UNIVERSIDADE FEDERAL DO RIO GRANDE

INSTITUTO DE OCEANOGRAFIA

PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

Avaliação do antioxidante alfa-ácido lipóico em pampo, *Trachinotus marginatus* (Pisces, Carangidae).

Acadêmico: Mateus Tavares Kütter

Orientador: Marcelo Borges Tesser

Co-orientador: José Maria Monserrat

Rio Grande, Março 2013.

Sumário

Resumo Geral 4

General Abstract 5

Introdução geral 7

Objetivo 20

Referencias 21

Capitulo 1- Efeito da concentração do antioxidante α-ácido lipóico no fígado e cérebro do pampo, *Trachinotus marginatus* (Pisces, Carangidae).

Resumo 30

Abstract 30

Introdução 31

Material e Métodos 34

Resultados 38

Discussão 44

Referências 47

Capítulo 2- Efeito da suplementação da dieta com α-ácido lipóico no crescimento, composição corporal e *status* antioxidante em pampo *Trachinotus marginatus* (Pisces, Carangidae).

Resumo 55

Abstract 55

Introdução 56

Material e Métodos 58

Resultados 66

Discussão 74

Referencias 77

Capítulo 3- Tempo de resposta do status antioxidante do peixe *Trachinotus marginatus* (Pisces, Carangidade) alimentado com dieta suplementada com α-ácido lipóico.

Resumo 86

Abstract 86

Introdução 87

Material e Métodos 88

Resultados 91

Discussão 94

Referencias 96

Capítulo 4- Ácido lipóico: antioxidante com potencial uso na aquicultura.

Resumo 101

Abstract 102

Introdução 102

Estresse oxidativo em organismos aquáticos 104

Consumo e toxicidade do ácido lipóico 106

Possiveis beneficios da utilização do ácido lipóico em organismos aquáticos 107

Conclusões e direcionamento futuro 109

Referências 110

Considerações finais 118

Agradescimentos

Aos meus orientadores Prof. Dr. Marcelo Tesser e Prof. Dr. José Monserrat pela paciência dedicação e amizade.

Aos colegas do Instituto de Ciências Biológicas e da Estação Marinha de Aquicultura pela participação e convívio durante o desenvolvimento do trabalho, que tornou o ambiente de trabalho agradável e que de alguma forma ajudaram para o meu crescimento profissional e pessoal.

Aos colegas de alojamento Diego, Clivea, Adriana, Mariana, Paula e Ricardo pelos divertidos momentos que passamos juntos.

Aos meus amigos Juan, Renato e Eduardo pelo companheirismo e auxilio no desenvolvimento deste trabalho.

A equipe do grupo de pesquisa em piscicultura marinha e estuarina pelo auxíliono desenvolvimento dos experimentos.

A professora Juliana e professor Elton pelas sugestões na elaboração do texto.

A minha família, especialmente a minha mãe e meus avós, por todo o ensinamento e carinho que me deram.

A minha esposa pelo companheirismo e incentivo.

Resumo Geral

O presente trabalho avaliou o efeito anti-oxidante do ácido α-lipóico (AL) no peixe *Trachinotus marginatus*. Foram determinados os efeitos agudo por meio da administração de diferentes concentrações de AL por injeção via intraperitoneal (i.p) (experimento I) e crônico por meio da alimentação (experimento II). Também foi realizado mais um experimento utilizando a dose (via alimentação) que apresentou as melhores respostas fisiológicas, avaliando-se as mudanças na capacidade antioxidante do peixe ao longo do tempo (experimento III). A administração via i.p demonstrou efeitos antagônicos dependendo do órgão estudado. No fígado no cérebro, as doses de 20 mg AL/kg e 40 mg AL/kg, respectivamente, melhoraram a capacidade antioxidante. No entanto, no fígado, as doses mais altas (40 e 60 mg AL/kg) apresentaram um efeito pro-oxidante com aumento da peroxidação lipídica e ferro livre, e redução da capacidade de desintoxicação. No experimento II, os peixes foram alimentados por 42 dias com dieta contendo AL. A administração por meio da alimentação alterou o metabolismo lipídico do organismo. Os peixes alimentados com as maiores doses (890 e 1367 mg AL/kg de ração) apresentaram redução no crescimento, na eficiência proteica e ingestão alimentar. Nestas doses ocorreu uma redução do teor de lipídio na carcaça em comparação aos peixes do grupo controle. A capacidade de desintoxicação foi maior na dose de 890 mg AL/kg de ração no cérebro. No músculo ocorreu redução da peroxidação lipídica em todos os tratamentos com AL, um efeito que pode ser correlacionado com a redução da concentração de lipídios na carcaça. No experimento III, os peixes foram alimentados com uma dieta contendo 524 mg AL/ kg de ração. Foram analisados atividade da glutationa-S-transferase e peroxidação lipídica aos 10, 20 e 30 dias no cérebro, músculo, fígado e brânquia. No músculo a redução da peroxidação lipídica ocorreu aos 30 dias. Considerando os resultados obtidos podemos sugerir que

