos de muitas células dos animais (Davis, 1990; Underwood e Suttle, 1999; NRC 2011).

A base das funções e das necessidades de minerais para os crustáceos é similar aos dos peixes. Como os peixes, os crustáceos marinhos são capazes de absorver minerais do ambiente aquático, e assim seus requerimentos podem, em parte, ser atendidos pela ingestão da água (Hertramp e Piedad-Pascual, 2000).

Os minerais são componentes necessários ao exoesqueleto dos crustáceos, sendo também necessários para a manutenção da pressão osmótica e para a regulação do pH dos fluidos corporais (Piedad-Pascual, 1989). No caso de camarões, para a ecdise periódica, é necessária a incorporação de uma mistura mineral de modo a compensar a perda inorgânica durante o processo. Assim, para os camarões, a disponibilidade de minerais na dieta é extremamente importante e essencial (CIBA, 2006).

Apesar da nutrição do *L. vannamei* ter sido amplamente estudada nos últimos anos, uma classe de nutrientes que não foi cuidadosamente investigada é a dos microminerais (Cai et al., 2017).

3. Microminerais

Os microminerais influenciam o crescimento e sobrevivência dos camarões e não são sintetizados por estes animais. Assim, os microminerais são necessários para satisfazer a demanda fisiológica dos organismos aquáticos (CIBA, 2006).

As possíveis rotas de absorção desses microminerais pelos organismos aquáticos são pela água ingerida, alimentação, tegumento e brânquias. Após a entrada do micromineral no organismo, rotas como a utilização deste em atividades metabólicas, detoxificação com estocagem temporária ou permanente e excreção podem ocorrer (Rainbow et al., 1999; Hashmi et al., 2002). Ao entrar no organismo, os microminerais são transportados ligados a proteínas, e acabam sendo acumulados em brânquias, tecido gorduroso, músculos e em tecidos hepáticos (Heath, 1995), resultando em várias alterações nos processos bioquímicos e fisiológicos dos animais.

Na década de 90, Davis e Gatlin (1996) recomendaram o uso de três microminerais (Cu, Se e Zn) para inclusão em dietas de camarões peneídeos. Posteriormente, poucos estudos foram realizados com nutrição de microminerais para melhorar o desempenho zootécnico de camarões, sendo recomendado pelo o NRC (2011) os microminerais: Cu, Mn, Se e Zn.

De acordo com Underwood e Suttle (1999), a concentração e as formas de armazenamento dos microminerais nos tecidos e fluidos do organismo podem sofrer alterações com a ingestão de dietas deficientes, desbalanceadas ou com excesso deles. Além disso, já foi demostrada a capacidade dos organismos aquáticos em bioacumular e biomagnificar elementos tóxicos na natureza (Clements, 1992). No entanto, na produção aquícola são poucos os trabalhos com microminerais, feitos em bioflocos (Prangnell et al., 2016; Kuhn et al., 2017).

De acordo com Sabry Neto et al. (2015), a inclusão de bioflocos (*ex situ*) na dieta parece ter um efeito favorável ao crescimento do *L. vannamei* influenciado por microminerais e outros compostos presentes no sistema.

Enquanto alguns elementos são necessários para o cultivo de camarão, experimentos de Kuhn et al. (2017) observaram que camarões alimentados com dietas contendo mais de 570 mg.kg^{-1} de Mn, composta por biofloco, apresentaram menores taxas de crescimento.

3.1.Microminerais considerados essenciais

3.1.1. Cobre (Cu)

O Cu é um mineral necessário na nutrição animal e humana, porem pode também ser um nutriente potencialmente tóxico. Alguns crustáceos podem regular a concentração de Cu corporal, que é necessário para a síntese de hemocianina para hemolinfa (White e Rainbow, 1982).

A hemolinfa do camarão, assim como de outros invertebrados, possui a molécula baseada em Cu, onde o oxigênio, nas brânquias, é então carreado para todo o corpo e está envolvida em funções especializadas no transporte de oxigênio, sendo responsável pelas trocas gasosas na respiração dos artrópodes (Burmester, 2004). Além disso, Sreenivasa e Anjaneyulu (2008) demonstraram a importância do Cu também na regulação hormonal do crescimento e na reprodução do *L. vannamei*.

Alguns estudos com balanço de Cu na produção intensiva de camarões mostram que o alimento é uma fonte importante desse mineral nos tanques de cultivo (Lacerda et al., 2006; Cheng et al., 2014). Lee e Shiau (2002) estudaram o efeito do Cu na produção de camarão *P monodon*, e observaram que camarões alimentados com dietas suplementadas com 10 e 20 m mg.kg^{-1}de Cu apresentaram aumento significativo ($p<0,01$) de ganho de peso, eficiência alimentar e relação de eficiência protéica em relação aos camarões que foram alimentados com a dieta controle sem suplementação de Cu, e com níveis maiores ou iguais à 40 mg.kg^{-1}.

Por outro lado, Yeh et al. (2004) observaram que concentrações de Cu na água igual ou superior à 1 mg/L, durante 24 horas, resultaram em diminuição da contagem total de hemócitos, atividade da enzima fenoloxidase, atividade fagocítica, eficiência de depuração e aumentou a susceptibilidade do *L. vannamei* à infecção por *Vibrio alginolyticus*, devido a uma depressão na capacidade imune.

Cheng et al. (2014) realizaram pesquisa com balanço de Cu na produção sob sistema de recirculação e observaram que das fontes deste mineral adicionadas ao sistema produtivo de forma artificial (75,7% da entrada total), a alimentação correspondeu a 21,0% da entrada, e a saída de Cu em biomassa de camarão representou 11,68% do total da entrada de Cu, sendo que a concentração de Cu nos camarões foi significativamente superior ao dos sistemas de animais controle. Segundo Lee e Shiau (2002), a concentração total de Cu em camarão geralmente aumenta à medida que aumenta a suplementação dietética de Cu.

Em relação à concentração de Cu no ambiente aquático, resultados indicam que pós-larvas de *L. vannamei* podem servir como um indicador sensível à presença desse mineral na água, que em excesso pode ser considerado como um poluente (Nookala et al., 2014).

Em sistema de recirculação de água o filtro mecânico associado foi a principal rota para remoção do Cu existente na água, e que mesmo águas com até 0,30 mg/L de Cu podem ser usadas para cultivo comercial de *L. vannamei*, sem perda de qualidade do camarão produzido (Cheng et al., 2014).

Resultados experimentais com dietas indicam que fontes de cobre quelatado tem maior biodisponibilidade que cobre de fonte inorgânica, em dietas contendo ácido fítico. Foi necessário de 3 a 4 vezes mais quantidade de cobre inorgânico, para produzir um crescimento comparável ao observado em camarões alimentados cobre quelatado, sendo seguro e eficaz para *L. vannamei* (Bharadwaj, et al., 2014).

3.1.2. Selênio (Se)

O Se é um micromineral necessário para a maioria dos animais, sendo que o pescado é uma das principais fontes de selênio para alimentação humana (Yamashita et al., 2013). Dentre os microminerais considerados essenciais, é o mais tóxico, uma vez que a diferença entre sua necessidade e toxicidade é muito pequena (Chapman, 1999).

Em animais aquáticos, o Se possui ação antioxidante e ajuda a proteger contra a ação tóxica de alguns metais como mercúrio (Hg) e cádmio (Cd), sendo que um teor relativamente elevado de Se pode ser acumulado adicionalmente, quando o Hg está presente no ambiente (Ganther et al., 1972; Yamashita et al., 2005).

Segundo Devlin (2007) como o efeito da vitamina E sobre a formação de peróxidos é limitado primariamente à membrana, tanto o Se quanto a vitamina E são necessários à eliminação eficiente dos peróxidos, sendo os principais agentes antioxidantes no organismo animal.

Geralmente dietas contendo acima de 15% de farinha de peixe, apresentam concentrações adequadas de Se, não necessitando de suplementação. No entanto, rações formuladas predominantemente com produtos de origem vegetal necessitam de suplementação desse mineral (Wang, 1997).

Davis (1990) observou maior crescimento de juvenis de *L. vannamei*, quando esses foram alimentados com dieta purificada contendo 0,20 $mg.kg^{-1}$ de Se, e que a suplementação de Se acima de 0,30 $mg.kg^{-1}$ deve ser evitada, devido ao seu efeito tóxico.

De forma semelhante, Wang et al. (2006) observaram que o mecanismo de toxicidade do nitrito no camarão *L. vannamei*, envolve a suplementação de dietas com níveis mais altos de Se, tornando-se prejudicial ao camarão devido ao aumento da toxicidade do nitrito. Segundo o NRC (2011), o nível de Se recomendado para camarões seria de 0,20 a 0,40 $mg.kg^{-1}$.

3.1.3. Manganês (Mn)

O Mn é o quinto elemento mais abundante na terra e é conhecido por ser necessário para animais (Suttle, 2010). Está envolvido em sistemas bioquímicos como cofator enzimático ou grupos ativos, como a Mn superóxido dismutase (MnSOD). Os sintomas da deficiência de Mn incluem crescimento lento, reprodução prejudicada e anormalidades esqueléticas (NRC, 1993; 2011).

Além disso, ele possui função em diversos processos bioquímicos como ativador de algumas metaloenzimas como a piruvato carboxilase, superóxido dismutase e a glicosiltransferase (Suttle, 2010).

Nos crustáceos, o hepatopâncreas é o órgão que excreta as enzimas digestivas, absorve nutrientes e armazena reservas. As funções do hepatopâncreas na absorção, desintoxicação e excreção de microminerais são essenciais à fisiologia dos crustáceos (Dall e Moriarty, 1983). Segundo pesquisa realizada por Cai et al. (2017), a concentração de Mn no hepatopâncreas e no músculo dos camarões, aumentou com os níveis crescentes de Mn na dieta, sendo que o acúmulo foi menor no músculo do que no hepatopâncreas.

Número limitado de estudos analisou a toxicidade do Mn em dietas para camarões, sendo mais comuns trabalhos relacionados à toxidade deste mineral para crustáceos via coluna d´água (Lasier et al., 2000; Frias-Espericueta et al., 2003). Porém, segundo Davis et al. (1992), o Mn e o Cu são microminerais tóxicos para o desenvolvimento de larvas de camarões marinhos, sendo que o Cu é mais tóxico do que o Mn, e sua toxicidade pode ser reduzida com a utilização de EDTA, que é um agente quelante.

Em juvenis de *L. vannamei*, a suplementação dietética de Mn não só afetou significativamente o desempenho do crescimento, mas também influenciou as atividades das enzimas antioxidantes, as concentrações de Mn no tecido e a expressão do mRNA cMnSOD (Cai et al., 2017).

Níveis crescentes de Mn avaliaram os impactos dietéticos deste micromineral no camarão. Embora o aumento das concentrações de Mn na dieta não tenha impactado significativamente a sobrevivência ($p>0,05$), comprometeu o crescimento e o desempenho ($p<0,05$) dos camarões, quando ofertado em concentrações superiores a 570 $mg.kg^{-1}$ de Mn na dieta (Kuhn et al., 2017).

3.1.4. Zinco (Zn)

O Zn atua como cofator em vários sistemas enzimáticos e é componente de um grande número de metaloenzimas, incluindo anidrase carbônica, carboxipeptidase A e B, álcool desidrogenase, desidrogenase glutâmica, D-gliceraldeído-3-fosfato desidrogenase, lactato desidrogenase, málico desidrogenase, fosfatase alcalina, aldolase, superóxido dismutase (SOD), ribonuclease e DNA polimerase (NRC, 1980; Lall, 2002).

O excesso de Zn pode causar redução no crescimento e na taxa de consumo do *L. vannnamei*, além de alterações nas condições nutricionais, possivelmente por não disponibilizar bioquimicamente os nutrientes da dieta, por meio do hepatopâncreas (Wu e Chen, 2005).

