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“JÚLIO DE MESQUITA FILHO”

FACULDADE DE MEDICINA VETERINÁRIA

CAMPUS DE ARAÇATUBA

BIOMASSAS MICROBIANAS NA ALIMENTAÇÃO DE TILÁPIAS

Thiago Luís Magnani Grassi

Médico Veterinário

ARAÇATUBA – SP

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Orientadora: Profa. Associada Elisa Helena Giglio Ponsano

Coorientador: Dr. Giovani Sampaio Gonçalves

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Tese apresentada à Faculdade de Medicina Veterinária de Araçatuba – Unesp, como parte das exigências para obtenção do título de Doutor em Ciência Animal (Medicina Veterinária Preventiva e Produção Animal).

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Araçatuba, 24 de agosto de 2018.

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DADOS CURRICULARES

THIAGO LUÍS MAGNANI GRASSI – Médico Veterinário e Mestre em Ciência Animal pela Faculdade de Medicina Veterinária (FMVA) - UNESP Araçatuba. Atualmente é doutorando, bolsista Fapesp, vinculado à área de Medicina Preventiva e Produção Animal, com ênfase em Tecnologia de Produtos de Origem Animal e Produção Animal, no programa de Pós-Graduação em Ciência Animal da FMVA, UNESP, sob orientação da professora Associada Elisa Helena Giglio Ponsano. Realizou doutorado sanduíche com bolsa PDSE da Capes na área de Produção Agrária (Aquicultura) na Escuela Técnica Superior de Ingenería Agrononómica, Alimentaría y de Biosistemas da Universidad Politécnica de Madrid (UPM) na Espanha, sob orientação do Prof. Dr. Morris Villarroel, durante 6 meses. Durante o período, contribuiu em pesquisas da Facultad de Veterinária da Universidad Complutense de Madrid, sob coorientação do Prof. Dr. Jesús de la Fuente Vazquez. Realizou estágio na área de nutrigenômica, análise de compostos antioxidantes e aquicultura na Facultad de Ciencias Agronómicas da Universidad de Chile (Santiago / Chile), sob orientação do Prof. Dr. Jurij Wacyk. Realizou estágio no laboratório de aquicultura na Facultad de Agronomía da Universidad de Buenos Aires (Buenos Aires / Argentina), sob orientação do Prof. Dr. Gabriel Alejandro Morales. Realizou pesquisas com animais de produção (tilápias, aves de corte e poedeiras, ovinos), com produtos de origem animal (carne, leite, ovos, mel, peixes e seus derivados) e tem experiência em análises laboratoriais, clínicas e de campo. Participou de diversos eventos nacionais e internacionais com apresentação de trabalhos derivados de suas pesquisas. Ministrou aulas para alunos de graduação em Medicina Veterinária e de pós-graduação em Ciência Animal e colaborou com a formação de residentes em Saúde Pública da FMVA - Unesp.

“Só se pode alcançar um grande êxito quando nos mantemos fiéis a nós mesmos.”

FRIEDRICH NIETZSCHE

“Veja! Não diga que a canção está perdida
Tenha fé em Deus, tenha fé na vida
Tente outra vez!

Queira! Basta ser sincero e desejar profundo
Você será capaz de sacudir o mundo
Tente outra vez!

Tente! E não diga que a vitória está perdida
Se é de batalhas que se vive a vida
Tente outra vez!**”**

RAUL SEIXAS

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BIOMASSAS MICROBIANAS NA ALIMENTAÇÃO DE TILÁPIAS

RESUMO – Biomassas microbianas produzidas a partir de resíduos e coprodutos industriais podem ser utilizadas como fonte de proteínas e de antioxidantes em dietas de peixes, reduzindo os custos para a indústria com tratamento e descarte e auxiliando na preservação do meio ambiente. O objetivo deste experimento foi avaliar o desempenho produtivo e o *status* oxidativo de tilápias alimentadas com diferentes biomassas microbianas e a qualidade de sua carne. Juvenis de tilápia-do-Nilo (26,84 ± 1,03 g) foram alimentadas com as dietas experimentais durante 76 dias. Os tratamentos incluíram uma dieta controle negativo (sem biomassa microbiana), uma dieta controle com 0,01% de vitamina E (VE) e as demais contendo três tipos de biomassa microbiana em duas concentrações (0,25 e 0,5%): *Rubrivivax gelatinosus* (RG25 e RG50), *Saccharomyces cerevisiae* (SC25 e SC50) e *Spirulina platensis* (SP25 e SP50). Foram avaliados o desempenho e o *status* oxidativo dos peixes e os parâmetros físicos, químicos e bromatológicos da qualidade da carne. A inclusão de biomassa microbiana nas dietas reduziu o *status* oxidante total do plasma, o malonaldeído e a atividade respiratória dos leucócitos, e aumentou o *status* antioxidante total, sem interferir nos parâmetros bioquímicos. Todos os tratamentos apresentaram resultado similar para os parâmetros de desempenho, exceto pela conversão alimentar aparente do RG50 que foi menor que do grupo controle negativo. No filé, o uso de biomassa microbiana diminuiu as substâncias reativas ao ácido tiobarbitúrico, incrementou a intensidade de vermelho (exceto por SC) e a deposição de carotenoides (exceto por SC25), aumentou a concentração de proteínas e melhorou a relação n-6/n-3, sem interferir na textura. Os tratamentos RG25, SC25 e SP50 aumentaram a concentração do ácido eicosapentaenoico dos filés e o RG50 apresentou efeito positivo sobre desempenho dos animais. Com isso, pôde-se concluir que o uso de biomassa microbiana provocou um efeito antioxidante no plasma e melhorou a conversão

alimentar dos animais, reduziu a oxidação lipídica e aumentou o teor de proteínas, a intensidade de vermelho e a concentração de carotenoides dos filés, sem influenciar negativamente na saúde dos animais.

Palavras-chave: *Oreochromis niloticus, Rubrivivax, Saccharomyces, Spirulina.*

MICROBIAL BIOMASS IN TILAPIA FEED

SUMMARY – Microbial biomass produced with industrial residues can be used as sources of proteins and antioxidants in fish feeds. The practice results in reduced amounts of waste to treat and discard and so brings benefits to the industry and to the environment. The aim of this experiment was to evaluate the growth and the oxidative status of tilapia fed microbial biomass and the quality of its flesh. Nile tilapia (26.84 ± 1.03 g) were fed one of experimental diets for 76 days. Treatments included a negative control diet (without microbial biomass), a control diet supplemented with 0.01% vitamin E (VE), and diets containing three types of microbial biomass in two concentrations (0.25 and 0.5%): *R. gelatinosus* (RG25 and RG50), *S. cerevisiae* (SC25 and SC50), and *S. platensis* (SP25 and SP50). The growth and the oxidative status of the animals and the physical, chemical and bromatological parameters of the flesh quality were evaluated. Adding microbial biomass to diets decreased plasma total oxidant status, malonaldehyde and leukocyte respiratory burst, increased the total antioxidant status and did not affect the blood biochemical parameters. All treatments had similar growth parameters, except feed conversion ratio that was lower for RG50 than for negative control group. In the flesh, the use of the microbial biomass lowered the thiobarbituric acid reactive substances and increased redness (except for *S. cerevisiae*), carotenoid deposition (except SC25) and protein content and improved the n-6/n-3 ratio of the fillets without interfering with the texture. RG25, SC25 and SP50 increased eicosapentaenoic acid on the fillets and RG50 had a positive effect on the animals' growth. So, it was concluded that the use of the microbial biomass provided an antioxidant effect in blood plasma and improved the feed conversion ratio of the animals, decreased the lipid oxidation and increased protein content, pigmentation and carotenoid deposition in the fish flesh, without imparting a negative impact on the animals` health.

Keywords: *Oreochromis niloticus, Rubrivivax, Saccharomyces, Spirulina.*

CAPÍTULO 1 - CONSIDERAÇÕES GERAIS

1 INTRODUÇÃO

A expansão no consumo do pescado vem ao encontro do aumento da população mundial, como forma de fornecimento de proteína animal de alto valor biológico. Aliado a isso, o perfil de ácidos graxos do pescado representa uma condição favorável à manutenção da saúde humana, pois está relacionado com a prevenção de doenças crônicas. Por outro lado, essa mesma característica é responsável pela principal reação química envolvida na limitação da vida de prateleira do pescado, a rancidez oxidativa.

Na criação de peixes, são adicionados antioxidantes à ração para controlar a reação de rancidez e, como eles são transferidos para a porção muscular do pescado, é razoável prever, também, uma ação antioxidante dessas substâncias, prevenindo a deterioração da carne por esse mecanismo. Os antioxidantes sintéticos são os mais utilizados para essa finalidade, porém, em função da toxicidade que podem apresentar ao consumidor, alguns países vêm revendo a legislação sobre o assunto, o que estimula a busca por fontes alternativas dessas substâncias.

A biotecnologia, em seu conceito clássico, utiliza-se de organismos vivos para a produção de bens e serviços. Em processos biotecnológicos, substâncias de interesse são recuperadas e purificadas por processos *downstream* e os microrganismos podem ser reaproveitados na forma de biomassa contendo proteínas e outras substâncias produzidas pelo metabolismo microbiano, tais como compostos com atividade antioxidante.

Neste trabalho de pesquisa foram utilizadas biomassas microbianas com potencial antioxidante na alimentação de tilápias como forma de aproveitar os resíduos gerados em processos biotecnológicos, diminuindo custos com descarte e evitando danos ambientais.

2 REVISÃO DA LITERATURA

2.1 Produção e consumo de pescado

Em 2016, a produção mundial de pescado atingiu 171 milhões de toneladas e foi a proteína animal mais produzida do mundo, apresentando vantagem considerável sobre as produções de carne de suíno (2ª colocada – 120 milhões de toneladas), de frango (3ª colocada – 118 milhões de toneladas) e de bovino (4ª colocada – 70 milhões de toneladas), segundo a Food and Agriculture Organization (FAO, 2018). De todo o pescado produzido, 47%, o que corresponde a 80 milhões de toneladas, foi proveniente da aquicultura, sendo 54,1 milhões de toneladas de peixe (FAO, 2018).

Em termos econômicos, estima-se que a pesca e a aquicultura movimentaram US$ 362 bilhões, sendo US$ 232 bilhões provenientes da atividade aquícola. Além de desempenharem um papel essencial para a segurança alimentar do planeta, a pesca e a aquicultura são responsáveis por gerar 59,6 milhões de empregos, que vão desde pequenos pescadores até trabalhadores de grandes fábricas de processamento de produtos pesqueiros, tanto em países desenvolvidos como em desenvolvimento (FAO, 2018).

A aquicultura é o cultivo de organismos aquáticos, incluindo peixes, moluscos, crustáceos e algumas algas e plantas. A atividade de cultivo implica na intervenção do homem para aumentar a produção, a reprodução, a alimentação e a proteção contra predadores (FAO, 2014). A produção por aquicultura cresceu 60% comparando os dados de 2007 e 2017 (de 50 para 80 milhões de toneladas), enquanto que a produção por pesca extrativa manteve-se estável (90 a 92 milhões de toneladas) (FAO, 2018). Segundo estimativas da FAO (2018), a partir de 2021 ou 2022, a aquicultura será a principal fonte de produção de pescado. Segundo as projeções do mesmo órgão, em 2030, a

produção de espécies de água doce (carpas, bagres e tilápias) crescerá dos atuais 58 para 62% de toda a produção aquícola.

O Brasil é o oitavo maior produtor mundial por aquicultura de água doce, atrás de China, Índia, Indonésia, Vietnã, Bangladesh, Egito e Myanmar (FAO, 2018). Apesar dos gargalos normais, ligados à questão ambiental e ao uso das águas da União, e das condições sazonais, tal como a intensa seca no Nordeste, a piscicultura brasileira apresentou crescimento de 8% em 2017, comparado ao ano anterior, demonstrando o avanço da atividade como agronegócio, representado por uma receita anual estimada em R$ 4,7 bilhões (PEIXE BR, 2018). Dentre todas as espécies de peixes produzidas no Brasil, a tilápia se apresenta como a principal, em termos produtivos e econômicos.

Segundo a Food and Agriculture Organization (FAO), o consumo mundial de peixe apresentou crescimento anual médio de 3,2%, levando em consideração o período de 1961 a 2016, superando o crescimento da população (1,6%) e do consumo de carne de todos os animais terrestres combinados (2,8%) (FAO, 2018). Em termos *per capita*, o consumo de peixe no Brasil foi de 14,4 kg em 2015 (BRASIL, 2017), o que está acima dos 12 kg recomendados pela Organização Mundial da Saúde (OMS), porém abaixo dos 20,2 kg descritos pela FAO como a média mundial.

2.2 Tilápia-do-Nilo

Tilápia é o nome mais utilizado para se referir a três gêneros de peixes da família *Cichlidae: Oreochromis, Sarotherodon e Tilapia* (WATANABE et al., 2002). A Tilápia-do-Nilo (*Oreochromis niloticus*), espécie de maior importância comercial, é natural da África, de Israel e da Jordânia e, devido a seu potencial para a aquicultura, teve sua distribuição expandida para as mais diversas regiões do mundo nos últimos 60 anos. No Brasil, a espécie foi introduzida em 1971 com

60 indivíduos proveniente da Estação de Piscicultura de Bouaké na Costa do Marfim, na região nordeste e, posteriormente, disseminou-se pelos demais estados brasileiros (CASTAGNOLLI, 1992; WATANABE et al., 2002).

Segundo dados da FAO (2018) relacionados à produção mundial de pescado, a tilápia é a segunda espécie de peixe mais produzida por aquicultura, sendo precedida apenas pelas carpas. A disseminação do cultivo da tilápia-do-Nilo pode ser explicada por características da espécie, tais como: a rusticidade; a precocidade; o hábito alimentar onívoro de amplo espectro, ou seja, que utiliza satisfatoriamente altos teores de proteína vegetal; a facilidade de reprodução e de obtenção de alevinos; a possibilidade de manipulação hormonal do sexo para produção de machos (reversão sexual); o excelente crescimento em sistema de cultivo intensivo e a resistência a doenças (MARENGONI, 2006).

Um levantamento realizado pela Associação Brasileira da Piscicultura (PEIXE BR, 2018) indicou que a produção de tilápia no Brasil foi de 357 mil toneladas em 2017, sendo a quarta maior do mundo, ficando atrás apenas de China, Indonésia e Egito. Segundo os números das principais espécies produzidas no Brasil, a tilápia representou 51,7% de toda a produção da piscicultura nacional, que foi de 691,7 mil toneladas em 2017.

A importância e a expansão da tilapicultura no Brasil decorrem da demanda de mercado, uma vez que a carne de tilápia apresenta boa aceitação pela população devido ao sabor, ao valor nutritivo e aos preços baixos (SIMÕES et al., 2007). Também colabora com essa expansão, a aptidão do país para a atividade aquícola, já que conta com disponibilidade de grãos para ração, clima favorável e abundância de recursos hídricos.

A tilápia-do-Nilo apresenta em sua composição Na, K, Ca, Mg, P, vitaminas lipossolúveis do complexo B e ácidos graxos poli-insaturados; por outro lado carece de colesterol (OGAWA; MAIA, 1999). Os ácidos graxos poli-insaturados (AGPI) do pescado o tornam mais susceptível ao processo de deterioração por oxidação lipídica. No entanto, a ingestão de fontes de AGPI

pode trazer benefícios ao consumidor, tais como a redução do colesterol de baixa densidade (LDL – low density lipid), e atividades antitrombótica, anti-inflamatória, antiarrítmica e de vasodilatação (LOMBARDO; CHICCO, 2006). Com isso, o consumo desse peixe pode auxiliar na prevenção de doenças coronarianas, hipertensão, diabetes tipo 2 e resistência à insulina (ZHANG et al., 2013). Além disso, a tilápia apresenta requisitos típicos dos peixes preferidos pelo consumidor, tais como carne branca de textura firme, sabor delicado e fácil filetagem, não tendo espinha em “Y” e nem odor desagradável (SOUZA, 2002).

Apesar da importância nutricional, o pescado é o alimento de origem animal com maior probabilidade de deterioração, por reunir características como pH próximo à neutralidade, elevada atividade de água nos tecidos, alto teor de nutrientes facilmente utilizáveis pelos microrganismos, acentuado teor de lipídeos e rápida ação destrutiva das enzimas presentes nos tecidos e nas vísceras do peixe (GASPAR et al., 1997; LEITÃO et al., 1997).

2.3 Estresse oxidativo e oxidação lipídica

O estresse oxidativo resulta de um desequilíbrio entre a geração de espécies reativas de oxigênio (EROs) e as defesas antioxidantes em organismos vivos, podendo causar danos diretos ou indiretos aos principais componentes celulares, tais como os lipídeos, as proteínas e o DNA (HUANG, 2004; NISHIDA, 2011; SCANDALIOS, 2005). Apesar do risco potencial das EROs, as células apresentam uma variedade de mecanismos de defesa para neutralizar os efeitos nocivos dos radicais livres (MONTEIRO et al., 2006). Sistemas antioxidantes enzimático (superóxido dismutase, catalase, glutationa peroxidase) e não enzimático (vitaminas e carotenoides) são essenciais para manter o *status* redox das células dos peixes e representam a barreira de defesa contra o estresse oxidativo (MONTEIRO et al., 2006; VINAGRE et al.; 2012).

As EROs podem ser produzidas por fontes celulares endógenas durante o metabolismo celular normal, seja durante uma resposta imune (leucócitos) ou mesmo pela respiração mitocondrial (SEVCIKOVA et al., 2011). Alguns processos, como a inflamação e o estresse ambiental (temperatura, elevadas densidades populacionais), aumentam a produção de EROs de forma significativa e acabam proporcionando um desequilíbrio no *status* oxidativo do organismo (LIU et al., 2018).

O uso de antioxidantes em dietas animais reduz o desperdício de matérias-primas e amplia a gama de gorduras que podem ser usadas na dieta, especialmente as de alta energia, aumentando a vida útil do produto (AKLAKUR, 2018). Os antioxidantes ajudam a evitar situações de estresse oxidativo e, quando transferidos para o músculo dos animais, podem, também, ser eficientes na conservação do pescado durante o processamento e a estocagem, inibindo a degradação dos lipídeos pela oxidação e, assim, aumentando o tempo de prateleira do produto final (CHEAH, 1995).

A oxidação lipídica é o processo primário de deterioração da qualidade dos peixes e seus produtos, manifestando-se por mudanças que impactam negativamente no odor, no sabor, na coloração, na textura e no valor nutritivo, e pela possível produção de compostos tóxicos (JENSEN et al., 1998; NUNES et al., 2007). Devido às alterações organolépticas que provocam aspecto de ranço e, consequentemente, rejeição nos consumidores, a reação também pode ser denominada de rancidez oxidativa. No caso dos peixes, as membranas celulares são mais suscetíveis à reação porque seus lipídeos possuem maior grau de insaturação (RUFF et al., 2004).

A rancificação da gordura causa a quebra das ligações duplas nas frações fosfolipídicas das membranas celulares, sendo o processo constituído por uma cadeia complexa de reações induzidas por oxigênio na presença de catalisadores, tais como calor, radicais livres, luz, pigmentos e íons metálicos (LAGUERRE et al., 2007). O processo é dividido em três etapas: iniciação,

propagação e terminação. Inicialmente, ocorre a formação do radical livre em condições favorecidas pela luz e calor (fase de iniciação), seguida pela formação de produtos primários, principalmente os peróxidos e hidroperóxidos (fase de propagação), e estes, quando expostos a condições de oxidação prolongada, dão origem a produtos secundários, que incluem aldeídos, cetonas, epóxidos, compostos hidroxi, oligômeros e polímeros (fase de terminação) (BARRIUSO et al., 2013; GORDON, 2001). O malonaldeído (MDA) é um dos aldeídos mais produzidos durante a fase de terminação e, provavelmente, o marcador mais utilizado para avaliar e quantificar a reação (BARRIUSO et al., 2013).

O período de indução é extremamente importante para o processo da oxidação lipídica, pois é caracterizado por uma alta sensibilidade a pequenas concentrações de componentes, como os pró-oxidantes, que encurtam esse período e aceleram o processo de deterioração, e os antioxidantes, que o prolongam e retardam a rancidez. De acordo com Del Ré e Jorge (2012), segundo o mecanismo de ação, os antioxidantes podem ser classificados em primários, que são os que atuam interrompendo a cadeia da reação através da doação de elétrons ou hidrogênio aos radicais livres (ADEGOKE et al., 1998), ou em secundários, que atuam na formação de complexos com metais, no sequestro de oxigênio, na decomposição de hidroperóxidos para formar espécie não radical, na absorção da radiação ultravioleta ou na desativação de oxigênio singlete (DECKER, 2002).

A concentração de agentes pró-oxidantes e antioxidantes pode ser manipulada pela dieta (REY et al., 2004). Os antioxidantes provenientes da nutrição (vitaminas C, K e E e carotenoides) e seus cofatores (cobre, manganês, zinco, selênio, ferro e riboflavina) mantêm o equilíbrio na formação e no controle das espécies reativas de oxigênio e têm sido utilizados com a finalidade de melhorar o crescimento, a resistência ao estresse e a doenças e a sobrevivência de peixes e camarões cultivados e, também, melhorar a qualidade do produto final desses pescados (HAMRE et al., 2004; HUO et al., 1999; SLANINOVA et al., 2009).

2.4 Antioxidantes na alimentação

Apesar de os antioxidantes sintéticos serem os mais utilizados em formulações de rações e produtos alimentícios, eles têm sido alvo de questionamentos quanto a sua inocuidade (ANESINI et al., 2006). Estudos revelam que alguns desses compostos podem implicar em vários riscos para a saúde, como toxicidade e desenvolvimento de câncer (HOU, 2003; PRIOR, 2004). Portanto, o uso desses produtos deve estar sob estrita e constante regulamentação e fiscalização (PARK et al., 2001). O Japão, por exemplo, suspendeu a compra de camarões produzidos na Índia por apresentarem elevados níveis de etoxiquina (AKLAKUR, 2018), e outros países têm seguido o mesmo caminho. Dentre os antioxidantes comerciais sintéticos mais utilizados estão o hidroxitolueno butilado (BHT), o hidroxianisol butilado (BHA), a terc-butil-hidroquinona (TBHQ), o propil galato (PG) e a etoxiquina (EQ) (AKLAKUR, 2018).

A produção de substâncias com papel antioxidante por via biotecnológica (microrganismos) pode representar uma alternativa aos produtos sintéticos utilizados atualmente. Exemplo disso são os carotenoides, tais como a astaxantina, a cantaxantina, a espiriloxantina, o β-caroteno e o licopeno que, além de seu papel como corantes naturais de alimentos, também apresentam propriedades antioxidantes, importantes para diminuir a deterioração do produto causada pela oxidação lipídica (BHOSALE, 2004; BHOSALE; BERNSTEIN, 2005; STAHL; SIES, 2003). Segundo Ponce-Palafox et al. (2004), há diversos exemplos de biomassas microbianas como fonte de carotenoides para a alimentação de animais aquáticos, como a levedura *Phaffia rhodozyma* e as microalgas *Haematococcus pluvialis* e *Dunaliella salina*.

As vitaminas também são exemplos de substâncias que podem ser produzidas por microrganismos, destacando-se a vitamina E que, segundo Gao et al. (2012), é utilizada em dietas animais por desempenhar um papel importante na prevenção da oxidação lipídica de diversas espécies de peixes, buscando

proteger a integridade dos tecidos (LEWIS-McCREA; LALL, 2007; PENG et al., 2009; TOCHER et al., 2002).

2.5 Biomassas microbianas

Rubrivivax gelatinosus (previamente chamada de *Rhodocyclus gelatinosus*) é uma bactéria fotossintetizante que possui a habilidade de crescer em efluente de pescado e de aves, reduzindo a carga poluente e produzindo biomassa rica em proteínas e em pigmentos carotenoides, tal como a espiriloxantina (LIMA et al., 2011; PONSANO et al. 2002, 2003, 2004, 2011). Um esquema ilustrativo da produção de biomassa de *Rubrivivax gelatinosus* em efluente de indústria de pescado está apresentado na Figura 1. Os oxicarotenoides produzidos por *R. gelatinosus* são assim classificados por apresentarem várias funções oxigenadas em sua cadeia carbônica, característica que possibilita a deposição nos tecidos dos animais que ingerem esses compostos na dieta (BAKER; GÜNTHER, 2004).

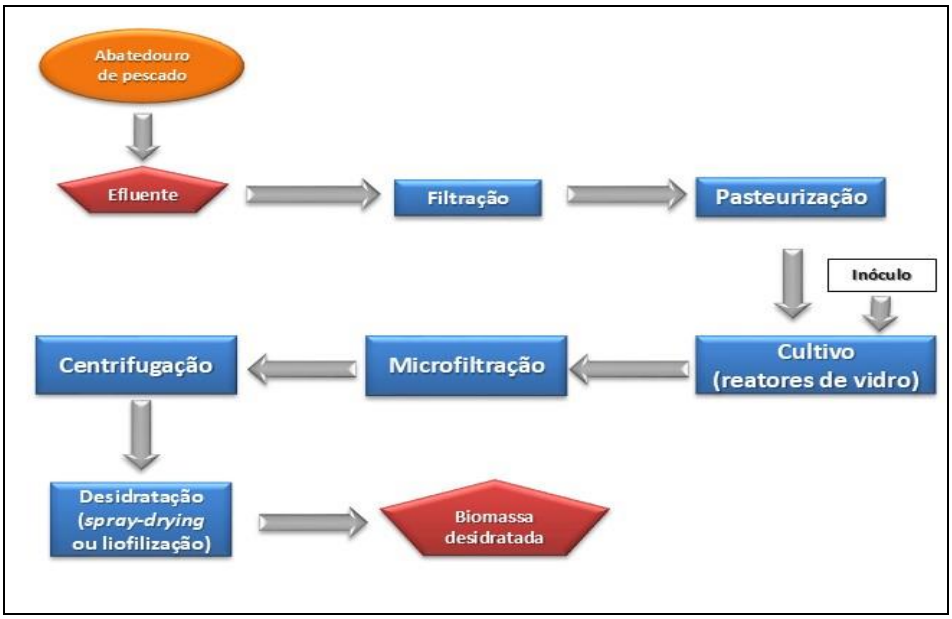

Figura 1 - Fluxograma da produção de biomassa de *R. gelatinosus.*

A biomassa da bactéria já foi utilizada em rações de aves e peixes e apresentou efeito pigmentante e antioxidante, além de aumentar a deposição de proteínas nos produtos finais. Santo et al. (2016) relataram retardo na oxidação lipídica e Grassi et al. (2016) descreveram maior concentração de carotenoides, de proteínas e intensidade de vermelho em filés de tilápias alimentadas com biomassa de *R. gelatinosus*. Segundo Polonio et al. (2010), as gemas de ovos de galinhas alimentadas com *R. gelatinosus* apresentaram pigmentação mais intensa e, conforme a análise sensorial, houve preferência dos consumidores por esses produtos. Avanço et al. (2014) encontraram efeito pigmentante semelhante para peito e coxa de frangos de corte.