Em estudo de toxicidade aguda de Zn e Cd, foi relatado que existe influência destes microminerais no consumo de oxigênio do *L. vannamei*, quando presentes em 3 mg/L. O aumento da concentração desses minerais no ambiente ocasionou a inibição no consumo de oxigênio, e que lesões branquiais resultaram na alteração da excreção de amônia e da pressão osmótica da hemolinfa (Wu e Chen, 2004).

Contudo, a espécie *L. vannamei* tem a capacidade de reverter lesões hepatopancreáticas causadas pelo excesso de Zn, que foram confirmadas pelas induções de dois marcadores bioquímicos, glutamato hemolinfático-oxalacetato transaminase e glutamato-piruvato transaminase (Wu et al., 2008).

Já em estudos nutricionais, indicadores imunológicos sugerem que concentração de 35 a 48 mg.kg^{-1} de Zn na dieta é ideal para melhorar as respostas imunes não específicas de *P. monodon*. No entanto, nas análises de regressão "broken-line" a inclusão de 32 a 34 mg.kg^{-1} de Zn na dieta teve a melhor resposta para o maior crescimento desta espécie (Shiau e Jiang, 2006).

Davis et al. (1992) mostraram que na ausência de fitato, um nível suplementar de 15 a 18 mg.kg^{-1} de Zn na dieta basal (33 de Zn total na dieta) satisfaz as exigências para o *L. vannamei*. Contudo, na presença de fitato é necessário um nível de 200 mg.kg^{-1} de Zn suplementar na dieta (218 mg.kg^{-1} de Zn total na dieta), uma vez que o fitato presente em ingredientes tem a capacidade de quelar os minerais indisponibilizando-os para o animal.

Apesar do maior custo de suplementação, maior biodisponibilidade, os quelatos já foram usados com o objetivo de verificar diferentes complexos quelatados de zinco, como fonte de mineral ao camarão *L. vannamei*. Sendo o zinco quelatado com metionina, dentre todos quelatos testados, o que propiciou melhora satisfatória na resposta imune em todo parâmetro analisado (Lin et al., 2013).

A suplementação dietética de Zn para organismos aquáticos geralmente excede o requerimento real, devido à sua baixa absorção. Dessa forma, ele é altamente concentrado na natureza e pode causar poluição ambiental em áreas de produção intensiva de aquicultura (NRC, 2011).

3.1.5. Ferro (Fe)

O Fe nos vertebrados possui como principal função o transporte de oxigênio no sangue por intermédio da hemoglobina (Suttle et al., 2010). No entanto, para os camarões, que possuem hemocianina no lugar da hemoglobina, o Fe não apresenta uma função característica bem estabelecida até o momento.

O requerimento de Fe na dieta de *L. vannamei* é inferior ao dos peixes (Davis et al., 1992). Embora alguns animais aquáticos tenham requerimento nutricional de Fe para seu crescimento (NRC, 2011), não há padrões para concentrações ideais desse mineral para maximizar a produção do *L. vannamei*, sendo necessárias pesquisas sobre requerimentos ótimos para minimizar o uso deste micromineral nas dietas (Wu e Yang, 2011).

Segundo pesquisa realizada por Davis et al. (1992) a suplementação de Fe dietético não teve efeito significativo no ganho de peso ou sobrevivência do camarão *L. vannamei.* Além disso, não houve sinal claro de deficiência observados nos camarões alimentados com dieta basal contendo 12 mg.kg^{-1} de Fe na dieta, e nem toxicidade aparente para suplementação de Fe até o limite de 80 mg.kg^{-1}.

O hepatopâncreas, órgão responsável pela absorção total ou temporária, desintoxicação e excreção dos minerais em crustáceos (Dall e Moriarty, 1983), teve sua concentração de Fe aumentada linearmente com a suplementação dietética desse mineral, sendo que o mesmo não ocorreu com os níveis desse mineral na carapaça (Davis et al., 1992).

A suplementação excessiva de Fe parece ter efeitos potencialmente adversos no crescimento de *Marsupenaeus japonicus* (Deshimaru e Yone, 1978; Kanazawa et al., 1984).

Na carcinicultura, a importância do Fe está em compor a proteína ferretina. Em pesquisas, a Ferretina recombinante via injetável exerceu papel protetivo contra o White Spot Syndrome Virus (WSSV) por supressão da replicação viral pela qual o Fe tem envolvimento (Ye et al., 2015).

O Fe, como outros minerais de difícil eliminação, quando em excesso é depositado nas células do sistema digestório e eliminado pelo trato intestinal (NRC, 2011). O requerimento dos microminerais necessários para espécie *L. vannamei,* segundo o NRC (2011), estão dispostos na tabela 1.

Tabela 1. Requerimento dietético de microminerais considerados para espécie *Litopenaeus vannamei*

Micromineral	Requerimento (mg.kg^{-1})
Cu	16-32
Fe	Dispensável
Se	0,2 -0,4
Zn	15 (32 total) (218 total) com fitato
Mn	Requerido

Cálculo em rações purificadas e semi-purificadas

Fonte: Adaptado de NRC, 2011.

4. Acúmulo de microminerais e contaminates em BFT

Estudos da década de 70 e 80 sobre bioacumulação de microminerias já demostravam a contaminação de algas e macroinvertebrados em sistemas aquáticos (Clements, 1992).

Os microminerais mais característicos estudados em contaminação de organismos aquáticos são: o arsênio (As), cádmio (Cd), chumbo (Pb), cromo (Cr), Cu, Fe, níquel (Ni), Mn, mercúrio (Hg) e Zn (Cui et al., 2011; Yi e Zang, 2012). Elementos como: rubídio (Rb); vanádio (V); estrôncio (Sr); molibdênio (Mo); prata (Ag); estanho (Sn); antimônio (Sb); césio

(Cs) e bário (Ba), são menos estudados, mas já detectados em estudos de bioacumulação em peixes de vida livre e camarões *P. monodom* de aquicultura (Tu et al., 2008; Tu et al., 2014).

Mesmo que todos estes elementos sejam conhecidos e de ocorrência natural em pequenas quantidades no ambiente aquático (Eisler, 2010), concentrações elevadas devido a fatores externos podem resultar na contaminação dos animais (Woo et al., 1993). Logo, em se tratando de carcinicultura intensiva, o reuso da água por ciclos contínuos de produção pode gerar a bioacumulação de microminerais e consequentemente a contaminação do sistema produtivo e do ambiente, com riscos a saúde humana e animal.

Recentemente, em levantamento desenvolvido na China, foi descoberto que as concentrações de arsênio (As), Cd, cromo (Cr) e Zn, em rações e adubos orgânicos para animais, aumentaram significativamente nos últimos 18 anos, em função do amplo uso de aditivos na dieta animal durante esse período (Wang et al., 2013).

Estudos com produtos a base de cinzas vulcânicas compostos por aluminossilicatos de cálcio e sódio hidratado, com presenças de elementos como cério (Ce), lantânio (La), rubídio (Rb), estrôncio (Sc) e tório (Th), geram resultados conflitantes e com melhora de diversos parâmetros tanto na produção de peixes como camarões (Tan et al., 2014; Mohamed et al., 2016), e parece incontestável a necessidade de estudos da influência de diversos microminerais e elementos potencialmente tóxicos na produção em BFT.

Poucos trabalhos discutem a importância dos microminerais nas rações de animais aquáticos (Berntssen et al., 2004). Como é previsto aumento na utilização de sistemas intensivos com pouca troca de água, visando aumentar a produção, minimizando o impacto no ambiente para um futuro próximo (Moss, 2002; Verdegem et al., 2006), torna-se importante avaliar a presença e a dinâmica dos microminerais, dos contaminantes e elementos potencialmente tóxicos, assim como os possíveis efeitos adversos à saúde humana, associados ao consumo de animais provenientes de sistemas produtivos como o BFT.

5. Análise por Ativação Neutrônica Instrumental (INAA)

A capacidade da técnica de INAA em detectar uma gama variada de elementos e quantificar suas concentrações em diversos insumos agropecuários já é conhecida (Oliveira,et al, 2013; Menezes et al, 2017). A técnica tem como base a irradiação de uma amostra por um feixe de nêutrons em um reator nuclear, e a consequente formação de radionuclídeos, cuja emissão gama é medida posteriormente e identifica os elementos químicos. Suas principais características são a análise simultânea de diversos elementos, identificação de elementos no nível de traços a percentagem, fornecendo resultados com exatidão e precisão, sendo indicada na certificação de amostras de referência (Greenberg et al., 2011; Weizhi et al., 2001). Para ser aplicada, não exige que as amostras sejam submetidas a preparo químico em etapa anterior à análise.

As amostras podem ser analisadas em maiores ou menores massas, considerando os procedimentos já estabelecidos no Laboratório de Análise por Ativação Neutrônica do Centro de Desenvolvimento da Tecnologia Nuclear em Belo Horizonte (Menezes et al. 2003; Menezes e Jacimovic, 2006, 2014). As amostras devem ser pesadas em frascos de polietileno apropriados à irradiação em reator nuclear de pesquisa TRIGA MARK I IPR-R1, que a 100 kW tem fluxo de nêutrons térmicos médio de 6,35x10^{11} nêutrons cm^{-2} s^{-1} (Menezes e Jacimovic, 2006). Na análise, as amostras devem ser acompanhadas por monitores de nêutrons compostos de liga de Al - Au (0,1%) certificadas, código IRMM-530R e fornecida pelo IRMM, *Institute for Reference Materials and Measurements*, Bélgica.

Após a irradiação é necessário tempo adequado para o decaimento dos radionuclídeos com meias vidas mais curtas e, assim, reduzir possíveis interferências. A espectrometria gama é executada em detector hiper puro, HPGe, eletrônica associada, em programa Genie 2000 CANBERRA para a aquisição dos espectros gama, o software HyperLab do Gamma spectroscopy software, HyperLabs Software, para a deconvolução dos espectros e, para o cálculo das concentrações elementares, o programa Kayzero para Windows® específico para o método k_0.

A técnica tem sua característica referencial de qualidade como técnica primária de análise (Weizhi et al., 2001; Greenberg et al., 2011). O controle de qualidade do método é realizado por meio da análise de amostra de referência certificada, seguindo a mesma metodologia de análise das amostras. Para avaliação da eficiência do método, é aplicado o teste E_n (ISO 13528:2005), que considera, nos cálculos, a incerteza expandida dos valores experimentais e certificados com um fator de cobertura k=2. Isso significa que os resultados verdadeiros têm 95% de probabilidade de estarem dentro do intervalo de confiança.

6. Espectrometria de Massa com Plasma Acoplado Indutivamente (ICP-MS)

É uma poderosa ferramenta e importante para determinação de elementos químicos, surgida na década de 80, e com melhores limites de detecção, variando de partes por bilhão a partes por trilhão para a maioria dos elementos. É uma técnica multielementar isotópica classificada rápida, precisa e exata, e usa pequenas quantidades de amostra (López-Alonso, 2012; Thomas, 2013).

O espectrômetro de massa é um equipamento que separa íons, e é constituído de cinco partes: Sistema de introdução de amostras; Fonte de íons; Interface; Sistema analisador e Sistema de detecção de íons (Taylor, 2001).

A técnica de via úmida requer preparo prévio com digestão da amostra, e posteriormente, de modo geral são nebulizadas após introdução no equipamento, e segue transportada por fluxo de gases específicos até o plasma, local que ocorre a dessolvatação, vaporização, atomização e ionização, para leitura no espectrômetro de massa (McLean et al., 1998; Thomas, 2013).