Spirulina (*Arthrospira*) platensis é uma microalga rica em proteínas, vitaminas, aminoácidos essenciais, minerais, ácidos graxos essenciais e pigmentos, tais como os carotenoides e as ficocianinas, o que lhe proporciona elevado valor nutricional e propriedades antioxidantes (LI et al., 2003; MORIST et al., 2001). Além disso, a biomassa da microalga apresenta efeito na imunomodulação de humanos e animais, onde amplia os mecanismos humorais e celulares do sistema imunitário (PANG et al., 1988; QURESHI et al., 1996; TAKEUCHI et al., 2002). Vários estudos também mostram que a *Spirulina* pode evitar ou inibir o câncer em seres humanos e animais (PANG et al., 1988; QURESHI et al., 1996).

Segundo Jafari et al. (2014), o uso de *Spirulina* na alimentação de peixes pode levar a alterações no perfil e na quantidade de ácidos graxos da carne, o que vai ao encontro da demanda dos consumidores por produtos nutritivos e que promovam a saúde humana (BELL; WAAGBØ, 2008). A *Spirulina* é classificada como GRAS (Generally Recognized as Safe) pelo Food and Drug Administration (FDA), órgão americano que regulamenta o uso de alimentos e medicamentos (ANDRADE; COSTA, 2008), o que atesta a segurança de seu consumo. *Spirulina* é capaz de crescer em diferentes substratos, incluindo efluentes industriais e meios de cultura simples e de baixo custo (Figura 2), o que torna sua produção totalmente viável (ARRUDA et al., 2006; CHANG et al., 2013).

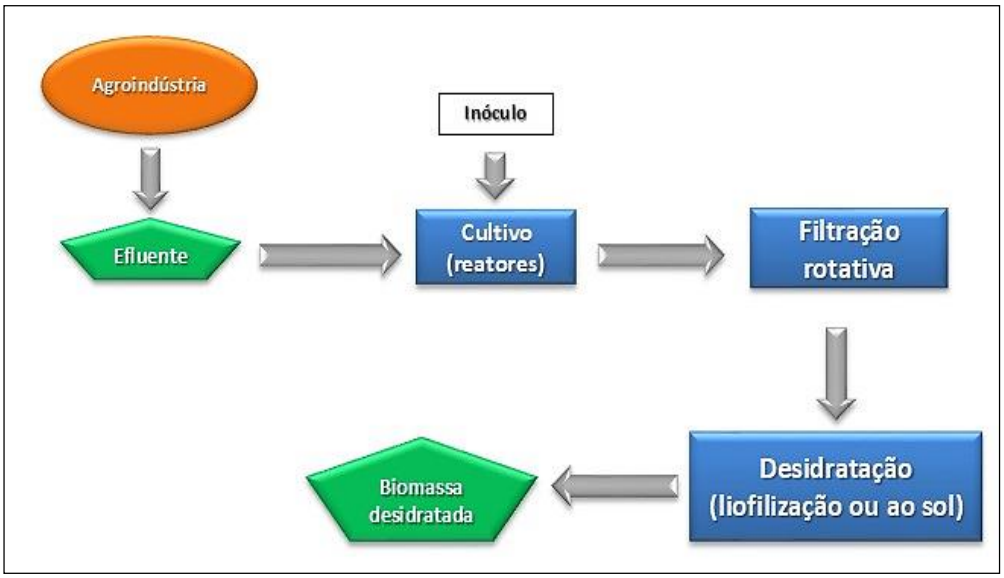

Figura 2 - Fluxograma da produção de biomassa de *S. platensis.* Fonte: Adaptado de Aquarone et al. (2001).

Saccharomyces cerevisiae é uma levedura frequentemente utilizada biotecnologicamente para a elaboração de diversos produtos e, na maioria das vezes, sua participação é apenas como agente biológico de transformação, uma vez que, ao término do processo produtivo, ela é recuperada, podendo ser utilizada como coproduto (Figura 3) (BATISTA, 2001). Na fabricação de etanol, por exemplo, a massa celular do microrganismo gerada ao final da produção pode encontrar várias aplicações devido a suas propriedades nutricionais e antioxidantes, tal como ingrediente de rações animais.

Isso se deve ao fato de a biomassa da levedura conter proteínas, e vitaminas, como a E e as do complexo B, enzimas, ácidos graxos voláteis, minerais quelatados, antibióticos naturais e peptídeos. Com essa composição, o uso da biomassa da levedura na alimentação de peixes confere melhor palatabilidade à ração e menor estresse aos animais (MACHADO, 1997), além de favorecer as respostas de desempenho produtivo, beneficiar o sistema imune não-específico e aumentar a resistência contra infecções bacterianas (REQUE et al., 2010; RODRIGUEZ et al., 2003). Esses efeitos podem estar relacionados a propriedades antioxidantes de componentes presentes na levedura.

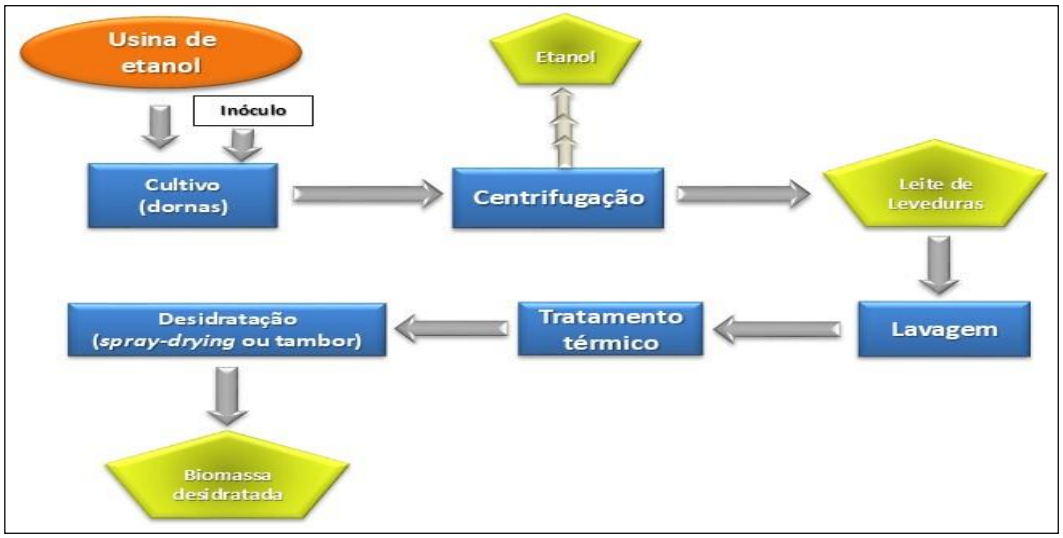

Figura 3 - Fluxograma da produção de biomassa de *S. cerevisiae.* Fonte: Adaptado de Aquarone et al. (2001).

Dessa forma, as características inerentes a essas biomassas desperta o interesse de seu uso em alimentação animal visando o controle do estresse oxidativo e da rancificação da carne, o que motivou a execução deste trabalho de pesquisa.

3 OBJETIVO

O objetivo desse estudo foi avaliar os efeitos da inclusão (0,25 e 0,5%) de biomassas microbianas (*Rubrivivax gelatinosus*, *Saccharomyces cerevisiae* e *Spirulina platensis*) na ração de Tilápias-do-Nilo sobre o desempenho e a saúde dos animais e a qualidade da carne.

4 CONCLUSÕES GERAIS

4.1 Em relação aos animais

4.1.1 Desempenho

Os animais alimentados com as rações contendo 0,5% de *Rubrivivax gelatinosus* apresentaram melhor conversão alimentar aparente que o grupo controle negativo.

4.1.2 Parâmetros bioquímicos

Não houve alteração nos parâmetros bioquímicos dos animais alimentados com as rações contendo biomassas microbianas.

4.1.3 NBT

O uso das rações contendo biomassas microbianas provocou um efeito imunomodulatório positivo nos animais, por meio da avaliação da atividade respiratória dos leucócitos.

4.1.4 *Status* oxidativo e TBARS

O uso das rações contendo biomassas microbianas aumentou o *status* antioxidante total e reduziu o *status* antioxidante e a oxidação lipídica (TBARS) no sangue dos animais.

4.1.5 Índices viscerais

O uso das biomassas microbianas não interferiu nos índices viscerais (hepatossomático, esplenossomático e digestivossomático) dos animais.

4.2 Em relação à carne

4.2.1 Cor instrumental

O uso das biomassas de *Rubrivivax gelatinosus* e de *Spirulina platensis* aumentou a intensidade de vermelho dos filés.

4.2.2 Concentração de carotenoides

O uso das biomassas de *Rubrivivax gelatinosus*, *Spirulina platensis* e *Saccharomyces cerevisiae* (0,5%) aumentou a concentração de carotenoides dos filés.

4.2.3 Textura

O uso das biomassas microbianas não influenciou a textura dos filés.

4.2.4 Composição proximal

O uso das biomassas microbianas nas rações aumentou o teor proteico dos filés.

4.2.5 Perfil de ácidos graxos

As biomassas *Rubrivivax gelatinosus* (0,25%), *Saccharomyces cerevisiae* (0,25%) e *Spirulina platensis* (0,5%) aumentaram a concentração de ácido eicosapentaenoico (EPA) dos filés. A alimentação dos peixes com as biomassas microbianas melhorou a relação de ácidos graxos n-6 / n-3.

4.2.6 Oxidação lipídica

Os filés dos peixes alimentados com as biomassas microbianas sofreram menor oxidação lipídica com até 60 dias de armazenamento.

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CAPÍTULO 2 – MICROBIAL BIOMASS AS AN ANTIOXIDANT FOR TILAPIA FEED

PERIÓDICO: AQUACULTURE RESEARCH (PUBLISHED)

Abstract

Microbial biomass (MB) produced by different industries is thought to be a beneficial supplement in fish feed due to high contents of antioxidants and pigments. However, little is known about their impact on fish health. In this experiment, 960 tilapia (26.84 ± 1.03 g) were fed one of eight experimental diets - a control diet with no MB (C), a control diet with vitamin E (VE), and six diets with three types of MB at two concentrations (0.25% and 0.5%): *Rubrivivax gelatinosus* (RG25 and RG50), *Spirulina platensis* (SP25 and SP50) and *Saccharomyces cerevisiae* (SC25 and SC50). Adding MB to diets decreased plasma total oxidant status, malonaldehyde and leukocyte respiratory burst, increased the total antioxidant status and did not affect the blood biochemical parameters. In flesh, the use of the MB lowered the thiobarbituric acid reactive substances and increased redness (except for SC) and carotenoid deposition (except SC25). So, it was concluded that the use of the MB provided an antioxidant effect in tilapia blood plasma, decreased lipid oxidation and increased pigmentation and carotenoid deposition in the fish flesh, without imparting a negative impact on the animals` health.

Key word: biochemical analysis; carotenoids; color; leukocytes respiratory burst; lipid oxidation; TBARS.

1. Introduction

Microbial biomass (MB) generated by biotechnological processes can be used to supplement fish feed (GRASSI et al., 2016; JAIME-CEBALLOS et al., 2006; REQUE et al., 2010), which helps to reduce costs and lessen environmental impacts, but few studies have considered the antioxidant potential of MB in tilapia (HERATH et al., 2016; LARA-FLORES et al., 2003). Two important MB with antioxidant effects currently produced in large quantities include *Saccharomyces cerevisiae*, a yeast used to elaborate many products (e.g., ethanol production) and that is normally discarded, and *Spirulina* (*Arthrospira*) *platensis*, a blue-green microalgae that can be produced in wastewater (CHANG et al., 2013). In addition, a relatively new candidate for fish is *Rubrivivax gelatinosus* (previously named *Rhodocyclus gelatinosus)*, a phototrophic bacterium produced in pasteurized wastewaters of fish slaughterhouse (LIMA et al., 2011; PONSANO et al., 2003) that has already been used for other animals raising (PONSANO et al., 2002, 2004).

Dietary antioxidants as the included in the above MB (e.g., carotenoids) help to maintain an equilibrium in the formation and control of reactive oxygen species, which in turn help improve growth performance, resistance to stress and disease and survival of cultivated fish and shrimp (HAMRE et al., 2004). Adding MB supplements with a high antioxidant potential to fish feeds can also help to substitute synthetic products (e.g., BHT and vitamin E, the most used in tilapia feed; see BHOSALE, 2004; BHOSALE; BERNSTEIN, 2005; STAHL; SIES, 2003), which may pose health risks for fish and consumers (ANESINI et al., 2006; HOU, 2003; PRIOR, 2004). More specifically, *Spirulina platensis* is regarded as a cheap and rich source of antioxidant pigments such as carotenoids and phycocianin (JAIME-CEBALLOS et al., 2006; LI et al., 2003; MORIST et al., 2001), with effects on humoral and cellular immunity (TAKEUCHI et al., 2002). *Saccharomyces cerevisiae* is a rich source of antioxidants such as vitamins and peptides, which increases growth, stimulates the innate immune system and improves fillet quality (RAN et al., 2015; REQUE et al., 2010; RODRIGUEZ et al.,

2003). *Rubrivivax gelatinosus* contains oxycarotenoids (xanthophylls), a group of carotenoids with antioxidant potential that also provide pigmentation through selective deposition on different animal tissues (BAKER et al., 2002; PONSANO et al., 2008). According to Grassi et al. (2016), using up to 0.14% *R. gelatinosus* in tilapia feed increases protein retention and redness on the fillets, but less is known about the potential effects of higher supplementation on fish health.

A high level of antioxidants in blood, indicated by high plasma total antioxidant status (TAS) and low plasma total oxidant status (TOS), as well as flesh with low TBARS (2-thiobarbituric acid reactive substances), can help to increase tilapia health and preserve product quality during processing and storage, inhibiting lipid degradation by oxidation and thus increasing shelf-life (CHEAH et al., 1995). Lipid oxidation is the most important chemical alteration of fish flesh post-mortem, leading to changes in odor, color, texture, nutritional value and eventually producing toxic compounds (BAKER, 2001; NUNES et al., 2007). In muscles, lipids are oxidized by chemical compounds or reactive oxygen species that break down the double bonds of phospholipidic fractions of cell membranes. Fish lipids are more susceptible to that reaction because of their high degree of unsaturation (RUFF et al., 2004). In this study, we used microbial biomasses with antioxidant potential in fish feeding to evaluate their effects on blood antioxidant activity, plasmatic biochemistry and flesh quality parameters.

2. Material and methods

2.1. Experimental design, animals and blood and flesh sampling

The procedures cited herein were approved by the Animal Ethics Committee, UNESP (CEUA/FOA 2016/00407). It was adopted a completely randomized design with 8 treatments and 3 replicates, totalizing 960 Nile tilapias (*Oreochromis niloticus*). Fish averaged 26.84 ± 1.03 g and were distributed into 24 tanks (1000 L) (40 fish/tank, 120 fish per treatment), in a water recirculation

system. Experimental diets (n=8, Table 1) were provided three times a day, *ad libitum*, for 76 days. Performance data (initial weight, final weight, weight gain, feed consumption, specific growth rate) were recorded along the trial.

Treatments included a control diet (C), a control diet supplemented with 0.01% vitamin E (VE), *R. gelatinosus* at 0.25% (RG25) or 0.5% (RG50), *S. cerevisiae* at 0.25% (SC25) or 0.5% (SC50), and *S. platensis* at 0.25% (SP25) or 0.5% (SP50). Vitamin E (Microvit® E Promix 50) was set at 0.01% as usual for commercial fish raising. In order to allow the inclusion of vitamin E and biomasses in the experimental diets, the following ingredients were partially substituted: ground corn (vitamin E), soybean meal (*S. cerevisiae* and *S. platensis* biomasses) and meat meal (*R. gelatinosus* biomass). These changes were necessary to make the rations isoproteic and isoenergetic. The calculated and analysed values of the nutrients in the diets are presented in the Table 1. *R. gelatinosus* levels adopted were based on a previous experiment with the biomass in which Santo et al. (2016) reported that its use in fish feed at 0.14% provided an antioxidant effect on the flesh. So, for the present experiment, we assayed slightly higher levels (0.25 and 0.5%) of that biomass and extended the same concentrations for *S. cerevisiae* and *S. platensis.* The biomasses contained live cells of the microrganisms and had the following proximal composition: 4% moisture, 46% protein, 17% lipid and 5% ash for *R. gelatinosus*; 4% moisture, 45% protein, 0.2% lipid and 5% ash for *S. cerevisiae* and 6% moisture, 46% protein, 3% lipid and 8% ash for *S. platensis*.

Sediments deposited on the bottom of the tanks were removed by siphoning once a week, and the water quality was monitored twice a week in each tank (temperature 27.0 ± 0.4 °C, pH 7.0 ± 0.1, DO 12.0 ± 1.0 mg/L, nitrite 0.0016 ± 0.0002 ppm, NH_3 0.0038 ± 0.0002, chloride 0.001 ± 0.0002). No mortality was observed during the experiment. At the end of the trial, nine fish from each treatment were anesthetized with benzocaine solution at 0.1 g/L (pH 7) and 0.5-1 mL of blood was collected from the caudal veins in tubes containing sodium

heparin. After 24 h of fasting, the fish were placed on ice and slaughtered by sectioning the gills, and the fillets were removed for the laboratory analyses.

2.2. Health parameters

Plasma biochemical analyses were performed in an automated spectrophotometer (BS 200, Shenzhen Mindray Bio-Medical Electronics Co., Nanshan, China) previously calibrated with a calibrator and control reagents (Biosystems, Barcelona, Spain). The levels of the following analytes were determined: total protein (biuret method), albumin (bromocresol green method), globulin ([total protein – albumin]), alanine aminotransferase (ALT, IFCC kinetic method), aspartate aminotransferase (AST, IFCC kinetic method), γ glutamyltranspeptidase (GGT, enzymatic UV urease/glutamate dehydrogenase method), alkaline phosphatase (ALP, diethanolamine method), uric acid (uricase/peroxidase method), creatinine (alkaline picrate kinetic method) and creatine kinase (CK, creatine phosphate method). All the biochemical reactions were performed at 37°C, following the manufacturer's protocol. The leukocyte respiratory burst was assayed with total blood optical density at 540 nm following Biller-Takahashi et al. (2013) methodology, in which the reduction of nitroblue tetrazolium (NBT) is measured according to the production of reactive oxygen species (ROS).

2.3. Oxidative stress of the blood

Plasma total antioxidant status (TAS) and plasma total oxidant status (TOS) were determined colorimetrically by the reduction of the ABTS cation (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid), expressed as mmol Trolox equivalent/L and by the oxidation of the iron ion, expressed as μmol hydrogen peroxide equivalent/L, respectively (EREL, 2004, 2005). Lipid peroxidation was quantified as thiobarbituric acid-reactive substancies (TBARS) according to Hunter et al. (1985) and expressed as μmol malonaldehyde (MA).

2.4. Lipid oxidation

Ten g of fish fillets were homogenized with 50 ml of trichloroacetic acid 7.5% in turrax, during 1 min. Then the mixture was filtered and 5 ml of the extract were transferred to a tube containing 5 ml thiobarbituric acid 0.02 M. The tubes were heated in a boiling water bath for 40 min and cooled in running water for 10 min, for the measurement of the 2-thiobarbituric acid reactive substances (TBARS) at 538 nm. The values were expressed as mg malonaldehyde/kg. The analyses were performed immediately after slaughter and repeated after 30 and 60 days of storage at - 30 °C.

2.5. Color attributes and ΔE

The CIE colors of fish fillets (L - lightness, a* - redness, b* - yellowness) were obtained in triplicate by using a portable MiniScan XE Plus colorimeter (Hunterlab) previously calibrated with white and black standards (10° observer angle, illuminant D65). Measurements were taken on the inner portion of the muscle above the lateral line, at three points (head, middle and tail), and the mean values were considered (CHOUBERT et al., 1997). ΔE was used to classify the color difference between treatments in categories (DEUTSCHE INSTITUT FÜR NORMUNG, 1979) – distinguishable (1.5 - 3.0), difficult to distinguish (< 1.5) and easily distinguishable (> 3.0) - and was measured as:

$$\Delta E = \sqrt{(\Delta L^2) + (\Delta a^2) + (\Delta b^2)}$$

2.6. Carotenoid content

Prior to the carotenoid extraction, the fish fillets were freeze-dried at -35 °C for 48 h and ground to powder. Then, samples of the lyophilized fillets were vortexed with dimethyl sulfoxide and sonicated for 15 min at 40 °C. The extraction was performed repeatedly with acetone. The phase separation was attained with diethyl ether and distilled water, and the supernatant was dried with N_2. The extract was suspended in acetone for the quantification of total carotenoids were at 475 nm which were calculated using 2500 as the coefficient of extinction. The analyses were performed according to Grassi et al. (2016).

2.7. Statistical analysis

No interaction between the times of storage and the diets was detect regarding to lipid oxidation of fillets. All data were subjected to analysis of variance, and the significant differences between means were checked with Tukey's test ($P<0.05$). Broken line regression was used to predict the relationship between redness and carotenoid content on the fillets. The significance level was set at 0.05.

3. Results

The results suggest that supplementing fish feed with MB increased the level of antioxidants in blood and fillets and the flesh color. Except for the respiratory activity of leukocytes, health parameters were similar among all treatments, indicating that MB supplementation did not impart detrimental effects on renal or hepatic functions and did not pose a health risk for the tilapia. As the initial weigth, final weigth, weigth gain, specific growth rates and feed consumption were the same among the treatments (Table 2), the results presented herein were ascribed to the different diets composition.

3.1. Health parameters

The respiratory activity of leukocytes was lower when the tilapias were supplemented with MB, as indicated by lower levels of NBT (Table 3). Plasma biochemical analysis showed no significant differences among the groups (Table 3).

3.2. Oxidative stress of blood

TAS and TBARS of MB groups were significantly higher and lower (respectively) than control group (Table 3) with no significant differences among

them. Except for *S. cerevisiae*, all the other treatments had significantly lower TOS than the control. The lowest TOS values were found in VE, RG50 and SP50.

3.3. Lipid oxidation

Fillets from fish fed MB had a lower lipid oxidation than those fed the control diet, at all times evaluated (Table 4). Among the treatments, there was only a difference between SC25 and SP50 after 60 days of storage, while no significant differences were observed at the other times.

3.4. Color attributes, carotenoid content and ΔE

Except for *S. cerevisiae* and vitamin E feeds, the color analysis showed significant differences in the redness between MB diets and the control (Table 5). The highest redness was due to the greater inclusion of *R. gelatinosus,* which differed from all the other treatments, except for the lower inclusion of *R. gelatinosus*. L* and b* values showed no significant differences among the groups (Table 5). Treatments with *R. gelatinosus* provided the highest contents of carotenoids in the fillets, followed by the treatments with *S. platensis* (Table 5). Except for SC25, the MB diets showed distinguishable or easily distinguishable ΔE, compared to the control (Table 6). The broken line regression analysis showed that the highest redness (7.27) can be reached when 11.08 mg/kg carotenoids are deposited in the fillets (Figure 1).

4. Discussion

4.1. Health parameters

Neutrophils and macrophages are phagocytic cells that generate unpaired oxygen molecules (free radicals) in the presence of antigens (DÜGENCI et al., 2003), causing increases in the NBT measurement. In our study, none of the

treatments implied a challenge (no antigens); however, NBT levels in MB tilapia were lower than in control, indicating either that the production of free radicals was decreased or that the antioxidant effect of the biomasses neutralized the free radicals produced by the leucocytes (reducing oxygen radicals and nitrous oxide). So, it is feasible to say that the carotenoids in *Rubrivivax* and *Spirulina* (JAIME-CEBALLOS et al., 2006; PONSANO et al., 2008) and the vitamins in *Saccharomyces* (RAN et al., 2015) neutralized the free radicals responsible for the reduction of NBT into formazan granules. Free radicals are toxic to microorganisms and host cells since their action is usually unspecific, therefore they need to be controlled or neutralized in the absence of antigens to protect host cells. Gallani et al. (2017) reported a decreased respiratory activity of leukocytes in pacus (*Piaractus mesopotamicus*) fed *R. gelatinosus* biomass. According to Sakai (1999), several components of microorganisms have immunomodulatory activity, which influence the leukocyte respiratory burst. Some authors found enhancement effects on the non-specific immune response of tilapia fed yeast nucleotides (XU et al., 2015) and yeast culture (RAN et al., 2015; ZHOU et al., 2009).

4.2. Oxidative stress of blood

The high TAS and low TOS in the blood of tilapia fed MB demonstrate that the antioxidants of the diets were absorbed and influenced the oxidative metabolism. Since dietary antioxidants have been shown to help fight against oxidative stress and immune pathologies (BABIN et al., 2015), additives containing carotenoids and vitamins may have positive effects on immunity, acting as a powerful antioxidant and potentially reducing the toxic effects of reactive oxygen species (KIM et al., 2011).

One of the end products of lipid peroxidation is malonaldehyde (MA). Metwally (2009) found that decreasing levels of MA in tilapia given garlic (rich in antioxidants) as compared to a control diet. Antioxidant compounds are thought to inhibit lipoxygenase enzymes, thereby increasing antioxidant capacity and

decreasing MA (SCHULZ et al., 2004). Along those lines, Abdelkhalek et al. (2015) found that MA levels decreased in liver, kidney and brachia of tilapia fed *S. platensis* and exposed to subacute deltamethrin intoxication, suggesting that *S. platensis* prevents membrane lesions in those organs. Xu et al. (2015) found lower MA level in the liver of juvenile hybrid tilapia fed dietary yeast nucleotides (0.6 and 1.2%), so indicating the enhancement of the antioxidant status.