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CAPÍTULO II

Avaliação de microminerais (Cu, Zn, Mn e Se) presentes em *Litopenaeus vannamei* cultivado em sistema BFT com água sabobra artificial

RESUMO - De acordo com a National Research Council (NRC), alguns microminerais são considerados essenciais ao desenvolvimento de crustáceos em cativeiro. No entanto, pouco se sabe sobre o destino desses elementos em sistemas que empregam pouca ou nenhuma troca de água no cultivo. O objetivo deste estudo foi analisar a concentração de microminerais (Cu, Zn, Mn e Se), presentes na ração, no biofloco e nos camarões comerciais criados em sistema superintensivo (400 camarões/m^2), distante da costa, e sem renovação de água em baixa salinidade. Na primeira etapa da pesquisa, foi feita análise multielementar dos insumos utilizados na criação de camarão, pela técnica de Ativação Neutrônica Instrumental (INAA), com base no método k0 usando reator nuclear. Na segunda etapa, a técnica de Espectrometria de Massa com Plasma acoplado (ICP-MS), também foi aplicada para medir a concentração dos microminerais na ração utilizada e, ambas as técnicas foram empregadas, após o tempo de cultivo, para analisar os referidos elementos em diferentes partes dos animais testados e no biofloco. Com base nos resultados obtidos, podemos afirmar que as duas técnicas empregadas se mostraram complementares para a determinação dos minerais presentes nas diferentes matrizes estudadas. Foi possível observar maior acúmulo de elementos no cefalotórax dos camarões quando comparado com o restante do corpo dos animais. Foi identificado o fator de transferência trófica (FTT) >1 de Cu e Se, via ração para amostras de animais inteiros. Em relação à ração comercial, o biofloco concentrou todos os elementos requeridos. Apesar dos resultados encontrados no presente estudo, nenhum valor obtido ultrapassou o limite permitido pela legislação brasileira. Podemos concluir que avanços e ajustes na nutrição micromineral são necessários em sistemas com baixa renovação de água, tendo em vista o acúmulo de elementos no sistema e seu reflexo na qualidade do ambiente produtivo ao longo dos ciclos sucessivos de cultivo.

Palavras-chave: Ativação Neutrônica Instrumental, BFT, camarão, ICP-MS.

Evaluation of microminerals (Cu, Zn, Mn and Se) present in *Litopenaeus vannamei* cultured in continental BFT system using artificial brackish water

ABSTRACT - According to the National Research Council (NRC) some microminerals are considered to be essentials for development of crustaceans. However, little is known about the fate of these elements in aquaculture systems that employ low or no water exchange such as the Biofloc Technology system (BFT). The objective of this study was to analyze the concentration of microminerals (Cu, Zn, Mn and Se) present in the aquafeed, biofloc and shrimp raised in superintensive system (400 shrimps/m^2), distant from the coast without water renewal in low salinity. The first step, a multielementary analysis of the aquacultural inputs used in shrimp farming was performed using the Instrumental Neutron Activation Analysis (INAA), based on the k0 method using nuclear reactor. In second step, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) technique was applied to measure the concentration of microminerals in the feed used during shrimp farming. Both techniques were used after the culture time to analyze the refered elements in different parts of the animals tested and in the biofloc composition. Based on the obtained results, we can affirm that the two techniques used were robust and complementary for elements present in the different matrices studied. We observed a greater accumulation of elements in the shrimp cephalothorax when compared with the rest of the body. Trophic Transfer Factor (TTF) was > 1 for Cu and Se, via feed for whole animal samples. In relation to commercial feed, the biofloc concentrated all the required elements. Despite the results found in the present study no value exceeded the limit permitted by Brazilian legislation. We can conclude that advances and adjustments in micromineral nutrition are necessary in BFT system, considering the accumulation of elements in the system and effects on successive cycles of production.

Keywords: BFT, ICP-MS, Neutron Activation Analysis, Shrimp.

INTRODUCTION

As a result of the decline in fishing resources most aquaculture systems have begun to intensify their production (Coloso, 2015; FAO, 2018).

The intensification of a traditional aquaculture production system inevitably leads to increased waste production and water use however as a solution to these concerns, aquaculture tends to the development of low or no water renewal production systems, with water reused, thus reducing nutrient discharges into the environment (Verdegem et al., 2006; Avnimelech, 2015, Samocha et al., 2017).

New aquaculture trends have been developed in the last decades such as tank reduction, zero water exchange, greenhouse production, recirculation systems, and increasingly closed designs. An example of such evolution in production systems is the Biofloc Technology System (BFT) which is characterized as a culture system, using high densities, with low or no water exchange resulting an increased productivity, and thus eliminating effluents into the environment (Moss, 2002; Burford et al., 2003; Samocha et al., 2017). This system is characterized by the formation of microbial aggregates, called biofloc, composed of microalgae, feces, exoskeletons, feed and debris, bacteria, rotifers, protozoa and other invertebrates (Emerenciano et al., 2013; Avnimelech, 2015).

Although the system provides advantages in aquaculture production, it is necessary to control the biofloc and the water quality, since cultivated animals can use the biofloc as a food source and which substances, such as microcontaminants and some minerals, can accumulate in the system, and possibly in animal tissues and in the biofloc (Kuhn et al., 2017; Samocha, 2017).

Usually analyzes performed for the detection of microminerals are obtained in atomic absorption spectrophotometer (López-Alonso, 2012). However, the Instrumental Neutron Activation Analysis (INAA) method has the advantages of better detection sensitivity (Corliss, 1963), multielementarity, which allows the simultaneous detection of different minerals in single sample without preparation (Heidarieh et al., 2013). The identification of elements providing results with accuracy and precision makes this analysis indicated in the certification of reference materials (Weizhi et al., 2001; Greenberg et al., 2011). Another multi-element analytical technique is Inductively Coupled Plasma Mass Spectrometry (ICP-MS) which allows the analysis of several minerals with low detection limits (López-Alonso, 2012).

The use of biofloc in the nutrition and feeding of shrimps and other aquatic organisms, has been investigated, however, information on the occurrence of microminerals in the culture environment still needs to be investigated. The objective of this study was to detect and quantify microminerals required - studied and established by the NRC (2011) for crustaceans - present in the aquacultural inputs, animals and biofloc of a *Litopenaeus vannamei* production system, maintained at low salinity, with no water exchange and far from the coast.

Material and methods

Preliminary analysis of aquaculture inputs

All supplies used in the present study (dolomite, molasses, probiotic, initial aquafeed and grow out aquafeed) were previously analyzed by Instrumental Neutronic Activation - INAA (De Soete et al., 1972; De Corte, 1987), by means of the k0-standardized method (De Corte, 1987; Menezes et al., 2003), for determining microminerals (Cu, Zn, Mn and Se) present in the samples. The technique consisted in the irradiation of a sample by a neutron beam in a nuclear reactor and the consequent formation of radionuclides, whose gamma emission was measured later and the chemical elements were then identified.

The different aquacultural inputs (200 to 500 mg samples) were analyzed considering the procedure established in the Nuclear Technology Development Center, in Belo Horizonte-MG, Brazil (Menezes et al., 2003; Menezes and Jacimovic, 2006). The samples were weighed in polyethylene flasks suitable for irradiation and irradiated for eight hours in the TRIGA MARK I IPR-R1 nuclear research reactor, which at 100 kW has a mean thermal neutron flux of 6.35×10^{11} $cm^{-2}s^{-1}$ neutrons (Menezes and Jacimovic, 2006). The samples were managed by neutron monitors composed of Al/Au alloy (0.1%) certified, code IRMM-530R and supplied by IRMM, Institute for Reference Materials and Measurements, Belgium.

After irradiation a suitable time was expected to decay of the radionuclides with shorter half lives. The gamma spectrometry was performed in hyper pure detector, HPGe, and associated electronics, using the program Genie 2000 (Canberra) for the acquisition of gamma spectra. The HyperLab program (Gamma spectroscopy) was used for the deconvolution of the spectra and, for the calculation of the elementary concentrations. The Kayzero program for Windows® (specific to the k0 method) was used.

The quality control of the method was performed through the analysis of certified reference samples, SRM 1573a, Tomato Leaves, NIST (National Institute of Standards and Technology) and BCR-320R (Channel Sediment, Reference Materials and Measurements), following the same methodology of analysis of the samples.

In order to evaluate the efficiency of the method, the test En (ISO 13528: 2005) was applied considering the expanded uncertainty of the experimental and certified values with a coverage factor $k = 2$. This means that true results are 95% likely to be within the confidence interval.

The second technique used was Inductively Coupled Plasma Mass Spectrometry (ICP-MS), for the accomplishment of this technique, all the samples were lyophilized, digested in a microwave oven, and 250 mg of sample were transferred to the PFA polymer flasks. Volumes of 3.5; 1.0 and 3.5 mL of concentrated HNO_3, H_2O and H_2O_2, respectively, were added to the vial containing the sample. After the samples were placed inside the oven of a microwave system (Ethos I, Milestone, Italy) equipped with temperature and pressure sensor (temperature program: 10 min "ramp" at 190°C and 20 min "hold" at 190°C). After digestion, the pressure of each vessel was carefully released and the resulting solutions were transferred to conical tubes, diluted to the 15 mL volume with water and subsequently analyzed. For analytical determination of minerals, the ICP-MS analyzer (7700x, Agilent Technologies, Japan) equipped with a peristaltic pump was used. The reference materials cited were used for the calibration of all analyzes.

Experimental system

The aquacultural inputs analysed were used in the shrimp production experiment in the BFT system. The nine-day old post-larvae of *Litopenaeus. vannamei* were kept in quarantine prior to the experiment and were tested by official methods recommended by the World Organization for Animal Health (OIE) for WSSV, IMNV, TSV, YHV and NHP.

After nursery period (60 days) juveniles of *L. vannamei*, with an average weight of 2.91 ± 0.93 g, were transferred to experimental tanks supplied with 100% reusing water (with biofloc), and from a formed biofloc of animals kept in the laboratory.

Thus, the experiment of shrimp production was performed with the animal density of 400 animals/m^2, 315 animals per tank, in three polyethylene tanks, filled with 500 L, with an area of 0.785 m^2. Feeding was offered twice a day (8:00 a.m. and 4:00 p.m.) and fed with a decreasing rate of feed ranging from 3 to 1% of the biomass, kept without water exchange for 100 days, with only evaporated water being replenished to maintain the original volume, at low salinity (10‰) obtained artificially (Grasshoff et al., 1983; Boyd, 2003), and with suspended solids controlled by clarification if necessary. Clarification was performed in each tank with sedimentable solids limit of 15 mL.L^{-1} using graduated Imhoff cone (1,000 mL) and providing sludge extraction throughout the culture for 2 to 4 hours per day ocassionaly. pH corrections were performed to maintain values close to 7.8 and alkalinity above 150 mg.L^{-1}, adding dolomite. The addition of molasses powder was based on the concentration of total ammonia (> 0.25 mg.L^{-1}), maintaining the C:N ratio of 6:1, considering the proportion of carbon in molasses of 45% (Avnimelech, 1999; Ebeling et al., 2006). Commercial probiotic was used to improve the culture environment. Air diffusers were positioned to keep the solids suspended in the tank (Samocha et al., 2017) and the photoperiod used was 12 h.

The water physicochemical monitoring was carried out daily for the following variables (mean ± standard deviation): Mean dissolved oxygen concentration (5.80 ± 0.34 mg.L^{-1}), using a digital oximeter (Hanna - model HI9146, República Czech); pH (7.62 ± 0.20) and temperature (28.82 ± 0.92°C) with multiparameter apparatus (Hanna - model HI98129, Czech Rep.), some parameters were checked weekly, such as: alkalinity (170.06 ± 24.27 mg $CaCO_3$.L^{-1}) according to APHA (1999); total ammonia (0.72 ± 0.42 mg.L^{-1}), nitrite (0.41 ± 0.23 mg.L^{-1}) (Unesco, 1983), and salinity with refractometer (Atago - model ATC-S / Mill 2440, Japan); suspended solids (14 ± 2 mL.L^{-1}) using graduated Imhoff settling cone, and total suspended solids (300 ± 20 mg.L^{-1}) by filtration according to Gaona et al. (2016).

After this complete cycle of shrimp culture in the three tanks, with a mean survival of 55.2 ± 5.1% with mean weight of 10.53 ± 2.8 g, the animals were dissected and parts of the animals and biofloc were analyzed using ICP-MS and INAA.