4.3. Lipid oxidation

Lipid oxidation in the MB fillets was delayed compared to control, at all sampling times, demonstrating the effectiveness of the antioxidant components. The only difference occurred between SC25 and SP50 after 60 days of storage. Santo et al. (2016) reported a lower TBARS in the fillets of tilapia fed 0.14% *R. gelatinosus* biomass after 20 days of storage. In our experiment we also found a lower TBARS in RG25 and RG50 but starting from day 0, probably because our supplementation levels were higher. Those results were confirmed by the significantly lower levels of TBARS in the blood of tilapia fed *R. gelatinosus*, along with the high TAS and low TOS, which suggests the existence of an antioxidant effect *in vivo* that remained post-mortem. According to Khan et al. (2005), in addition to being a source of protein, *S. platensis* holds antioxidant and immunomodulatory effects (phycocyanin and allophycocyanin), as evidenced by the results of lipid oxidation and NBT, respectively. *S. cerevisiae* also delayed lipid oxidation, suggesting the presence of antioxidants, although they still lack identification. So, it can be said that MB supplementation delayed sensory alterations and the production of substances that pose a risk to human health (aldehydes, ketones, esters, hydrocarbons and other compounds), thereby prolonging the shelf life of fillets, and bringing benefits to consumers and the fish processing industry (ORDOÑEZ, 2005).

4.4. Color attributes, carotenoids content and ΔE

Except for *S. cerevisiae*, MB supplementation increased redness on the fillets, due to their supply of carotenoids. That was confirmed by the polynomial

regression analysis (Fig. 1), which assigns 88.4% of the increase of the fillets redness to the increase of the carotenoids deposited in the muscles. The pigmentation effect of *R. gelatinosus* oxycarotenoids (spiriloxanthin, spheroidene and OH-spheroidene) was previously described by Grassi et al. (2016), who reported mean redness (a*) of 2.55 in the fillets of tilapia fed 0.14% of that biomass. So, it can be said that increasing the level of *R. gelatinosus* biomass in the diets also increases redness.

Thus, we believe that *R. gelatinosus* biomass should be tested in salmonids as a natural alternative to synthetic pigments (such as astaxanthin and canthaxanthin), which are more costly and, according to TEIMOURI et al. (2013) and PONCE-PALAFOX et al. (2004), may have negative effects on human health.

Pigmentation effects were also previously assigned to the presence of carotenoids and phycocyanin in *S. platensis* (JAIME-CEBALLOS et al., 2006). As found in this study, Teimouri et al. (2013) also reported higher redness in trout fillets fed that biomass. Nevertheless, as *S. cerevisiae* contains small amounts of carotenoids, no differences were detected for the redness of the SC fillets, when compared to control. According to the DIN 6174 (DEUTSCHE INSTITUT FÜR NORMUNG, 1979), ΔE is used to detect the color perception by consumers and, in our study, its measurements revealed that the fillets colors were easily distinguishable among MB treatments and control (except for SC25).

4.5. General considerations

The utilization of tilapia in this study was justified for the widespread cultivation of this species all over the world. In Brazil, it is fully adapted to the climate (location of experiment) and is the most cultivated species. However, we believe that more experiments should be conducted with other species with the aim of checking whether the positive results found in this study could be extended to others, such as salmonids and shrimps.

5. Conclusion

From the results found in our study, we concluded that adding microbial biomasses to tilapia diets provided antioxidant effects on blood plasma and flesh and increased pigmentation (except for *S. cerevisiae*) and carotenoid deposition (except for *S. cerevisiae* at 0.25%) in the fillets, without imparting a negative impact on the fish health.

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TABLE 1
Basal diet for tilapias.

Ingredients (%)	C	VE	RG25	RG50	SC25	SC50	SP25	SP50
Feather meal	2	2	2	2	2	2	2	2
Ground corn	33.06	33.05	33.06	33.06	33.06	33.06	33.06	33.06
Poultry meal by-products	6	6	6	6	6	6	6	6
Soybean meal	10.44	10.44	10.44	10.44	10.19	9.94	10.19	9.94
Wheat meal	15.88	15.88	15.88	15.88	15.88	15.88	15.88	15.88
Meat meal	17.14	17.14	16.89	16.64	17.14	17.14	17.14	17.14
Blood meal	12	12	12	12	12	12	12	12
NaCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Chicken oil	2	2	2	2	2	2	2	2
Premix[a]	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Methionine	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Vitamin E	0	0.01	0	0	0	0	0	0
R. gelatinosus	0	0	0.25	0.5	0	0	0	0
S. cerevisiae	0	0	0	0	0.25	0.5	0	0
S. platensis	0	0	0	0	0	0	0.25	0.5
Total	100	100	100	100	100	100	100	100
Nutrients/Energy – calculated values								
Digestible energy (kcal/kg)	3000	3000	3000	3000	3000	3000	3000	3000
Digestible protein (%)	27.63	27.63	27.63	27.63	27.63	27.63	27.63	27.63
Ether extract (%)	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Crude fiber (%)	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral composition (%)	10.48	10.48	10.48	10.48	10.48	10.48	10.48	10.48
Total calcium (%)	2.74	2.74	2.74	2.74	2.74	2.74	2.74	2.74
Total phosphorus (%)	1.57	1.57	1.57	1.57	1.57	1.57	1.57	1.57
Starch (%)	27.03	27.03	27.03	27.03	27.03	27.03	27.03	27.03
Available phosphorus (%)	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Arginine (%)	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Lysine (%)	2.33	2.33	2.33	2.33	2.33	2.33	2.33	2.33
Threonine (%)	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Tryptophan (%)	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Methionine (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Vitamin C (mg/kg)	700	700	700	700	700	700	700	700
Nutrients – analysed values (%)								
Protein	32.29	32.18	32.26	32.39	32.26	32.22	32.41	32.34
Ether extract	8.11	8.15	8.32	8.25	8.14	8.11	8.18	8.09
Ash	9.79	9.84	9.91	9.77	9.68	9.82	9.69	9.84
Crude fiber	3.62	3.65	3.62	3.68	3.57	3.72	3.62	3.59
Moisture	10.75	10.63	10.79	10.82	10.65	10.85	10.93	10.86

C - control diet, VE - control diet supplemented with 0.01% vitamin E, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. [a] Composition per kg of product - Choline 83,333 mg, vitamin A 2,083 UI, vitamin D3 500 UI, vitamin K3 2,500 mg, vitamin B1 4,167 mg, vitamin B2 4,167 mg, vitamin B6 3,333 mg, vitamin B12 7,500 μg, niacin 12,500 mg, calcium pantothenate 8,152 mg, pantotenic acid 7,500 mg, folic acid 833 mg, biotin 125 mg, vitamin C 116,667 mg, inositol 12,500 mg, iron specific source 12,500 mg, Fe 12,500 mg, Cu 2,500 mg, Mn 7,500 mg, Zn 16,667 mg, Co 42 mg, I 125 mg, Se 67 mg.

TABLE 2

Means (± SD) of growth of tilapias fed microbial biomasses.

	Treatment									
	C	VE	RG25	RG50	SC25	SC50	SP25	SP50	P	CV (%)
Initial weigth (g)	28.25 ± 0.40	26.48 ± 2.04	27.04 ± 1.50	27.42 ± 1.36	26.23 ± 1.31	26.52 ± 1.54	25.04 ± 1.03	27.36 ± 1.40	0.2609	5.53
Final weigth (g)	229.29 ± 7.25	219.20 ± 18.38	229.98 ± 9.70	244.09 ± 10.61	229.13 ± 10.21	228.32 ± 15.31	233.45 ± 8.59	233.03 ± 6.06	0.4098	5.03
Weigth gain (g)	201.04 ± 7.34	192.72 ± 18.40	202.95 ± 9.83	216.66 ± 10.74	202.90 ± 10.45	201.80 ± 13.88	208.41 ± 8.80	205.66 ± 5.04	0.4050	5.59
Specif growth rates (g/day)	2.65 ± 0.10	2.54 ± 0.24	2.67 ± 0.13	2.85 ± 0.14	2.67 ± 0.14	2.66 ± 0.18	2.74 ± 0.12	2.71 ± 0.07	0.4050	5.59
Feed consuption (g)	260.49 ± 5.54	241.66 ± 26.15	255.98 ± 15.45	253.57 ± 16.56	249.58 ± 12.73	248.17 ± 9.94	259.53 ± 3.71	254.66 ± 3.12	0.7306	5.11

C - control diet, VE - control diet supplemented with 0.01% vitamin E, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. *CV* - coefficient of variation.

TABLE 3
Means (± SD) of the blood plasma analyses of tilapias fed the experimental diets.

	Treatments								*P*	*CV (%)*
	C	VE	RG25	RG50	SC25	SC50	SP25	SP50		
TAS (mmol/L)	0.76 ± 0.03^{b}	0.91 ± 0.06^{a}	0.90 ± 0.03^{a}	0.90 ± 0.03^{a}	0.92 ± 0.03^{a}	0.91 ± 0.04^{a}	0.92 ± 0.05^{a}	0.89 ± 0.03^{a}	0.0019	5.97
TOS (μmol/L)	62.23 ± 1.13^{a}	39.98 ± 4.79^{c}	46.43 ± 3.48^{bc}	38.82 ± 4.97^{c}	56.18 ± 2.29^{ab}	54.13 ± 1.03^{ab}	50.69 ± 4.30^{b}	48.47 ± 4.63^{bc}	< 0.0001	16.06
NBT (Abs)	0.52 ± 0.03^{a}	0.17 ± 0.01^{c}	0.28 ± 0.06^{bc}	0.32 ± 0.05^{bc}	0.22 ± 0.05^{bc}	0.26 ± 0.04^{bc}	0.25 ± 0.13^{bc}	0.34 ± 0.04^{b}	0.0001	35.78
TBARS (μmol/L)	36.97 ± 0.37^{a}	24.46 ± 0.61^{b}	25.96 ± 0.38^{b}	24.72 ± 1.03^{b}	26.68 ± 0.91^{b}	24.46 ± 1.11^{b}	25.04 ± 1.20^{b}	26.15 ± 0.67^{b}	< 0.0001	15.63
Albumin (g/L)	13.70 ± 0.73	13.81 ± 2.26	13.88 ± 1.08	13.78 ± 0.97	14.05 ± 0.95	13.33 ± 2.00	13.75 ± 0.46	13.63 ± 0.59	0.9988	1.53
Globulin (g/L)	22.19 ± 0.66	19.94 ± 1.57	20.07 ± 0.29	20.30 ± 1.07	21.11 ± 0.22	19.84 ± 0.33	21.42 ± 0.44	20.23 ± 2.12	0.1376	4.07
Total protein (g/L)	35.89 ± 1.34	33.75 ± 3.77	33.95 ± 1.17	34.08 ± 1.11	35.16 ± 0.84	33.17 ± 2.27	35.17 ± 0.33	33.80 ± 2.31	0.6859	2.68
ALT (UI/L)	42.34 ± 18.92	29.36 ± 6.01	55.29 ± 33.41	46.68 ± 11.82	30.96 ± 6.09	46.67 ± 15.59	50.02 ± 17.68	41.71 ± 23.80	0.684	20.87
AST (UI/L)	140.62 ± 43.08	88.07 ± 34.36	143.17 ± 65.01	108.57 ± 32.79	140.94 ± 26.84	119.81 ± 18.41	143.50 ± 58.59	110.94 ± 86.46	0.8131	16.71
GGT (UI/L)	0.63 ± 0.21	0.50 ± 0.12	0.26 ± 0.16	0.35 ± 0.18	0.32 ± 0.11	0.51 ± 0.20	0.32 ± 0.08	0.33 ± 0.11	0.0986	31.95
ALP (UI/L)	16.58 ± 3.03	18.82 ± 4.57	16.84 ± 7.85	17.47 ± 3.94	19.62 ± 3.23	23.51 ± 5.14	14.53 ± 3.70	20.02 ± 1.75	0.4047	14.79
Uric acid (mg/dL)	0.87 ± 0.11	0.75 ± 0.14	0.75 ± 0.14	0.78 ± 0.20	0.83 ± 0.16	0.66 ± 0.07	0.78 ± 0.03	0.65 ± 0.08	0.4169	10
Creatinine (mg/dL)	0.13 ± 0.05	0.12 ± 0.04	0.14 ± 0.04	0.11 ± 0.03	0.12 ± 0.01	0.12 ± 0.08	0.11 ± 0.07	0.11 ± 0.02	0.9929	8.71
CK (UI/L)	4256.89 ± 260.11	3809.10 ± 1252.67	4371.54 ± 1106.80	3329.64 ± 1008.82	4193.27 ± 1212.44	4135.00 ± 202.21	3974.91 ± 695.62	2083.99 ± 1376.01	0.1647	20.03

C - control diet, VE - control diet supplemented with 0.01% vitamin E, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. *CV* - coefficient of variation; ^{abc} Means followed by different letters are significantly different (P<0.05).

TABLE 4

Means (± SD) of the lipid oxidation (mg malonaldehyde/kg) in the fillets of tilapias fed the experimental diets.

	Treatments								*P*	*CV (%)*
Day	C	VE	RG25	RG50	SC25	SC50	SP25	SP50		
0	0.057 ± 0.004^{a}	0.028 ± 0.015^{b}	0.019 ± 0.008^{b}	0.017 ± 0.002^{b}	0.020 ± 0.005^{b}	0.026 ± 0.007^{b}	0.024 ± 0.009^{b}	0.021 ± 0.003^{b}	0.0003	48.53
30	0.061 ± 0.005^{a}	0.032 ± 0.011^{b}	0.032 ± 0.008^{b}	0.029 ± 0.003^{b}	0.027 ± 0.004^{b}	0.035 ± 0.002^{b}	0.037 ± 0.014^{b}	0.025 ± 0.004^{b}	0.0007	32.58
60	0.085 ± 0.001^{a}	0.054 ± 0.002^{bc}	0.054 ± 0.003^{bc}	0.053 ± 0.005^{bc}	0.057 ± 0.005^{b}	0.048 ± 0.004^{bc}	0.050 ± 0.004^{bc}	0.047 ± 0.001^{c}	< 0.0001	21.77

C - control diet, VE - control diet supplemented with 0.01% vitamin E, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. *CV* - coefficient of variation; ^{abc} Means followed by different letters are significantly different (P<0.05).

TABLE 5
Means (± SD) of color attributes and concentration of carotenoids in the fillets of tilapias fed the experimental diets.

	Treatments								*P*	*CV (%)*
	C	VE	RG25	RG50	SC25	SC50	SP25	SP50		
Lightness (L)	57.33 ± 0.54	57.03 ± 1.50	57.36 ± 1.86	60.41 ± 1.71	57.87 ± 1.10	57.80 ± 0.34	58.36 ± 2.12	59.83 ± 2.19	0.1423	3.02
Redness (a*)	4.25 ± 0.53^{d}	5.43 ± 0.35^{cd}	6.97 ± 0.25^{ab}	7.27 ± 0.42^{a}	4.85 ± 0.40^{cd}	5.34 ± 0.28^{cd}	5.61 ± 0.42^{c}	5.93 ± 0.85^{bc}	< 0.0001	18.26
Yellowness (b*)	11.95 ± 0.79	11.43 ± 0.42	11.64 ± 0.78	11.98 ± 0.63	12.46 ± 0.84	12.88 ± 1.10	13.52 ± 1.84	13.01 ± 1.38	0.2595	9.13
Carotenoids (mg/kg)	2.66 ± 0.41^{e}	4.21 ± 1.17^{de}	8.70 ± 1.40^{b}	13.27 ± 1.00^{a}	3.46 ± 0.61^{de}	5.57 ± 0.94^{cd}	7.56 ± 1.07^{bc}	7.63 ± 0.89^{bc}	< 0.0001	51.13

C - control diet, VE - control diet supplemented with 0.01% vitamin E, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. *CV* - coefficient of variation; ^{abcde} Means followed by different letters are significantly different (P<0.05).

TABLE 6
Human perception for the colors differences on tilapia fillets.

ΔE		
Difficult to distinguish (< 1.5)	Distinguishable (1.5 – 3.0)	Easily distinguishable (> 3.0)
C – VE (1.49)	C - SC50 (1.51)	C - RG25 (3.04)
C - SC25 (0.95)	C - SP25 (2.22)	C - RG50 (4.11)
SC25 - SC50 (0.65)	VE - RG25 (1.71)	C - SP50 (3.19)
SC25 - SP25 (1.30)	VE - SC25 (1.53)	VE - RG50 (3.68)
SC50 - SP25 (0.86)	VE - SC50 (1.66)	VE - SP50 (3.23)
	VE - SP25 (2.48)	RG25 - RG50 (3.08)
	RG25 - SC25 (2.61)	RG25 -SP50 (3.13)
	RG25 - SC50 (2.34)	RG50 -SC25 (3.34)
	RG25 - SP25 (2.81)	RG50 - SC50 (3.21)
	RG50 - SP25 (2.99)	
	RG50 - SP50 (1.57)	
	SC25 - SP50 (2.30)	
	SC50 - SP50 (2.12)	
	SP25 - SP50 (1.63)	

C - control diet, VE - control diet supplemented with 0.01% vitamin E, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*.

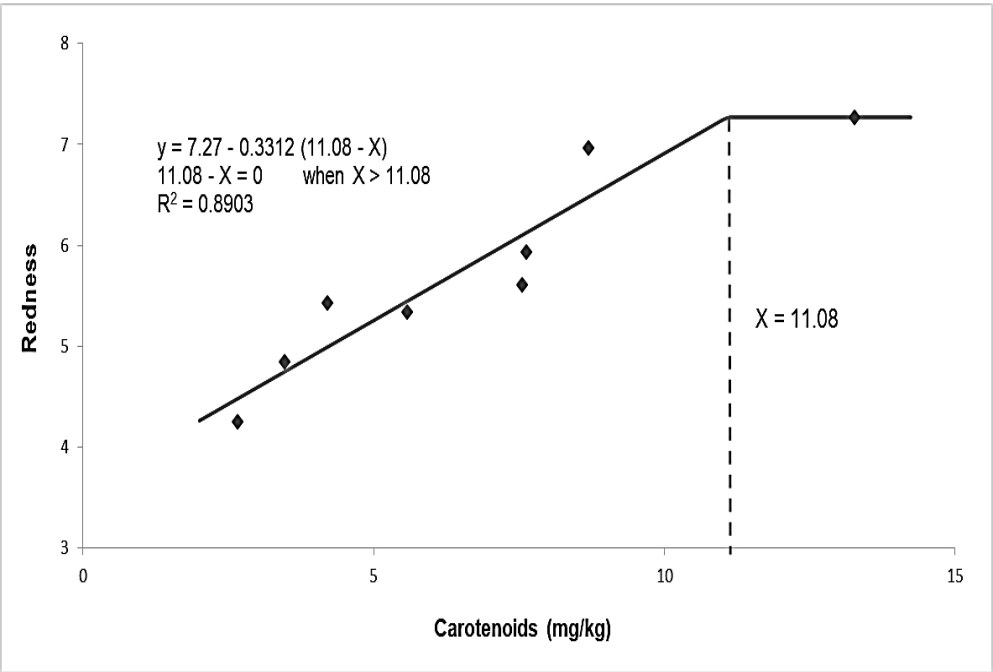

FIGURE 1 - Broken line plot of redness (a*) as a function of the carotenoid content in fillets of tilapias fed the experimental diets.

CAPÍTULO 3 – GROWTH, FATTY ACID COMPOSITION AND FLESH QUALITY OF TILAPIA FED MICROBIAL BIOMASS
PERIÓDICO: AQUACULTURE

Abstract

Microbial biomass (MB) produced with industrial residues can be used as sources of proteins and antioxidants in fish feeds. That results in reduced amounts of waste to treat and discard and so brings benefits to the industry and to the environment. In this experiment, 840 tilapia (26.84 ± 1.03 g) were fed one of seven experimental diets with different levels and types of MB: a control diet with no MB and three types of MB at two concentrations (0.25% and 0.5%); *Rubrivivax gelatinosus* (RG25 and RG50), *Saccharomyces cerevisiae* (SC25 and SC50) and *Spirulina platensis* (SP25 and SP50). The use of MB increased the protein content and improved the n-6/n-3 ratio of the fillets without interfering with the texture. RG25, SC25 and SP50 increased eicosapentaenoic acid on the fillets and RG50 had a positive effect on the animal's growth.

Key word: additives; protein; proximate composition; texture.

1. Introduction

The demand for animal protein for human consumption is currently on the rise and is mostly provided by terrestrial farm animals, although aquaculture shows up as an increasingly important alternative (EL-HAROUN et al., 2006). Global tilapia cultivation is growing quickly, with annual yields increasing 12% in recent years (JU et al., 2017; OGELLO et al., 2014). As a result, producers need high-quality feeds with the necessary nutrients and additives to keep fish healthy, promote favourable growth and improve flesh quality (EL-HAROUN et al., 2006).

A vast array of protein sources and additives have been evaluated for this purpose (ABDEL-TAWWAB, 2012; GRASSI et al., 2016; HERATH et al., 2016; RICHTER et al., 2003), however, the industrial by-products have received special attention in the last years (XU et al., 2007). Using industrial residues to produce the microbial biomass helps making the productive process more sustainable by reducing the costs of discarding waste and minimizing environmental damage.

Rubrivivax gelatinosus (previously called *Rhodocyclus gelatinosus)* is a phototrophic bacterium that grows readily in heat treated wastewaters of poultry and/or fish slaughterhouse, consuming organic compounds and producing a biomass high in protein and oxycarotenoids that have been used in animal feeds (LIMA et al., 2011; PONSANO et al., 2002, 2004). Microalgae are also a rich source of protein, essential fatty acids and antioxidants (JAIME-CEBALLOS et al., 2006; LI et al., 2003; MORIST et al., 2001). *Spirulina* (*Arthrospira*) *platensis*, a blue-green microalgae can be cultivated in municipal, agricultural or industrial wastewater (CHANG et al., 2013). Yeasts are another source of biomass with excellent nutrient profiles, since they are rich in protein, enzymes and fatty acids (REQUE et al., 2010; RODRIGUEZ et al., 2003), and relatively cheap to be produced. For example, *Saccharomyces cerevisiae*, a fungus used in the baking and ethanol industries (normally commercialized with ethanol co-product) has been used as a feed supplement for various animals, including fish (ABDEL-TAWWAB, 2012). Although microbial biomasses seem to be an economically

sound and sustainable ingredient for aquafeeds, few of them have been tested in growth trials and little is known about their effect on the fatty acid composition of fish and on the flesh quality, two aims of the current study.

2. Material and Methods

2.1. Experimental design, animals and flesh sampling

The procedures used in the research were approved by the Animal Ethics Committee, UNESP (CEUA/FOA 2016/00407). It was adopted a completely randomized design with 7 treatments and 3 replicates per treatment. Eight hundred and forty Nile tilapia (*Oreochromis niloticus*), averaging 26.84 g (SD = ± 1.03 g), were distributed among 21 tanks (1000 L) (40 fish/tank, 120 fish per treatment) linked by a water recirculation system. Experimental diets (n=7, Table 1) were provided *ad libitum* three times a day for 76 d and comprised a control diet (C) and six diets supplemented with different MB: *R. gelatinosus* at 0.25% (RG25) and 0.5% (RG50), *S. cerevisiae* at 0.25% (SC25) and 0.5% (SC50), and *S. platensis* at 0.25% (SP25) and 0.5% (SP50). *R. gelatinosus* biomass had 4% moisture, 46% protein, 17% lipid and 5% minerals; *S. cerevisiae* biomass had 4% moisture, 45% protein, 0.2% lipid and 5% minerals and *S. platensis* had 6% moisture, 46% protein, 3% lipid and 8% minerals. To allow the inclusion of biomasses in the experimental diets, the following ingredients were partially substituted: soybean meal (*S. cerevisiae* and *S. platensis* biomasses) and meat meal (*R. gelatinosus* biomass). These changes were necessary to make the rations isoproteic and isoenergetic. The calculated and analyzed values of the nutrients in the diets are presented in the Table 1. Grassi et al. (2016) described an increase in the protein levels on the fillets of tilapia fed until 0.14% *R. gelatinosus.* Therefore, we believed that larger biomass inclusions might cause larger protein increases, so we defined 0.25 and 0.5% as the inclusions levels of

the biomasses to be tested. The same concentrations were used for all MB, because of their similar protein content.

The sediments deposited on the bottom of the tanks were removed by siphoning once a week and the water quality was monitored twice a week (temperature 27 °C, pH 7, dissolved oxygen > 12 mg/L, nitrite < 0.002 ppm, NH_3 < 0.004 ppm, chloride < 0.001 ppm). No mortality was observed during the experiment. At the end of the trial, 9 fish from each treatment were emerged in ice and slaughtered by sectioning the gills, then the fillets and viscera were removed for the laboratory analyses and the visceral indexes assignment, respectively.

2.2. Growth performance and visceral indexes

All fish were weighed individually at the beginning and the end of the trial. Growth performance was assessed based on initial weight, final weight, weight gain, specific growth rate (SGR = weight gain / days of experiment), feed consumption and feed conversion ratio (FCR = feed consumption / weight gain). Liver, spleen and digestive tract (stomach, intestine, cecum, liver, gallbladder and pancreas without visceral fat) were weighed to calculate the hepatosomatic (HSI), splenosomatic (SSI) and digestivesomatic (DSI) indexes, respectively, as:

$$\text{Index (\%)} = \frac{\text{Viscera weight (g)}}{\text{Fish body weigth (g)}} \text{x } 100$$

2.3. pH, proximate and fatty acids composition

For the determination of the pH, 10 g of the fish muscles were homogenized using 100 mL of deionized water and measured with a pHmeter at 25 °C. Proximate chemical composition analyses of the fish fillets included

moisture (oven at 105 °C), crude protein (micro Kjeldahl) and total minerals (oven at 550 °C) and were performed according to Horwitz and Latimer Jr. (2006). Total lipids were determined according to Folch et al. (1957). The fatty acids were analyzed by capillary gas chromatography, according to Horwitz and Latimer Jr. (2006).

2.4. Texture

For the texture profile analyses (TPA), the compression test was performed on the central point of the fillets (crossing between the middle of craniocaudal sense and the middle of dorsoventral sense) and was carried out in a texture analyzer (TAXT2, Stable Micro Systems, Surrey, United Kingdom) coupled with a 25 mm diameter cylindrical stainless-steel probe. Samples were compressed twice at the following conditions: a pre-test speed of 2.0 mm/s, a test speed of 1.0 mm/s, a post-test speed of 2.0 mm/s and a compression of 50%. Data collected included hardness, springiness, cohesiveness, chewiness and resilience.

Shear force (SF) was measured on muscle samples (without skin) of approximately 1 cm high × 1 cm wide. The downward probe speed was 2 mm/s with a 5 g force trigger to commence recording. The samples were positioned such that the Warner Bratzler blade cut the sample block in half. The probe made a single cut before returning to the start position. The raw output from the texture analyzer was a force/distance curve that was transformed to force/area and expressed in g/cm^2.