Samples collected and statistics

The animals were sampled at the end of experiment to ascertain the final composition of microminerals. A total of 60 shrimps (total of three tanks) were collected, consisting of pools of 12 whole animals; 12 animals dissected in fragments composed of all parts of the cephalothorax (with heart, stomach, hepatopancreas, pereiopods, antennae and antennulas); 12 animals muscle with exoskeleton plus telson and uropod and without head; 12 animals exoskeleton (with telson, uropods and without head) and 12 animals only with muscle (or filet). The collected samples were kept refrigerated according to Sarmani et al. (1993) and biofloc samples (15 g of freeze-dried biofloc) separated were analyzed in triplicate for ICP-MS analysis and duplicate for INAA.

The data obtained from the mineral concentrations were analyzed in the InfoStat/S and the descriptive analysis was done and compared with data from the literature. For the analysis of the data, we calculated the Trophic Transfer Factor (TTF), which is the ratio of the concentration of the substance in the body tissue to the concentration found in the diet items (Rashed, 2001; De Forest et al., 2007) and calculated as 30% of biofloc and 70% of feed, estimated in the daily diet of the animals (Abreu et al., 2007), adapted from Qiu (2015).

Results and Discussion

The results of Cu, Zn, Mn and Se present in the aquacultural inputs used in the shrimp production in BFT are presented in table 1.

None of the minerals required by *L. vannamei* were detected in the molasses samples. However, Salles et al. (2016), in a study carried out at the CDTN/CNEN with INAA observed a varied range of contaminants (including Zn, other mineral required) at very low concentrations in samples of different types of sugar and industrial refining in several countries. In the present study, the dolomite used in the BFT system was a considerable source of Zn, unlike the probiotic by the total volume of product used. In others aquacultural inputs, it was not possible to detect, within the limits of INAA matrices for all essential microminerals present, except for Se with an average value of 0.58 mg.kg^{-1} for the probiotic.

The complementarity of the techniques used for the detection of the microminerals required by the animal present in the feed as possible to be observed, as an example, the Mn and Cu that were not detected and quantified by the INAA technique, were quantified by ICP-MS. Zn was detected by the two techniques in the grow out aquafeed (159.95 mg.kg^{-1} for INAA and 155.29 mg.kg^{-1} for ICP-MS) and Cu only detected by ICP-MS (47.51 mg.kg^{-1}), the recommendation of Cu by the NRC (2011) for *L. vannamei* is 16 to 32 mg.kg^{-1} in purified diet.

The values of Zn found in the commercial diet (160 mg.kg^{-1} range) are considered above the optimal for supplementation (32 mg.kg^{-1}), but below when in the presence of phytate (218 mg.kg^{-1}), or in the absence of phytase in the diet composition (NRC, 2011).

Mean values of Se = 1.6 mg.kg^{-1} were detected in the initial diet and 1.1 mg.kg^{-1} in the grow out aquafeed (Table 1). These values are above that recommended by the NRC (2011) (0.2 to 0.4 mg.kg^{-1}) and above the levels found by Davis (1990), who observed higher growth of *L. vannamei* juveniles when they were fed with a purified diet containing 0.20 mg.kg^{-1} of Se, and that the supplementation of this mineral above 0.30 mg.kg^{-1} should be avoided due to its toxic effect. Similarly, Wang et al. (2006) concluded that supplementation of diets with high levels of Se, increases and potentiates nitrite toxicity in *L. vannamei*. On the other hand, Li et al. (2014) recommended the inclusion of 0.98 $mg.kg^{-1}$ of Se for optimal growth of *L. vannamei* with a semi-purified diet.

Mn and Cu present in the diet were detected only by ICP-MS with concentrations of 92.99 $mg.kg^{-1}$ and 47.51$mg.kg^{-1}$, respectively (Table 1). Kuhn et al. (2017) evaluated diets with increasing levels of Mn and their effect on the *L. vannamei* production. Although the increase in Mn concentrations in the diet did not affect the survival of the animals ($p> 0.05$), the growth and performance impairment ($p <0.05$) of the shrimps were reported at concentrations above 570 $mg.kg^{-1}$ of Mn in the diet. Although considered as required by the NRC (2011), the recommendation of Mn for *L. vannamei* is not yet established.

In analysis of the commercial inputs for shrimp production, the aquafeeds used presented inclusions of these microminerals in disagreement with those recommended by the NRC in purified diets (2011). It is usual that in practical diets, the mineral levels are higher due to the presence of phytates and fibers, which can reduce the bioavailability of the microminerals.

The variation in the recommendations of microminerals should be related to several factors such as diet composition, age and sex of the animals, species used and type of aquaculture system. In this study in a experimental closed system, the concentration of Cu, Zn, Mn and Se in the tissues of *L. vannamei* shrimp raised in a biofloc system is shown in figure 1.

Cephalothorax was the shrimp part that presented the highest mean values for all the elements surveyed (Cu, Zn, Mn and Se) and were within the levels presented in studies that analyzed the organs separately, such as gills, antennal organ (Paez-Osuna and Tron-Mayen, 1996), hepatopancreas (Paez-Osuna e Tron-Mayen, 1996; Wu e Yang, 2011; Espericueta et al., 2015) and viscera (Silva et al., 2016), which are described in table 2.

The results found in the *L. vannamei* muscle for elements such as Cu and Zn were close to those observed by studies (Lacerda et al., 2009 and Silva et al., 2016) with vannamei production in Brazil (Table 2).

When comparing the concentration of the microminerals in the different types of samples, a greater accumulation in the biofloc was observed in relation to the animal tissues, except for the concentration of Cu in the shrimp cephalothorax (202,65 $mg.kg^{-1}$), (figure 1) which was higher than that found in the biofloc (72.30$mg.kg^{-1}$), which suggests a great ability of the animal to concentrate this micromineral.

The biofloc condition to concentrate certain micronutrients is not yet well studied in aquaculture production systems. However, Crab et al. (2012) reported that bioremediation studies such as coupled water treatment systems, and tank design changes to control sludge or suspended solids are necessary to improve shrimp production in the BFT system.

In this study, it was possible to observe that Zn was the micromineral with greater accumulation in the biofloc, followed by Mn, Cu and Se, and that this accumulation was proportional to the inclusion of these microminerals in the diet, that is, the microminerals with higher concentration in the diet had greater accumulation in the biofloc, in the order of 2.6 times for Zn, 1.5 for Cu, 2.0 for Mn and 1.5 times for Se.

The importance of monitoring microminerals has also been reported by Prangnell et al. (2016), who compared experimental diets with balanced commercial diets and evaluated factors limiting the production of marine shrimp in biofloc (in situ), concluded that studies are necessary to understand the environmental changes, in the animal and also on the accumulation and absorption of some microminerals in the production system.

Kuhn et al. (2017) observed high levels of mineral matter in the suspended solids in the BFT system, and in diets containing concentration up to 570 $mg.kg^{-1}$ (composed of biofloc), had lower growth rates. Wang et al. (2017) evaluating diets without the inclusion of vitamins and minerals, observed decreasing survival of the shrimp with increasing water exchange. So in zero of water exchange or with low renewal it is possible not to supplement the diets, improving the performance of *L. vannamei* in a closed recirculation system.

Considering the concern with the quality of the environment in the BFT system, in the present study TTF was calculated via feed and biofloc for the required elements. According to De Forest et al. (2007) TTF is a general term that may include bioconcentration, where the concentration of the substance in the body is greater than concentration in the environment and its diet, respectively; and biodilution when the concentration of substance in an organism is lower than in its diet .

Results of TTF in Table 3 shows that only Cu obtained the value higher than the others minerals in the feed used (TTF > 1.0), even with the estimated consumption of only 30% of biofloc. Changing this proportion of consumption between feed (70%) and biofloc (30%), it is possible that the biofloc that is already concentrated with Mn, Cu, Zn and Se derived from aquafeed can contribute to increase the accumulation of these microminerals in the animals present in the system, and further studies are needed to understand this dynamics in BFT systems.

According to some authors (Uotila, 1991; Serra et al., 1996), Zn and Cu can be found in high concentrations in diets for aquatic organisms and may accumulate in the aquaculture system. As a consequence of the accumulation of certain elements in closed production systems with the consequent transfer to the cultivated organisms, Silva et al. (2016) found levels of Se in some tissues of *L. vannamei* above the levels allowed in foods for human consumption based on Brazilian Health Regulatory Agency (Anvisa) and Ministry of Agriculture, Livestock and Food Supply (MAPA) by resolution RDC nº 42/2013.

By means of estimated calculations, considering the diet of the animals 100% supplied by the artificial aquafeed, without counting the natural supplementation in the biofloc, when applied both the Se and the Cu would have high TTF.

Studies on the bioaccumulation of several elements via feed in BFT become important to evaluate the storage and distribution of these elements in the animal and environment, and the transfer of these elements by the trophic chain using intensive and super intensive production, besides identifying potential accumulations in the animals, biofloc and diets.

Advances in the attempt to understand the dynamics of microminerals required in closed intensive or low water renewal intensive systems (such as BFT) are important to identify deleterious levels in the system minimizing use of microminerals in diets for *L. vannamei* when possible and maximize water reuse by avoiding accumulation of these elements in the system.

For the first time in the shrimp production using BFT system a study with all microminerals required by shrimp *L. vannamei* identified an accumulation of these in the biofloc, and coming from the balanced commercial diet offered during the laboratory experiment.

Conclusion

Among the animal parts analyzed, the cephalothorax presented the highest values of microminerals (Cu, Zn, Mn and Se).

The required microminerals (Cu, Zn, Mn and Se) during the production of *L. vannamei* in a BFT system can be concentrated in the biofloc present in the culture environment.

Cu and Se presented the highest TTF values via aquafeed for shrimp.

Concentration of different compartments in descending order was: Animal > biofloc > aquafeed for Cu; Biofloc > animal > aquafeed for Se; and for Mn and Zn were biofloc > aquafeed > animal.

Specific aquafeeds for production of *L. vannamei* in BFT system can be developed taking into account the accumulation of these microminerals in the closed aquaculture system.

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Table 1. Mean concentrations of Zn, Se, Mn and Cu in samples of supplies used to produce *Litopenaeus vannamei* in a biofloc system

Supplies	**Zn**	**Se**	**Mn**	**Cu**
molasses [a]	< l.d	< l.d	< l.d	< l.d
dolomite [a]	35.83	< l.d	< l.d	< l.d
Probiotic [a]	76.34	0.58	< l.d	< l.d
initial aquafeed [a]	159.95	1.60	< l.d	< l.d
grow out aquafeed [a]	159.95	1.10	< l.d	< l.d
grow out aquafeed [b]	155.29	< l.d	92.99	47.51
req. NRC (2011)[1]	32.0	0.2-0.4	-	16.0-32.0

<l.d = less than the detection limit for used matrix; [a] the technique used: neutron activation (duplicate); [b] technique used: ICP-MS (triplicate); [1]values found for purified diet. Values in $mg.kg^{-1}$ in the dry matter.

Table 2. Concentrations of Cu, Zn, Mn and Se, compiled in the literature, on different tissues of cultivated *Litopenaeus vannamei*.