2.5. Statistical analysis

All data collected were subjected to normality test followed by analysis of variance, and the significance of differences between means was tested by Tukey's test ($P<0.05$).

3. Results and Discussion

3.1. Growth performance and visceral indexes

All treatments had similar initial weight, final weight, weight gain, specific growth rates and feed consumption, except FCR that was lower for RG50 than for control group (Table 2). This result showed that tilapia fed RG50 took better advantage of the ration, reaching weight with less ration ingested. Grassi et al. (2016) found that adding 0.14% *R. gelatinosus* to tilapia feed had no effect on growth, which is similar to our results for RG25. However, the double level of *R. gelatinosus* had a positive effect on FCR, probably because of the high protein content and the probiotic potential of *R. gelatinosus*. The polynomial regression analysis showed that the increase of *R. gelatinosus* in the diets explained 78.72% of the decrease on FCR (Figure 1). Other authors also found that microbial biomass tends to reduce FCR in tilapia in the early stages of growth, such as Abdel-Tawwab (2012) who fed tilapia (initial average weight = 0.34 g) with 0.2% *S. cerevisiae* (35% crude protein) and Velasquez et al. (2016) who fed tilapia (initial average weight = 0.93 g) with 75% *S. platensis*. Nevertheless, at the present study, in which tilapia were fed the MB in later stages (juveniles with 26.84 ± 1.03 g), the results reached by *S. cerevisiae* and *S. platensis* were similar.

The visceral indexes were similar among the treatments (Table 2), implying that the MB did not cause negative effects on the organs growth. Herath et al. (2016) reported similar HSI values (2 to 3%) in tilapia fed corn co-products. Velasquez et al. (2016) reported similar HSI for tilapia fed up to 30% of *S. platensis* and control group and Asadi Rad et al. (2012) related the same for tilapias fed up to 2% of *S. cerevisiae*. Santo et al. (2016) reported no differences in the histological evaluation of liver, spleen and intestines of tilapias fed *R. gelatinosus*. DSI is an indicative of fish digestive capacity and it did not differ among the treatments.

3.2. pH, proximate and fatty acids composition

Flesh pH depends on many factors such as feeding protocols, pre-slaughter handling and storage (PACHECO-AGUILAR et al., 2000) but we did not find any significant differences in terms of MB treatments (Table 3). The fillets of fish fed MB had higher protein contents than control fillets ($P<0.05$) (Table 3) but no significant differences among MB treatments was detected. The increased protein content on the fillets of fish fed *S. platensis* can be explained by the increased protein synthesis and somatic growth caused by the biomass, as noted in other species such as red sea bream (MUSTAFA et al., 1994), African sharptooth (PROMYA; CHITMANAT, 2011) and Indian carp (NANDEESHA et al., 2001). Abdel-Tawwab and Ahmad (2009) reported protein values of 17.2 and 17.3% in tilapia carcasses fed *S. platensis* 0.25% and 0.5%, respectively, for 120 days. *S. cerevisiae* is also a rich protein source provided with probiotic effect, as widely described in the literature, which can favour the deposition of proteins in muscle (LARA-FLORES et al., 2003). Abdel-Tawwab (2012) found 14.8% protein in the flesh of Nile tilapia fed 0.5% *S. cerevisiae* for 12 weeks. The use of RG25 and RG50 also increased the protein contents of the fillets in 7.14 and 10.62%, respectively, as compared to the control group. According to Grassi et al. (2016), it is reasonable to believe that the biomass exerts some probiotic effect on the utilization of the nutritional components of the diets, which may have occurred in this experiment, although the elucidation for this mechanism was not the aim of the study. However, the higher inclusion levels of all MB tested indicated tendencies of increases in the fillets protein content and significant differences could possibly be found if higher levels of MB were included in the diets. The polynomial regression analysis showed that the increases of *R. gelatinosus*, *S. cerevisiae* and *S. platensis* in the diets explained 82.51, 77.83 and 86.67%, respectively, of the increases on the protein contents of the fillets (Figure 2). Although moisture, lipids and minerals in fillets are related to feeding, they were not significantly different among the groups (Table 3).

Dietary lipids affect the fatty acid composition of tilapias. In this study, RG25, SC25 and SP25 increased the levels of EPA. Although tilapias can bioconvert plant-oil-based alpha-linolenic fatty acids (ALA) to long-chain fatty

acids such as DHA and EPA (SARKER et al., 2016), Nile tilapias show a limited capacity for the *de novo* synthesis of these PUFA from ALA provided by the diet (KARAPANAGIOTIDIS et al., 2007). As the FA profile of MB diets was not revealed in this study, it is not possible to say whether the increase in EPA was due to deposition or to synthesis from ALA.

All treatments with MB lowered the ratio n-6/n-3 and these decreases were explained at 88.99, 94.3 and 88.97%, respectively, by the increases of *R. gelatinosus*, *S. cerevisiae* and *S. platensis* in the diets (Figure 3). Since the conversion of n-3 and n-6 fatty acids share the same series of enzymes, they act like competitors, making the excess of one to cause the decrease in the other (SCHMITZ; ECKER, 2008). High contents of n-3 fatty acids are desirable in food because they play an important role in the prevention of cardiovascular and inflammatory diseases and cancer in humans (BRASKY et al., 2010; SIRIWARDHANA et al., 2012). On the other hand, excessive consumption of n-6 fatty acids are associated with the prevalence of various chronic diseases (SIMOPOULOS, 2002). That is why recommendations for that ratio are prescribed, varying from 2:1 to 10:1 (MARTIN et al., 2006). In this study, all the treatments with MB reached n-6/n-3 within the ratios recommended by Simopoulus (2002), the most rigorous one (2:1 – 3:1).

3.3. Texture

Texture, evaluated by SF and TPA attributes (hardeness, springiness, cohesiveness, chewiness and resilience), was not significantly different among treatments (Table 5). According to Abbott et al. (1997), instrumental measurements of texture are preferred over sensory evaluations due to the reduced variation among measurements that may happen with humans. In the fish industry, the firmness of raw muscle is a critical parameter that determines the acceptability of fish products, with soft fillets being penalized by consumers (ASHTON et al., 2010; LIN et al., 2012). The firmness of fish fed MB (evaluated in terms of hardness and SF) was not different from the control. Wu et al. (2017)

reported TPA results similar to ours, with 0.54 to 0.57 for springiness, 0.43 to 0.50 for cohesiveness and 0.25 to 0.31 for resilience in tilapias fed different concentrations of vitamin E. The proximate composition and the physical structure of muscle can affect its texture properties (JONSSON et al., 2001), so, it is reasonable to believe that the higher protein content in the flesh of fish fed MB could alter some texture attributes, but that did not happen in this trial.

4. Conclusion

Feeding tilapia microbial biomass helped to increase the fillets protein content and improved the n-6/n-3 ratio, with no negative side-effects to the animals' growth and organ weights or to the flesh texture. Providing RG25, SC25 and SP50 increased eicosapentaenoic acid concentration in fillets and RG50 had a positive effect on growth, lowering the FCR.

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Table 1
Basal diet for tilapias.

Ingredients (%)	C	RG25	RG50	SC25	SC50	SP25	SP50
Feather meal	2	2	2	2	2	2	2
Ground corn	33.06	33.06	33.06	33.06	33.06	33.06	33.06
Poultry meal by-products	6	6	6	6	6	6	6
Soybean meal	10.44	10.44	10.44	10.19	9.94	10.19	9.94
Wheat meal	15.88	15.88	15.88	15.88	15.88	15.88	15.88
Meat meal	17.14	16.89	16.64	17.14	17.14	17.14	17.14
Blood meal	12	12	12	12	12	12	12
NaCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Chicken oil	2	2	2	2	2	2	2
Premix*	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Methionine	0.08	0.08	0.08	0.08	0.08	0.08	0.08
R. gelatinosus	0	0.25	0.5	0	0	0	0
S. cerevisiae	0	0	0	0.25	0.5	0	0
S. platensis	0	0	0	0	0	0.25	0.5
Total	100	100	100	100	100	100	100
Nutrients/Energy – calculated values							
Digestible energy (kcal/kg)	3000	3000	3000	3000	3000	3000	3000
Digestible protein (%)	27.63	27.63	27.63	27.63	27.63	27.63	27.63
Ether extract (%)	7.50	7.50	7.50	7.50	7.50	7.50	7.50
Crude fiber (%)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Mineral composition (%)	10.48	10.48	10.48	10.48	10.48	10.48	10.48
Total calcium (%)	2.74	2.74	2.74	2.74	2.74	2.74	2.74
Total phosphorus (%)	1.57	1.57	1.57	1.57	1.57	1.57	1.57
Starch (%)	27.03	27.03	27.03	27.03	27.03	27.03	27.03
Available phosphorus (%)	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Arginine (%)	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Lysine (%)	2.33	2.33	2.33	2.33	2.33	2.33	2.33
Threonine (%)	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Tryptophan (%)	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Methionine (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Vitamin C (mg/kg)	700	700	700	700	700	700	700
Nutrients – analysed values (%)							
Protein	32.29	32.26	32.39	32.26	32.22	32.41	32.34
Ether extract	8.11	8.32	8.25	8.14	8.11	8.18	8.09
Ash	9.79	9.91	9.77	9.68	9.82	9.69	9.84
Crude fiber	3.62	3.62	3.68	3.57	3.72	3.62	3.59
Moisture	10.75	10.79	10.82	10.65	10.85	10.93	10.86

C - control diet, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. *Composition per kg of product - Choline 83,333 mg, vitamin A 2,083 UI, vitamin D3 500 UI, vitamin K3 2,500 mg, vitamin B1 4,167 mg, vitamin B2 4,167 mg, vitamin B6 3,333 mg, vitamin B12 7,500 μg, niacin 12,500 mg, calcium pantothenate 8,152 mg, pantotenic acid 7,500 mg, folic acid 833 mg, biotin 125 mg, vitamin C 116,667 mg, inositol 12,500 mg, iron specific source 12,500 mg, Fe 12,500 mg, Cu 2,500 mg, Mn 7,500 mg, Zn 16,667 mg, Co 42 mg, I 125 mg, Se 67 mg.

Table 2
Growth and visceral index of tilapias fed microbial biomasses.

	Treatment								
	C	RG25	RG50	SC25	SC50	SP25	SP50	P	CV (%)
Initial weigth (g)	28.25 ± 0.40	27.04 ± 1.50	27.42 ± 1.36	26.23 ± 1.31	26.52 ± 1.54	25.04 ± 1.03	27.36 ± 1.40	0.1409	5.39
Final weigth (g)	229.29 ± 7.25	229.98 ± 9.70	244.09 ± 10.61	229.13 ± 10.21	228.32 ± 15.31	233.45 ± 8.59	233.03 ± 6.06	0.5256	4.26
Weigth gain (g)	201.04 ± 7.34	202.95 ± 9.83	216.66 ± 10.74	202.90 ± 10.45	201.80 ± 13.88	208.41 ± 8.80	205.66 ± 5.04	0.4980	4.72
SGR (g/day)	2.65 ± 0.10	2.67 ± 0.13	2.85 ± 0.14	2.67 ± 0.14	2.66 ± 0.18	2.74 ± 0.12	2.71 ± 0.07	0.5353	4.92
Feed consumption (g)	260.49 ± 5.54	255.98 ± 15.45	253.57 ± 16.56	249.58 ± 12.73	248.17 ± 9.94	259.53 ± 3.71	254.66 ± 3.12	0.7671	3.97
Feed conversion ratio	1.30 ± 0.03^{a}	1.26 ± 0.01^{ab}	1.17 ± 0.05^{b}	1.23 ± 0.03^{ab}	1.23 ± 0.04^{ab}	1.25 ± 0.04^{ab}	1.24 ± 0.03^{ab}	0.0310	3.8
HSI (%)	2.27 ± 0.20	2.70 ± 0.60	2.22 ± 0.36	2.37 ± 0.64	2.11 ± 0.60	2.02 ± 0.37	2.04 ± 0.08	0.5746	19.62
SSI (%)	0.14 ± 0.04	0.18 ± 0.02	0.14 ± 0.03	0.13 ± 0.02	0.16 ± 0.04	0.18 ± 0.05	0.16 ± 0.04	0.4117	22.44
VSI (%)	10.73 ± 0.99	11.69 ± 1.57	10.53 ± 1.42	12.09 ± 2.47	9.13 ± 1.27	10.76 ± 1.02	12.60 ± 0.40	0.1433	14.67

C - control diet, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. HSI - hepatosomatic index, SSI - splenosomatic index, DSI - digestivosomatic index. *CV* - coefficient of variation; ^{ab} Means followed by different letters are significantly different (P<0.05).

Table 3
pH and proximate composition of fillets of tilapias fed microbial biomasses.

	Treatment							P	CV (%)
	C	RG25	RG50	SC25	SC50	SP25	SP50		
pH	6.65 ± 0.05	6.65 ± 0.04	6.67 ± 0.08	6.62 ± 0.02	6.64 ± 0.04	6.57 ± 0.09	6.54 ± 0.08	0.1481	1.03
Moisture (%)	77.10 ± 0.35	77.79 ± 1.14	77.97 ± 0.07	77.22 ± 0.23	77.70 ± 0.77	77.51 ± 0.71	77.20 ± 0.23	0.5163	0.78
Minerals (%)	1.17 ± 0.08	1.28 ± 0.20	1.10 ± 0.10	1.08 ± 0.10	1.23 ± 0.01	1.08 ± 0.12	1.24 ± 0.05	0.1863	10.21
Lipids (%)	3.85 ± 0.58	3.38 ± 0.62	3.25 ± 0.35	3.92 ± 0.37	3.68 ± 0.84	3.31 ± 0.34	3.59 ± 0.28	0.6746	14.11
Protein (%)	15.82 ± 0.21[b]	16.95 ± 0.59[a]	17.50 ± 0.26[a]	16.87 ± 0.48[a]	17.00 ± 0.30[a]	17.00 ± 0.41[a]	17.13 ± 0.16[a]	0.0034	3.43
Relative protein gain (%)*	-	7.14	10.62	6.64	7.46	7.46	8.28	-	-

C - control diet, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. *CV* - coefficient of variation; [ab] Means followed by different letters are significantly different (P<0.05). *Increase on the protein content of MB treatments as compared to the control group.

Table 4
Fatty acids of fillets of tilapias fed microbial biomasses.

	Treatments							P	CV (%)
	C	RG25	RG50	SC25	SC50	SP25	SP50		
MUFAs	5.96 ± 0.76	5.24 ± 0.76	4.83 ± 0.96	6.53 ± 0.51	5.01 ± 1.08	5.59 ± 0.16	5.92 ± 0.45	0.1302	14.99
SFAs	5.61 ± 0.78	5.05 ± 0.76	4.68 ± 0.81	5.95 ± 0.70	4.81 ± 0.98	5.04 ± 0.15	5.35 ± 0.32	0.3356	13.92
TFAs	0.02 ± 0.002	0.02 ± 0.003	0.02 ± 0.005	0.02 ± 0.001	0.02 ± 0.003	0.02 ± 0.003	0.02 ± 0.003	0.1011	18.58
UFAs	9.23 ± 1.19	8.20 ± 1.00	8.09 ± 0.54	9.70 ± 0.70	7.75 ± 1.43	8.51 ± 0.30	9.07 ± 0.67	0.1711	11.71
PUFAs	3.27 ± 0.42	2.96 ± 0.24	3.26 ± 0.48	3.17 ± 0.26	2.74 ± 0.35	2.92 ± 0.17	3.15 ± 0.22	0.3922	10.79
C20:4 n-6 (AA)	0.29 ± 0.013	0.28 ± 0.010	0.28 ± 0.011	0.28 ± 0.005	0.28 ± 0.011	0.28 ± 0.008	0.28 ± 0.007	0.7481	3.19
C20:5 n-3 (EPA)	0.004 ± 0.0005^{b}	0.006 ± 0.0009^{a}	0.005 ± 0.0003^{ab}	0.006 ± 0.0003^{a}	0.005 ± 0.0005^{ab}	0.005 ± 0.0008^{ab}	0.006 ± 0.0005^{a}	0.017	14.95
C22:6 n-3 (DHA)	0.09 ± 0.004	0.09 ± 0.006	0.09 ± 0.005	0.10 ± 0.002	0.09 ± 0.006	0.10 ± 0.004	0.09 ± 0.002	0.4344	4.76
Σn-6	0.29 ± 0.013	0.28 ± 0.010	0.28 ± 0.011	0.28 ± 0.005	0.28 ± 0.011	0.28 ± 0.008	0.28 ± 0.007	0.748	3.19
Σn-3	0.09 ± 0.005	0.10 ± 0.005	0.10 ± 0.005	0.11 ± 0.002	0.10 ± 0.006	0.10 ± 0.005	0.10 ± 0.003	0.255	4.780
n-6/n-3	3.02 ± 0.04^{a}	2.81 ± 0.05^{b}	2.80 ± 0.04^{b}	2.63 ± 0.05^{c}	2.78 ± 0.05^{b}	2.77 ± 0.07^{b}	2.78 ± 0.01^{b}	<0.0001	4.17

C - control diet, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. MUFAs - mono unsaturated fatty acids, SFAs - saturated fatty acids, TFAs - trans fatty acids, UFAs - unsaturated fatty acids, PUFAs - polyunsaturated fatty acids. C20:5 n-3 = eicosapentaenoic acid, C22:6 n-3 = docosahexaenoic acid, C20:4 n-6 = arachidonic acid. *CV* - coefficient of variation; ^{abc} Means followed by different letters are significantly different (P<0.05).

Table 5

Texture Profile Analyses (TPA) and Shear Force (SF) of fillets of tilapias fed with microbial biomasses.

		Treatment							P	CV (%)
		C	RG25	RG50	SC25	SC50	SP25	SP50		
TPA	Hardness	23.88 ± 3.39	21.53 ± 2.79	24.41 ± 2.13	21.88 ± 1.90	25.95 ± 1.16	24.09 ± 2.46	24.40 ± 2.61	0.0823	10.58
	Springiness	0.66 ± 0.05	0.60 ± 0.06	0.66 ± 0.07	0.63 ± 0.10	0.76 ± 0.08	0.68 ± 0.03	0.72 ± 0.11	0.2625	11.88
	Cohesiveness	0.46 ± 0.07	0.47 ± 0.06	0.39 ± 0.01	0.44 ± 0.03	0.37 ± 0.03	0.40 ± 0.06	0.37 ± 0.03	0.0945	13.38
	Chewiness	6.09 ± 0.44	5.20 ± 1.60	6.93 ± 1.15	6.07 ± 0.97	8.17 ± 1.36	6.45 ± 0.43	5.65 ± 1.26	0.1158	20.52
	Resilience	0.19 ± 0.05	0.20 ± 0.04	0.15 ± 0.01	0.18 ± 0.01	0.14 ± 0.02	0.16 ± 0.03	0.14 ± 0.03	0.1212	20.34
SF (g/cm^2)		366.78 ± 35.79	334.92 ± 38.77	328.93 ± 56.37	310.70 ± 19.54	344.43 ± 40.86	304.84 ± 31.55	314.75 ± 27.22	0.7477	14.17

C - control diet, RG25 - control diet supplemented with 0.25% *R. gelatinosus,* RG50 - control diet supplemented with 0.5% *R. gelatinosus*, SC25 - control diet supplemented with 0.25% *S. cerevisiae*, SC50 - control diet supplemented with 0.5% *S. cerevisiae*, SP25 - control diet supplemented with 0.25% *S. platensis* and SP50 - control diet supplemented with 0.5% *S. platensis*. TPA - texture profile analysis and SF - shear force. *CV* - coefficient of variation.

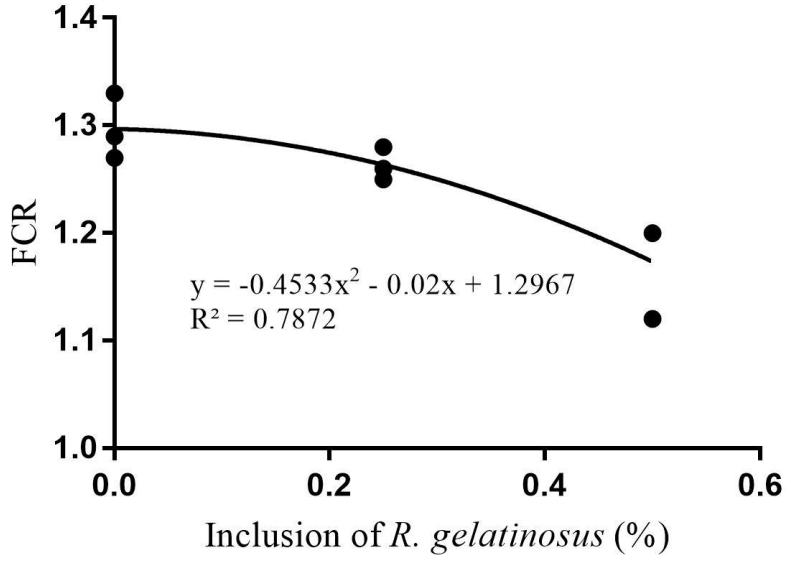

FIGURE 1 - Quadratic regression of FCR of tilapia as a function of the inclusion of *R. gelatinosus* in the experimental diets.

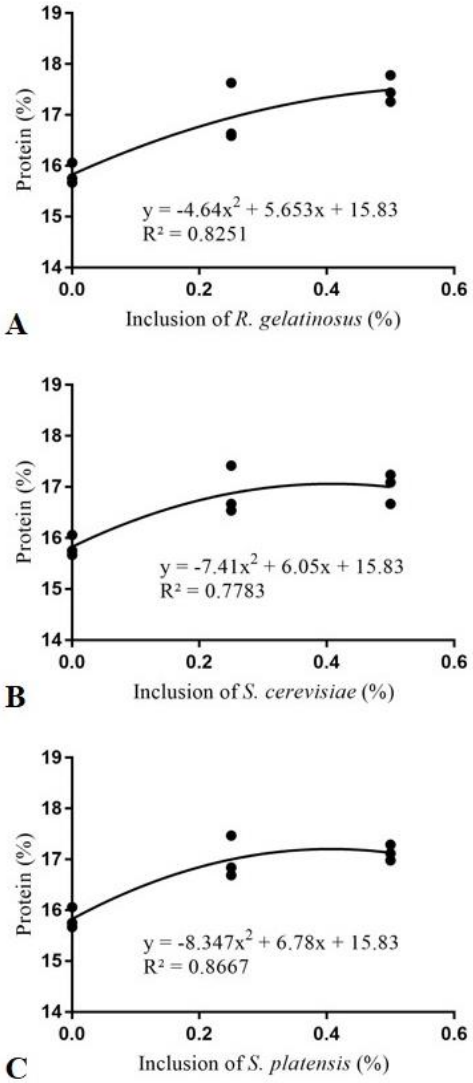

FIGURE 2 - Quadratic regression of protein of fillets as a function of the inclusion of *R. gelatinosus* (A), *S. cerevisiae* (B) and *S. platensis* (C) in the experimental diets of tilapia.

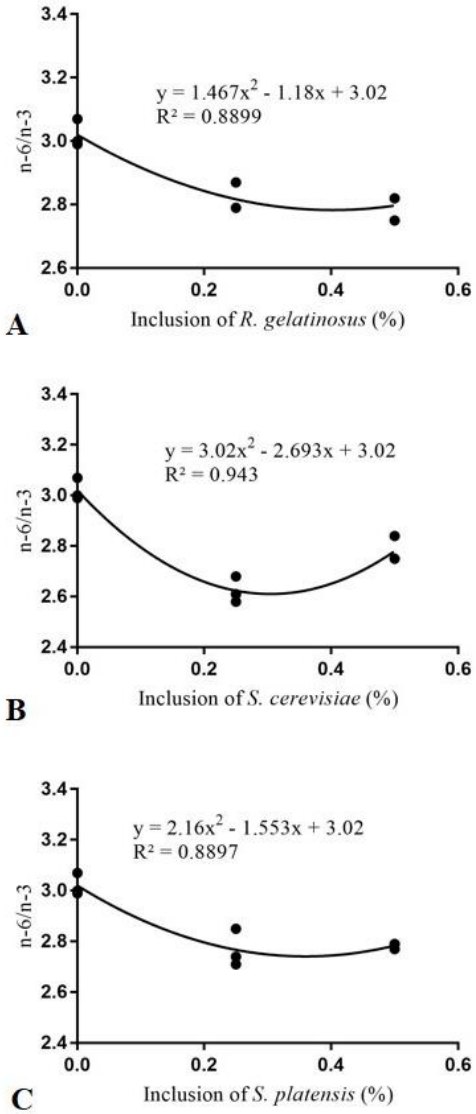

FIGURE 3 - Quadratic regression of the ratio n-6/n-3 as a function of the inclusion of *R. gelatinosus* (A), *S. cerevisiae* (B) and *S. platensis* (C) in the experimental diets of tilapia.

ANEXO

Certificado da Comissão de Ética no Uso de Animais (CEUA)

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Certificamos que o Relatório Final do trabalho intitulado **"Biomassa bacteriana para alimentação de tilápias"**, Processo FOA nº 2016-00407, sob responsabilidade de Elisa Helena Giglio Ponsano e colaboração de Thiago Luis Magnani Grassi, Lia Ferraz de Arruda Sucasas, Giovani Sampaio Gonçalves, Leandro Kanamaru Franco de Lima, Ricardo Borghesi, Paulo Cesar Ciarlini, Dayse Lícia de Oliveira, Natália Mingues Paiva, Izabela Pazzoto Alves Senna, Ane Maria Bosco e Ariana Aparecida Ferreira Pereira foi aprovado pela CEUA em 17 de Maio de 2017.

CERTIFICATE

We certify that the study entitled **"Microbial biomass for tilapia feeding"**, Process FOA nº 2016-00407, under the supervision of Elisa Helena Giglio Ponsano and collaboration of Thiago Luis Magnani Grassi, Lia Ferraz de Arruda Sucasas, Giovani Sampaio Gonçalves, Leandro Kanamaru Franco de Lima, Ricardo Borghesi, Paulo Cesar Ciarlini, Dayse Lícia de Oliveira, Natália Mingues Paiva, Izabela Pazzoto Alves Senna, Ane Maria Bosco and Ariana Aparecida Ferreira Pereira had its the Final Report approved by the CEUA on May 17, 2017.

Prof. Dr. Leonardo Perez Faverani
Coordenador da CEUA
CEUA Coordinator

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