Tissue	Cu	Zn	Mn	Se	References
Whole shrimp	67.69	52,33	-	-	Prangnell et al. (2016)
	17.62	18.43	0.98	-	Tacon et al. (2002)
Muscle	23.99	44.60	-	-	Espericueta et al. (2015)
	18.85	55.00	2.85	-	Paez-Osuna and Tron-Mayen (1996)
	22.16	39.42	-	-	Prangnell et al. (2016)
	(24.8-33.9)	(36.6-43.8)	(0.36-3.17)	(0.82-3.97)	Silva et al. (2016)
	23.2 a 63.4	-	-	-	Lacerda et al. (2009)
	27.66	267.46	5.35	-	Wu and Yang (2011)
Exoskeleton	(55.9-77.6)	(38.0-53.7)	(0.38-20.7)	(0.08-7.07)	Silva et al. (2016)
	31.71	54.21	13.46	-	Wu and Yang (2011)
	(49.9 - 112.2)	-	-	-	Lacerda et al. (2009)
	68.75	32.50	26.75	-	Paez-Osuna and Tron-Mayen (1996)
Hepatopancreas	223.70	121.46	-	-	Espericueta et al. (2015)
	64.40	122.00	21.30	-	Paez-Osuna and Tron-Mayen (1996)
	194.21	126.12	11.66	-	Wu and Yang (2011)
Gills	258.50	86.00	24.40	-	Paez-Osuna and Tron-Mayen (1996)
Viscera	(81.9-152.0)	(16.8-109.4)	(0.97-24.4)	-	Silva et al. (2016)
Antenal Organ	162.00	52.5	26.50	-	Paez-Osuna and Tron-Mayen (1996)

Results on dry matter ($mg.kg^{-1}$)

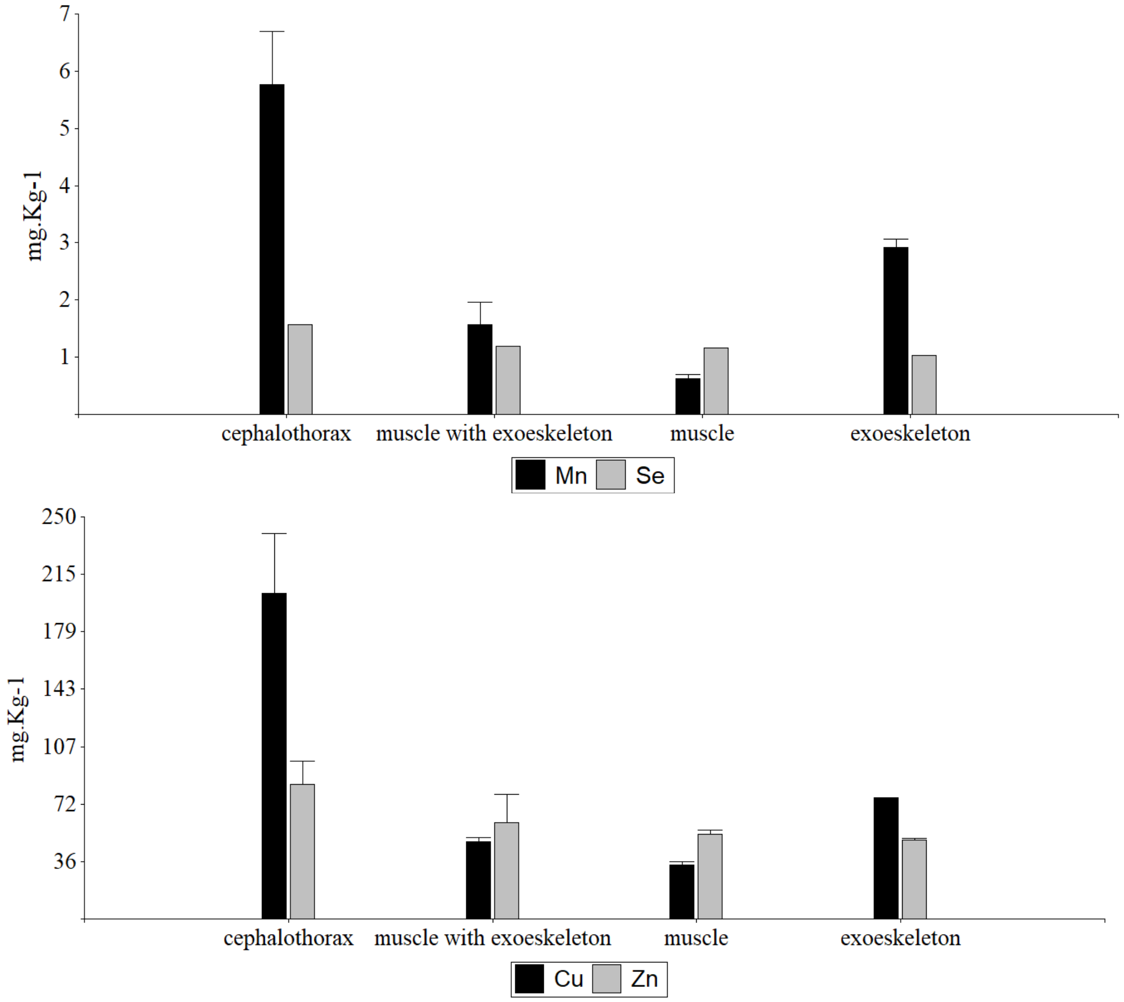

Figure 1. Mean concentrations of Cu, Zn, Mn and Se in mg.kg^{-1} (dry matter) in different tissues of *Litopenaeus vannamei* produced in BFT system, without water exchange (triplicate for Cu and Mn, obtained by ICP-MS; Duplicate for Se and Zn analyzed by INAA).

Table 3. Concentration of Mn, Cu, Zn and Se with mean values in mg.kg^{-1} of dry weight and Trophic Transfer Factor (TTF) in biofloc and aquafeed, from *Litopenaeus vannamei* culture in BFT system with no water exchange.

Element/Technique	**Sample**	**Mean**	**TTF**	**TTF^{e}**
	Whole shrimp	2.69		
Mn /ICP-MS	Biofloc	194.02		*0.004*
	Aquafeed	92.99	*0.029*	*0.020*
	Whole shrimp	86.06		
Cu /ICP-MS	Biofloc	72.30		*0.357*
	Aquafeed	47.51	*1.811*	*1.268*
	Whole shrimp	72.0		
Zn /INAA	Biofloc	419.0		*0.051*
	Aquafeed	159.95	*0.450*	*0.315*
	Whole shrimp	1.24		
Se /INAA	Biofloc	1.7		*0.218*
	Aquafeed	1.1	*1.107*	*0.77 5*

Based on the dry matter; ICP-MS = Coupled Plasma Mass Spectrometry; INAA = Instrumental Neutron Activation; TTF = Trophic Transfer Factor considering aquafeed (100% of the balanced artificial diet used); TTF^{e} = estimated trophic transfer factor, considering estimated total consumption of 30% of biofloc and 70% of feed, by whole animal (triplicate for ICP-MS and duplicate for INAA);

CAPÍTULO III

Análise elementar após a produção de *Litopenaeus vannamei* no sistema BFT - Uma possível ameaça aos seres humanos ou uma nova maneira de melhorar insumos específicos em sistemas fechados?

RESUMO - Este estudo teve como objetivo identificar os elementos minerais presentes na produção de camarões em sistema de biofloco (BFT), com baixa salinidade. Foram analisadas inicialmente as rações comerciais para fase inicial e engorda, artêmia, probiótico, calcário, melaço, usados no experimento, e uma farinha de peixe importada e outra nacional. Após estas análises, os animais foram povoados em tanques com água clara, em alta densidade com baixa salinidade, e produzidos até o tamanho comercial em BFT, e analisados ao término do cultivo por Ativação Neutrônica Instrumental. Neste trabalho diversos elementos classificados como essenciais e contaminantes potencialmente tóxicos ao sistema produtivo foram identificados. Também foram observadas concentrações variadas de elementos lantanídeos e actinídeos em floco microbianos. Elementos como césio (Cs), bromo (Br) e selênio (Se) obtiveram Fatores de Transferência Trófica (FTT) da ração usada para o animal >1. Dos 28 elementos presentes nos insumos, 23 concentraram no sistema produtivo via bioflocos e 16 elementos incorporaram a composição do tecido animal. Tanto insumos, como animais estão dentro dos limites exigidos pela legislação brasileira. Atenção especial deve ser dada a pesquisas em sistemas mais fechados de produção aquícola, que é tendência para o suprimento do pescado no futuro, pois existe uma gama variada de elementos e os efeitos do acúmulo destes ao longo dos ciclos sucessivos de produção são pouco conhecidos na literatura.

Palavras-chave: Análise de Ativação Neutrônica Instrumental; bioflocos; camarão; sanidade aquícola

Elemental analysis after the production of *Litopenaeus vannamei* in BFT system - A possible threat to humans or a new way to improve specific supplies in closed systems?

ABSTRACT - This study aimed to identify the mineral elements present in the production of shrimp in a biofloc system (BFT), with low salinity. The supplies analyzed included: commercial aquafeed for initial and grow out phases, artemia, probiotic, dolomite, molasses, used in the experiment, and the imported and national fish meal. After analyzes, animals were stocked in tanks with clear water, in high density with low salinity, and produced until the commercial size in BFT system, and analyzed at the end of the cultivation by Instrumental Neutronic Activation Analysis. In this research, several elements, classified as potentially toxic to the production system, were identified. Concentrations of lanthanide and actinide elements in biofloc were also observed, elements such as cesium (Cs), bromine (Br) and selenium (Se) obtained Trophic Transfer Factors (TTF) from aquafeed used to animals > 1. Of the 28 elements present in the inputs, 23 were concentrated in the production system via suspended solids and 16 elements were incorporated into the animal tissue composition. Both supplies and animals were within the limits required by Brazilian legislation. Special attention should be given to research into closed aquaculture systems, as there are a variety of elements that can accumulate along the production cycles and their effects on cultured animals and human beings are little known.

Keywords: Aquaculture health; bioflocs; Instrumental Neutron Activation Analysis; shrimp; minerals

INTRODUCTION

Aquaculture activities offers more than a half of all fish produced since 2016 (FAO 2018). This growing segment of global food production has increased rapidly in the last decades, with an annual average growth rate of over 6.0% (FAO, 2018).

In response to increased demand for aquaculture products, most production systems have intensified their productivity (Coloso, 2015). Of the species featured in shrimp farming, *L. vannamei* in 2014 accounted for 80% of the world production of farmed shrimp (4,580,768 t) and 46.5% of all shrimp marketed worldwide (farmed and caught shrimp). This increase in the *L. vannamei* production occurred due to advances in cultivation techniques and their intensification (ABCC, 2016; FAO 2018).

With the evolution of traditional production systems, the Biofloc Technology (BFT) emerged as sustainable and environmentally friendly technique (Burford et al., 2003). This system is characterized by the formation of microbial aggregates, called biofloc, composed of bacteria, algae, protozoa and zooplankton (Avnimelech, 2015), which is being explored with respect to its nutritional quality, when it is formed within the culture system (*in situ*) by addition of carbon, and in *ex situ*, when produced in a reactor, with the use and reuse of biofloc from different species. In addition, reuse of water and biofloc can enable marine shrimp production far from the coast.

Emerenciano et al. (2013) report that when water is reused some risks such introduction of pathogens and escape of exotic species and the discharge of wastewater (pollution) are reduced and even eliminated. However, according to Samocha et al. (2017), some microminerals can be accumulated in water, biofloc and in animals if the culture water is reused for some cycles. Thus, the future scenario signals the reduction of the water exchange to no or minimal water exchange, besides the removal of solids.

Nevertheless, as already described, that BFT systems can concentrate inorganic matter contaminating the farmed animals. For this reason studies must be performed to know what happens in relation to the compounds accumulated in the aquaculture environment after each cycle of production with water reuse. This knowledge will enable the development of new strategies such as multitrophic culture to avoid deleterious effects of pollutants.

The present study aimed to characterize by means of the multielementary Instrumental Neutron Activation Analysis (INAA) the distribution, accumulation and possible repercussion to animal and human health of diverse elements present in supplies used during the production of *L. vannamei* in BFT system, using artificial brackish water.

Material and methods

Preliminary analysis of supplies

All supplies used during the shrimp production in the BFT system were obtained in the Brazilian market, some of national origin and some imported. Samples of 1,000 g were taken from each commercial package (from five different points) and 100 g were ground in an electric mill with the purpose of obtaining a granulometry for each of the analysis.

Samples weighing around 5 to 10 g each supply, used in the shrimp production (Brazilian artemia, probiotic, dolomite, molasses), feed ingredients (national and imported fish meal not used in the experiment), and commercial aquafeeds (initial and grow out) were sent to the instrumental laboratory to be analyzed.

All supplies needed for commercial production used in this study (dolomite, molasses, probiotic, initial ration and grow out ration) were analyzed by INAA (De Soete et al., 1972; De Corte, 1987), by means of the k0-standardized method (De Corte, 1987; Menezes et al., 2003), for the determination of the microminerals present in the different samples. The basis of the technique consisted in the irradiation of a sample by a neutron beam in a nuclear reactor, and the consequent formation of radionuclides, whose gamma emission was measured later and the chemical elements were then identified.

In this study, the different inputs (200 to 500 mg samples) were analyzed considering the procedure already established in the Nuclear Technology Development Center, in Belo Horizonte-MG, Brazil (Menezes et al., 2003; Menezes and Jacimovic, 2006). The samples were weighed in polyethylene flasks suitable for irradiation and irradiated for eight hours in the TRIGA MARK I IPR-R1 nuclear research reactor, which at 100 kW has a mean thermal neutron flux of $6.35\times10^{11}cm^{-2}s^{-1}$ (Menezes and Jacimovic, 2006). The samples were monitored by neutron monitors composed of Al/Au alloy (0.1%) certified, code IRMM-530R and supplied by IRMM, Institute for Reference Materials and Measurements, Belgium.

After irradiation a suitable time was allowed for the decay of the radionuclides with shorter half lives, thus avoiding possible interferences. The gamma spectrometry was performed in Hyper Pure Germanium, HPGe, radiation detector and associated electronics, using the program Genie 2000 (Canberra) for the acquisition of gamma spectra. The HyperLab program (Gamma spectroscopy) was used for the deconvolution of the spectra, and the Kayzero program for Windows® was used for the calculation of the elementary concentrations, specific to the *k0* method.

The quality control of the method was performed through the analysis of certified reference samples, SRM 1573a, Tomato Leaves, NIST (National Institute of Standards and Technology) and BCR-320R, Channel Sediment (trace elements), Reference Materials and Measurements, following the same methodology of analysis of the samples.

In order to evaluate the efficiency of the method the test En (ISO 13528: 2005) was applied in the calculations, which considered, and the expanded uncertainty of the experimental values and certified with a coverage factor k = 2. This means that true results are 95% likely to be within the confidence interval.

The following equations were used in the calculation of En:

$$E_n = \frac{Value_{Lab} - Value_{Certified}}{\sqrt{U_{Lab}^2 + U_{Certified}^2}} \qquad (1)$$

Where *ULab* and *UCertified* were expanded uncertainties *(k = 2)* of the experimental results and the certified values respectively, and,

$$U_{Experimental} = 2 \cdot u_{Lab\_Comb} \qquad (2)$$

$$u_{Lab\_Comb} = \sqrt{u_{area}^2 \quad + u^2{}_{Method}} \qquad (3)$$

Where the u_{area} is the uncertainty of the net area under the peak interest range and a method is the total uncertainty of method k0 established in the CDTN as 3.5% with a coverage factor of *k = 1*. This is the value recommended by the software Kayzero for Windows®. The evaluation of the performance of the method is performed by the criterion $|E_n| \leq 1$, meaning that the performance was satisfactory, that is, the results produced by the method are within the 95% confidence interval. If $|E_n| > 1$, indicates that the method performance was unsatisfactory. Table 1 below shows experimental results and certified values as well as the calculated En-number for certified values. As the obtained En-number values were ≤ 1, it means that the performance of the *k0* method was adequate, generating results within the 95% confidence interval.

Experimental system

After the previous analysis of the supplies they were used in the shrimp grow out experiment in the BFT system. The 9-day post-larvae of *L. vannamei* (3 mg) were kept in quarantine prior to the start the nursery phase (45 days). They were tested by official methods recommended by the World Organization for Animal Health (OIE) for the following diseases: WSSV, IMNV, TSV, YHV and NHP.

The shrimp from the juvenile production phase, with an average weight of 2.91 ± 0.93 g, were transferred to experimental grow out tanks supplied with 100% water volume (with biofloc) from the initial cultivation with 60 days of use.

Thus, the shrimp grow out experiment was carried out with the distribution of 400 shrimp/m^2 animals, 314 animals per tank, in three polyethylene tanks, filled with 500 L of water, with an area of 0.785 m^2.

The mean values of the physicochemical variables of the experimental test are described in Table 2. Daily were measured: dissolved oxygen using a digital oximeter (Hanna-model HI9146, Czech Rep.); pH and temperature with multiparameter apparatus (Hanna-model HI98129, Czech Rep.). Alkalinity (APHA, 1999), total ammonia and nitrite (UNESCO, 1983), salinity (Atago - model ATC-S / Mill 2440, Japan) were measured every seven days; suspended solids using graduated Imhoff settling cone, and total suspended solids by filtration according to Gaona et al. (2016).

The photoperiod used was 12 h. Feeding was offered twice a day (8:00 a.m. and 4:00 p.m.) and the animals were fed a decreasing feeding rate ranging from 3 to 1% of the biomass, kept without water renewal for 100 days, being replenished with freshwater only the evaporated water to maintain the volume, in low salinity (10‰). The water was obtained by the addition of different salts (Grasshof et al., 1983; Boyd, 2003), and with solids control by means of clarifier (decanter) when necessary for maintenance of the solids concentration < 15 $mL.L^{-1}$. Clarification was performed in each tank with sedimentable solids were higher than the referred concentration using graduated Imhoff sediment cone (1,000 mL) and providing sludge extraction (using a submersible water pump) for 2 to 4 hours when necessary. pH corrections were performed to maintain values close to 7.8 and alkalinity above 150 $mg.L^{-1}$, adding dolomite. The addition of molasses powder was based on the concentration of total ammonia > 0.25 $mg.L^{-1}$, maintaining the C:N ratio of 6:1, considering the composition of carbon in molasses of 45% (Avnimelech, 1999; Ebeling et al., 2006). Commercial probiotics were used to aid in the integrity of the culture environment.

After the complete cycle of cultivation in the three tanks, with an average survival of 55.2 ± 5.1%, the shrimps (10.53 ± 2.8 g) and bioflocs were analyzed by INAA.

The biofloc was collected at the end of the cultivation, as well as the clarifier residue. The waste material was drained (by siphoning) after five days without oxygenation. All samples were analyzed in duplicate by INAA.

Statistics

The data obtained from the concentrations of the minerals were analyzed in the InfoStat/S and the descriptive analysis was made and calculated the Trophic Transfer Factor (TTF), which is the ratio of the concentration of the substance in the body tissue by the concentration found in the diet items (Rashed, 2001; De Forest et al., 2007).

Results and discussion

The elemental composition of aquacultural inputs used in the experiment was characterized. We determined 28 chemical elements distributed in inputs used and the results are presented in Table 3.

Of the supplies used for the production of shrimp in BFT, without water renewal, the elements calcium (Ca), cobalt (Co), chromium (Cr), iron (Fe), rubidium (Rb) and scandium (Sc), cesium (Cs), thorium (Th) and samarium (Sm) were detected in all samples with exception imported fish meal.

The elements europium (Eu), hafnium (Hf), antimony (Sb) and ytterbium (Yb) were not detected in the initial aquafeed, at the limit of detection of the technique, but were present in samples of grow out aquafeed. Arsenic (As) was detected only in the initial aquafeed with a maximum value of 1.8 $mg.kg^{-1}$.

About national and imported fish meal, the results were a quiet similar, with the exception of Ca, where the national fish meal obtained values twice as high, and with the additional presence of elements such as: selenium (Se), zinc (Zn), barium (Ba), Sm, Sb, cesium (Cs),Thorium (Th) and mercury (Hg), undetected in meal from imported origin.

The qualitative difference found in the analysis of microminerals between artemia and probiotic was the detection of Ba, cerium (Ce), Sb, Se, tantalum (Ta), Se and terbium (Tb), only in artemia.

On the routine supplies used in the BFT system, such as: dolomite and molasses, the most striking differences were the macroelements, such as sodium (Na) and potassium (K), and the microminerals As, bromo (Br), Eu, lanthanum (La), neodymium (Nd) and Ta, detected in molasses and absent in dolomite samples analyzed.

In general an potential sources of contamination of elements in environment come from the leaching of the materials and especially feed used (Deviller et al., 2005; Sapkota et al., 2008).

There are many factors involved in the elaboration of aquafeed for farmed species. Many ingredients may lead to contamination of diet, through the addition of premix (Granados-Chinchilla et al., 2015). In this study based on the concentration of many elements in inputs we visualize that to avoid their toxicity to animals is necessary deep knowledge of the elemental composition of the inputs used during the production period.

High concentration of microminerals in the biofloc can generate toxicity and when included in the diet, these elements demand attention and a correct balance for offer to *L. vannamei* (Kuhn et al., 2017).When the diet exceeds normal levels, there is a drop in performance due to toxicity even by minerals considered essential by the NRC (2011).

The ability of the INAA technique to detect elements and quantify low concentrations in several agricultural supplies is already known (Oliveira et al, 2013; Menezes et al., 2017). Additionally, the referential characteristic as the primary technique of analysis was reported by works published (Weizhi et al., 2001; Greenberg et al., 2011). In our study INAA was efficiently applied to animal tissues and solid waste (lyophilized) from shrimp production such biofloc and clarification residue, during the last week of cultivation, as well as drainage residues (via bottom drainage, simulating a partial discharge) in an anoxic environment for five days after harvesting and the end of the cultivation.

Most of the detected elements found and concentrated in the biofloc with varied values (minimum of 0.07 mg.kg^{-1} of Tb and maximum of 1,746.00 mg.kg^{-1}

for Fe). For example, silver element (Ag), which was not detected in the supplies, was concentrated in the biofloc during the last days of cultivation and was detected and quantified by the technique limits, which proves the accumulation trends of these minerals in the culture system (0.25 mg.kg^{-1} in the biofloc) (Tab. 3, Fig. 1). And yet, in the case of Ag, it was not possible to trace which input resulted in the accumulation of this element.

In a different way it was possible to observe that the uranium (U) present in the molasses and calcareous (0.54 and 0,9 mg.kg^{-1}) had the capacity to be concentrated, mainly through the solid residue of the clarifier with values higher than that found in the supply (Tab. 3, Fig. 1). U is a radioactive substance and has effects on human health and the ecosystem can be observed if concentrations exceed levels naturally found in the environment. Human daily intake has been estimated to range from 0.9 to 1.5 µg/day of U (ATSDR, 2013). Even U was only found in solid wastes, this finding enables a possible new small source of exploitation with the great potential to remove it from the food chain.

None of the lanthanides accumulated in animal tissues were characterized only by concentrating within the experimental system via biofloc (Fig. 1) as inert elements. Some rare earth metals are already used in nutrition studies to assess passing rate in fish (Austreng et al., 2000) and crustaceans (Cox et al., 2011). For this reason, Eu, La, Sm, Ta, Tb and Yb elements accumulated only in biofloc and not in the final composition of the animal produced, so that nutritional studies could be used as possible new markers for the *L. vannamei*.

These rare earth metals are not known to be elements required for crustaceans and should not affect their metabolism (Austreng et al., 2000). The lanthanides in aquaculture supplies reached 5.89 ± 3.0 mg.kg^{-1} for the group of aquafeeds used and 3.18 ± 1.65 mg.kg^{-1} for supplies related to water quality (Tab. 3).

In minerals required by *L. vannamei*, Davis (1990) observed higher growth of juveniles when fed a purified diet containing 0.20 mg.kg^{-1} of Se and that supplementation of Se above 0.30 mg.kg^{-1} should be avoided due to its toxic effect.

Similarly, Wang et al. (2006) observed that the nitrite toxicity mechanism in *L. vannamei* shrimp involves the supplementation of diets with higher levels of Se, making it detrimental to shrimp due to increased nitrite toxicity. According to the NRC (2011), the level of Se recommended for shrimps in purified diets ranges from 0.20 to 0.40 mg.kg^{-1}. In this study in a biofloc system, the high concentration of Se (1.7 mg.kg^{-1}) in the biofloc was linked to the diets used, since it was not detected in supplies for water quality.

Although some aquatic animals have a nutritional requirement of Fe for their growth (NRC, 2011) there are no ideal concentrations of this mineral to maximize the *L. vannamei* production and research on optimal requirements is required to minimize the use of this mineral in diets (Wu and Yang, 2011).

According to Davis et al. (1992), dietary Fe supplementation had no significant effect on weight gain or survival of shrimp *L. vannamei*. In addition, there were no clear signs of deficiency observed in shrimp fed with basal diet containing 12 mg.kg^{-1} of Fe in the diet, and no apparent toxicity for Fe supplementation up to the limit of 80 mg.kg^{-1}.

In the present study concentrations of Fe in biofloc more than doubled to feed content, this reinforces the idea of an accumulation that should be carefully studied, also high contents were found in others supplies (molasses and calcareous) and may bring toxicity to animals kept in a closed culture system if the biofloc is in large quantity, or a biofloc with great period of use, or water reused by several cycles of production. It was also observed that high average concentrations of Fe in supplies, such as ration (614 mg.kg^{-1}) and dolomite (1,025 mg.kg^{-1}), contributed to a significant accumulation of the element in the biofloc.

Likewise, Zn excess can cause a reduction in *L. vannnamei* growth and feed consumption rate, as well as changes in nutritional conditions, possibly because the dietary nutrients are not biochemically available through hepatopancreas (Wu and Chen, 2005). In this study, all elements considered essential (Zn and Se) and necessary for the development of *L. vannamei* were concentrated in the biofloc, except copper (Cu), which was below the limit of detection for animal tissue and biofloc (ld > 87mg.kg^{-1} and ld > 312 mg.kg^{-1}, respectively).

The highest concentrations of the detected elements were observed in the culture environment, that is, in the biofloc, which is also a food source rich in both essential and some inert minerals and some that can be considered toxic to aquatic animals.

Biofloc concentrated 23 elements detected and descending order was: Ca>Na>Fe>Br>Sr>Zn>Ba>Cr>Rb>Ce>La>Se>Co>Sc>Th>Sm>Cs>Ag>Eu>Ta>Hf>Sb>Tb, among 28 detected in the supplies used. Some of them at high levels (Fig. 2).

In the clarifier residue no Ag was detected but the concentration of U and Yb (totaling 24 elements) was observed while in the decanted residue for 5 days after treatment, 26 elements were present, with the exception of Nd and K, detected in the used supplies but not in the residue samples after shrimp production, at detection limits of 7.00 and 7,840.00 mg.kg^{-1}, respectively. Despite the non-detection of U in the shrimp, studies with more than one production cycle should be carried out to give an idea of a possible accumulation in the animal to be consumed.

Knowledge of microminerals dynamics contributes both to the production of a safe food and to develops multitrophic systems integrated to the BFT production, using possible excesses present in the system in a more balanced and sustainable way.

Hg was not detected in the used supplies within the limit of the technique for the matrices used, but were detected later, in the animal tissue. This reinforces that there is concentration of these elements in different productive compartments, besides the biofloc, as for example in the animal tissue that presented 0.14 mg.kg^{-1} of Hg (Tab. 3, Tab. 4, Fig. 1).

Since Hg was not detected in the experiment supplies it was not possible to calculate its TTF in the system, however the elements that had their TTFs calculated and greater than 1, include Br, Cs and Se (Tab. 4).Tacon and Metian (2008) infer that the main contaminants in feeding aquatic animals were microorganisms, their toxins, and metals.

In a survey carried out on the industry linked to animal production in China, the levels of certain accumulated elements increased significantly in the last 18 years due to the wide use of additives in the animal diet (Wang et al., 2013).

There are few studies evaluating the presence of microminerals in the BFT system. The published studies showing the ash contents present in the biofloc (Wasielesky Jr et al., 2006; Azim et al., 2008; Emerenciano et al., 2013; Sabry Neto et al., 2015), with minimum values of 2.8% of mineral matter and 64.9% in the composition of the biofloc, indicate the importance of understanding that these numbers may have an impact on the results in nutritional research using the biofloc in the diet and *in situ*. By the physiological role of the few known minerals, we can say that for the adjustment of the system, microminerals must be measured in the productive compartments (feed, biofloc and animals).

The importance of monitoring microminerals has also been reported by Prangnell et al. (2016), who compared experimental diets with balanced commercial diets and evaluated factors limiting the production of marine shrimp in biofloc (*in situ*), concluded that studies are necessary to understand the environmental changes in the animal and also on the accumulation and absorption of some microminerals in the production system.

According to Leffler and Brunson (2014), the natural dynamics of the biofloc makes the BFT system susceptible to several sudden changes that impact the stability, reproducibility and replicability of the field evaluations performed in some tests.

Our preliminary study shows that the production in a system with no water exchange, over a long period, results in the concentration of varied elements in the constitution of the biofloc and in the animals, as shown in Table 3 and Table 4.

The performance of animals in biofloc production is still little studied and with the diversity of elements detected and quantified for the first time, it is possible to visualize that the control of solids is important for the success of production of animals safe for the consumer. Future research is needed focusing on the quality of supplies and supplemention.

Wang et al. (2017) evaluating diets without vitamins and minerals observed that with zero or low renewal is possible do not supplement *L. vannamei* in a closed recirculation system. In this study, the great difference found in the concentrations of the elements should be taken into account in the formulation of mineral supplements and in the endogenous content available in the raw materials, since the interaction effect macros and micronutrients present in the animal diet is not yet well-known.

Aquafeed and animals produced after one cycle of production in BFT are within the limits allowed by Brazilian Health Regulatory Agency (Anvisa), Resolution RDC nº 42/2013, internalized by Ministry of Agriculture, Livestock and Food Supply (MAPA), in National plan for Residues and Contaminants Control (PNCRC). however, under conditions with low water exchange some essential and potential toxic elements may concentrate with sucessive cycles of production.

The effects of accumulation of these on BFT and in *L. vannamei* and humans are not known. For this reason, attention should be given to the results found in this study, since a range of elements were detected and quantified.

Conclusion

From 28 elements detected in supplies 23 concentrated in biofloc and 16 in whole shrimp.

The concentration of essential elements (Zn and Se) in bioflocs was higher than shrimp feed.

High rates of trophic transfer were observed for deleterious elements such as Br, Cs, as well as for Se, which is required by shrimp *L. vannamei*. As a consequence, the transfer of these elements to humans may occur.

Inert elements (Lanthanides) found only in the supplies and the biofloc are candidates for future markers in nutritional studies.

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Tabela 1. Experimental results and certified and *En*-number values for certified reference materials SRM 1573a, *Tomato Leaves* e BCR-320R, *Channel Sediment*

Elem.	SRM 1573a, *Tomato Leaves*			BCR-320R, *Channel Sediment*		
	Experimental results k=1 mg kg^{-1}	Cerfified values k=2 mg kg^{-1}	E_n-number	Experimental Results k=1 mg kg^{-1}	Cerified values k=2 mg kg^{-1}	E_n-number
As	< 1	NR	-	24 ± 1	21.7 ± 2	0.94
Ca	47180 ± 1922	50500 ± 900	-0.84	< 10000	NR	-
Co	0.55 ± 0,02	0.57 ± 0.02	-0.43	11 ± 1	9.7 ± 0.6	0.61
Cr	< 1	NR	-	67 ± 6	59 ± 4	0.58
Fe	378 ± 12	368 ± 7	0.41	27825 ± 6833	25700 ± 1300	0.28
Hg	< 1	NR	-	1.01 ± 0,07	0.85 ± 0.09	0.95
K	27190 ± 492	27000 ± 500	0.17	< 1000	NR	-
Na	133 ± 2	136 ± 4	-0.49	< 100	NR	-
Rb	15 ± 1	14.89 ± 0.27	-0.22	< 10	NR	-
Sb	0.063 ± 0.003	0.063 ± 0.006	0.03	< 0,05	NR	-
Sc	< 1	NR	-	6 ± 1	5.2 ± 0.4	0.21
U	< 1	NR	-	1.6 ± 0.5	1.56 ± 0.2	0.00
Zn	< 50	NR	-	352 ± 124	319 ± 20	0.13

Elem., Elements; NR, Not Reported; <, less than.

Table 2. Monitoring of the physicochemical parameters of the experimental system water.

Physicochemical variables	Mean values (± standard deviation)
Dissolved oxygen	5.80±0.34 mg.L^{-1}
pH	7.62±0.20
Total ammonia	0.72±0.42 mg.L^{-1}
Nitrite	0.41±0.23 mg.L^{-1}
Suspended Solids	14±2 mL.L^{-1}
Total Suspended Solids	300±20 mg.L^{-1}
Temperature	28.82±0.92°C

Table 3. Concentration of elements (mg.kg^{-1}) found in the supplies used during a production cycle of *L. vannamei* in BFT system with no water exchange

Element	**molasses**			**dolomite**			**initial aquafeed**			**grow out aquafeed**			**probiotic**			**artemia**			**fish meal nat.***			**fish meal imp.***		
	Mean	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**
Ag	<0.2		0,2	<0.2		0.2	<0.2		0.2	<0.2		0.2	<0.2		0.2	<0.2		0.2	<0.2		0.2	<0.2		0.2
As	0.95	0.04	0.2	<2		<2	1.8	0.1	0.3	<2		2	0.66	0.05	0.1	5.3	0.3	0.1	2.2	0.1	0.5	0.5	0.1	0.4
Au	<0.0001		0.0001	<0.0001		0.0001	<0.0001		0.0001	<0.0001		0.0001	<0.0001		0.0001	<0.0001		0.0001	<0.0001		0.0001	<0.0001		0.0001
Ba	9	1	6	46	2	6	9.8	1.3	8	22	2	5	<1		1	27	2	5	10	1	2	<10		10
Br	8.6	0.2	0.1	<2		2	12.6	0.3	0.2	12.2	0.3	2	5.6	0.3	1	217	5	1	25	1	1	50.3	1.2	0.2
Ca	57495	1479	500	223550	5693	500	31835	871	940	35420	903	1000	2091	294	100	1803	326	1000	87855	2217	1000	38750	1004	1000
Cd	<1		1	<1		1	<1		5	<5		5	<5		5	<5		5	<5		2	<2		2
Ce	1.4	0.1	0.3	2.1	0.1	0.2	1.1	0.1	0.4	3.6	0.1	1	<1		1	3.2	0.1	0.5	<1		1	<0.5		0.5
Co	0.78	0.02	0.03	0.58	0.02	0.02	0.52	0.01	0.02	1.9	0.1	1	0.36	0.01	0.02	0.61	0.02	0.02	0.15	0.01	0.1	0.07	0.01	0.03
Cr	1.5	0.1	0.4	6	0.2	0.5	2.9	0.1	0.4	2.7	0.1	0.5	1.3	0.1	0.4	2.5	0.1	0.4	3.8	0.1	0.04	2.2	0.1	0.42
Cs	0.23	0.01	0.03	0.57	0.01	0.03	0.069	0.003	0.02	0.11	0.01	0.04	0.73	0.02	0.02	0.11	0.01	0.02	0.05	0	0.01	<0.03		0.03
Cu	<100		100	<100		100	<140		140	<100		100	<100		100	<100		100	<100		100	<100		100
Eu	0.07	0.01	0.01	<10		10	<0.01		0.01	0.14	0.02	0.01	<0.01		0.01	<0.01		0.01	<0.1		0.1	<0.01		0.01
Fe	364	10	25	1045	27	50	729	19	15	614	16	26	470	13	16	1293	34	16	357	9	10	409	11	25
Ga	<20		20	<0.005		0.005	<30		30	<1		1	<1		1	<1		1	<0.05		0.05	<20		20
Hf	0.1	0.01	0.03	0.078	0.004	0.01	<0.02		0.02	0.09	0.01	0.05	0.07	0.01	0.02	0.18	0.01	0.02	<0.1		0.1	<0.03		0.03
Hg	<0.1		0.1	<0.5		0.5	<0.2		0.2	<0.5		0.5	<0.1		0.1	<0.1		0.1	0.24	0.01	0.01	<0.4		0.4
K	25180	804	500	<1000		1000	6954	1186	1000	11485	840	1000	22095	851	1000	<1000		1000	3928	465	500	8114	1068	500
La	0.7	0.02	0.01	<0.1		0.1	0.59	0.02	0.03	2	0.1	0.2	0.14	0.01	0.05	1.7	0.1	0.1	0.2	0.01	0.01	<0.03		0.03
Mo	<0.5		0.5	<2		2	<1		1	<2		2	<2		2	<0.05		0.05	<0.05		0.05	<0.5		0.5
Na	189	5	5	<300		300	4998	270	100	3977	100	500	1278	32	100	108800	5058	100	3326	83	10	12515	311	25

Element	molasses			dolomite			initial aquafeed			grow out aquafeed			probiotic			artemia			fish meal nat.*			fish meal imp.*		
	Mean	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**	**Mean**	**Un**	**DL**
Nd	1	0.1	0.84	<1		1	<2		2	<1		1	<1		1	<1		1	<1		1	<2		2
Rb	27	1	1	5.5	0.2	1	8.4	0.3	1	14	1	1	62	2	1	7.6	0.4	0.5	2.6	0.2	0.04	2.8	0.2	1
Sb	0.04	0.005	0.01	0.06	0.01	0.03	<0.02		0.02	0.09	0.01	0.04	0.05	0.004	0.02	<0.5		0.5	0.044	0.004	0.01	<0.03		0.03
Sc	0.44	0.01	0.1	0.34	0.01	0.1	0.114	0.003	0.001	0.5	0.01	0.01	0.078	0.002	0.001	0.24	0.01	0.02	0.053	0.001	0.01	0.009	0.001	0.001
Se	<0.1		0.1	<0.1		0.1	1.6	0.1	0.3	1.1	0.1	0.4	<0.5		0.5	0.58	0.04	0.001	2.2	0.1	0.1	<0.4		0.4
Sm	0.16	0.01	0.01	0.15	0.01	0.1	0.1	0.01	0.01	0.26	0.01	0.1	0.026	0.003	0.01	0.22	0.01	0.05	0.027	0.003	0.01	<0.01		0.01
Sr	353	9	15	253	7	10	107	3	10	214	6	20	<11		11	41	3	1	400	11	10	153	5	15
Ta	0.059	0.002	0.02	<0.05		0.05	0.029	0.002	0.01	0.25	0.01	0.02	<0.05		0.01	0.047	0.003	0.01	<0.05		0.05	<0.02		0.02
Tb	0.038	0.001	0.01	0.033	0.003	0.01	<0.01		0.01	<0.05		0.05	<0.05		0.01	0.025	0.003	0.01	<0.05		0.05	<0.01		0.01
Th	1.21	0.03	0.03	0.466	0.012	0.01	0.06	0.01	0.03	0.12	0.01	0.05	0.05	0.01	0.03	0.39	0.01	0.01	0.07	0.01	0.01	<0.03		0.03
U	0.54	0.02	0.04	0.9	0.1	0.01	<0.1		0.1	<0.5		0.42	<1		1	<0.03		0.03	<1		1	<0.1		0.1
W	<0.4		0.4	<100		100	<0.7		0.7	<20		20	<50		50	<1		1	<1		1	<1		1
Yb	0.11	0.01	0.02	0.13	0.01	0.03	<0.02		0.02	3.7	0.3	1	<0.1		0.1	<50		50	<1		1	<0.1		0.1
Zn	<40		40	36	1	10	160	4	1	165	4	1	76	2	1	32	1	4	127	3	10	527	13	1
Zr	<20		20	<20		20	<20		20	<20		20	<20		20	<20		20	<20		20	<20		20

Un =Uncertainty; DL = Detection Limit;

All elements were identified by the Instrumental Neutronic Activation Analysis – INAA;

* supplies not used in experimental test; Fish meal Nat *National; Fish meal Imp* Imported

All results in dry weight ;

Initial aquafeed (crude protein 41,17%; ash 14,06% e phosphorus 2,46%);

Grow out aquafeed (crude protein 38,10%; ash 14,33% e phosphorus 2,57%);

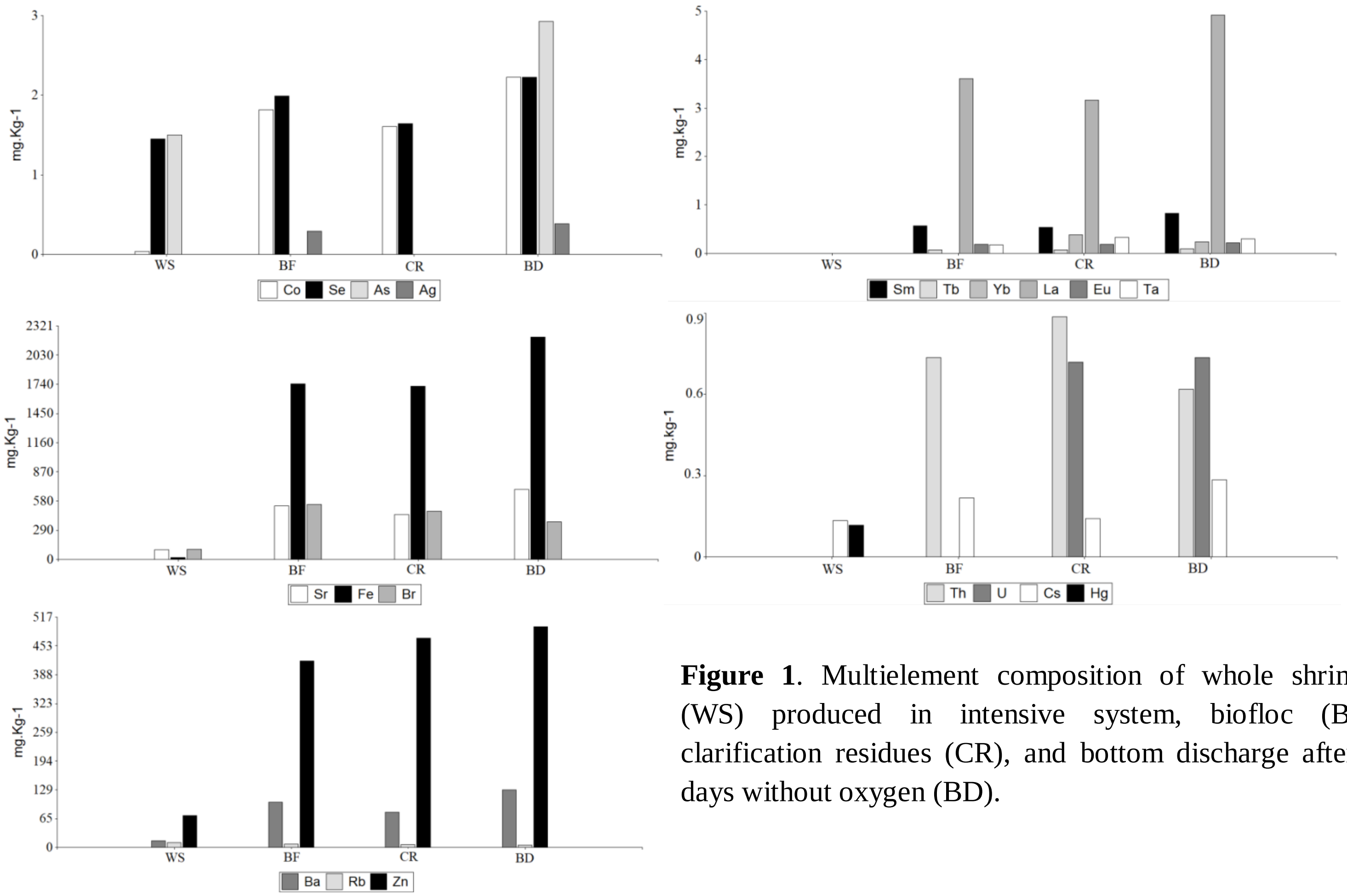

Figure 1. Multielement composition of whole shrimps (WS) produced in intensive system, biofloc (BF), clarification residues (CR), and bottom discharge after 5 days without oxygen (BD).

Figure 2. Descending order of the concentration of elements detected by INAA between sample types from the BFT system (biofloc and whole body of *L. vannamei*)

Items detected by INAA	**Decreasing concentration per sample (dry weight)**
Ba, Ca, Sc, Sr, Br, Na, Co, Fe, Zn, Se, Sb, Cs	biofloc > whole shrimp
Rb	whole shrimp > biofloc
Ce, Eu, La, Cr, Hf, Ta, Tb, Sm, Th, U	biofloc[1]
K, As, Hg	whole shrimp[2]

INAA = Instrumental Neutron Activation Analysis;
[1] = Not detected in the whole shrimp;
[2] = Not detected in the biofloc.

Table 4. Trophic Transfer Factor (TTF) of commercial ration applied in experimental intensive BFT system

Elements	Tested aquafeed (mg.kg^{-1})	Whole shrimp (mg.kg^{-1})	TTF (ration/animal)
Cs	0.11	0.16	1.45
Br	12.16	103	8.47
Se	1.12	1.24	1.11

TTF = Trophic Transfer Factor calculated by concentration in whole animal on feed concentration.

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CAPÍTULO IV

CONSIDERAÇÕES FINAIS

As informações dos microminerais são muito limitadas comparadas com demais nutrientes e as exigências ou necessidades de diversos elementos não estão definidas, e nesse contexto, surgem sistemas de produção cada vez mais sustentáveis (com reuso da água), onde acúmulos de minerais no meio produtivo ocorrem, como comprovado por este estudo.

Conforme apresentado, tanto elementos necessários como elementos potencialmente tóxicos foram identificados em insumos e aplicados no experimento. Os insumos comerciais com gama variada de elementos traços presentes resultaram em níveis de acúmulos variados no sistema, via biofloco, animal e em resíduos pouco estudados na literatura aquícola.

Excesso de suplementação em BFT pode causar toxicidade ao reuso de água por ciclos contínuos, além disso, causa desperdício e poluição do meio ambiente, já que frequentemente é feita em quantidades superiores para assegurar o bom desempenho, prática que também onera o custo da ração para manter a reserva mineral na dieta.

Os estudos futuros devem ser direcionados para qualidade de insumos, e o particionamento desses elementos no ambiente de produção de forma multitrófica, uma vez que os sistemas de produção são cada vez mais intensivos, com menor renovação de água e com utilização de rações e insumos. Sendo importante avaliar que os microminerais tem macroresponsabilidades e estão envolvidos tanto na nutrição animal, como na sanidade aquícola, meio ambiente e saúde humana.

O estudo elementar em sistema fechado de produção em bioflocos pode nortear as pesquisas de nutrição do *L. vannamei*, proporcionando conhecimentos para sanidade dos animais cultivados e melhoria de monitoramento ambiental para os resíduos sólidos, bem como para mensurar o risco de contaminantes do produto final para consumo.

Manter saúde ambiental, humana e animal são pilares para manutenção do ciclo de vida do sistema de produção em biofloco. A economia de água entre ciclos deve ser analisada com critério e conhecimento da composição dos insumos que entram no sistema de produção. Provavelmente, para a qualidade final desejada insumos específicos para o sistema podem ser elaborados.

Insumos eventualmente podem estar com suplementação em excesso ou contaminados com diversos elementos como demonstrado no presente estudo, e que ao longo do tempo de cultivo podem acumular e trazer prejuízos ao sistema produtivo quando presente no sistema (biofloco), e ao ser humano quando o acúmulo ocorre no produto final (camarão).

Pesquisas com ciclos sucessivos e reuso do biofloco são necessárias para conhecer a composição do resíduo sólido excedente e maneja-lo adequadamente, uma vez que o acúmulo e efeito da interação dos diversos elementos detectados neste trabalho ainda não são conhecidos em literatura.