juvenis de *T. marginatus* sejam tratados com AL com uma dose i.p 20 mg/kg pois em doses maiores ocorreu efeito pró-oxidante do AL no fígado. A inclusão de AL na dieta deve ser fixada entre 316 e 524 mg AL/ kg de ração, pois apresentou os melhores resultados em termos de quimioproteção, crescimento e metabolismo proteico e lipídico. O tratamento com AL na dieta apresentam aumento da capacidade de detoxificação e redução do dano lipídico a partir de 30 dias. Embora o uso de AL em animais aquáticos esteja restrito a estudos experimentais os resultados obtidos demonstram que a utilização deste composto apresenta potencial uso na aquicultura.

Palavras-chave: ácido lipóico, glutationa-S-transferase, peroxidação lipídica, peixe.

General Abstract

The present study evaluated the effect of antioxidant lipoic acid (LA) in the fish *Trachinotus marginatus*. Were tested acute and chronic effects of different concentrations of LA. The acute test used LA injected intraperitoneal (experiment I) and chronic test used diet LA supplemented (experiment II). Moreover, the time necessary to enhance the antioxidant capacity of fish fed with diet supplemented with LA were evaluated (experiment III). The acute test exhibited antagonist effects-depending of the organ and concentration. In liver and brain, acute doses of 20 mg LA/kg and 40 mg LA/kg, respectively, increase antioxidant capacity. However, in liver higher doses (40 and 60 mg LA/kg) showed a prooxidant effect with increase in lipid peroxidation and free iron and reduction of detoxification capacity. In experiment II fish were fed during 42 days with diet containing different concentrations of LA. The diet LA supplementation exhibited effect in lipid metabolism. The high doses (890 and 1,367 mg LA/ kg dry food) showed reduction in growth, specific growth rate, protein

efficiency ratio and food intake. These doses exhibited reduction in lipid composition of carcass in comparison to control group. The muscle showed reduction in lipid peroxidation in all treatments with LA. In experiment III fish were fed with diet containing 524 mg LA/ kg dry food. The glutathione-S-transferase activity and lipid peroxidation at 10, 20 and 30 days in brain, liver, gill and muscle were analysed. In muscle at 30 days occurred reduction in lipid peroxidation. Considering the results of present study we could suggest that *T. marginatus* can be treated with a single intraperitoneal injection of 20 mg/kg body weight, since that higher doses cause prooxidant effect in liver. In the diet LA can be supplemented in doses between 316 and 524 mg LA/kg dry food. These doses presents best results of growth, antioxidant status and lipid and protein metabolism. However, the use of LA in aquatic organism is restricted to experimental researches the beneficial proprieties of LA reported in current researches with aquatic organisms turn this compound a potential substance to be used in aquaculture.

Key-words: fish, glutathione-S-transferase, lipid peroxidation, lipoic acid.

Introdução Geral

Ao longo da história da humanidade o desenvolvimento da produção animal foi um fator determinante para o progresso das grandes civilizações (Diamond 2001). Por meio do desenvolvimento de tecnologias que permitiram domesticar e melhorar a produção animal foi possível, entre outros fatores, que em 2011 chegássemos aos 7 bilhões de habitantes no mundo (UN 2011). Nos últimos anos tem-se dirigido este foco para espécies marinhas, com intuito de suprir a crescente demanda por fonte proteica (Duarte *et al*. 2007). A cada ano novas espécies são adicionadas a lista de espécies criadas. No entanto, as condições de cativeiro trazem consigo a maior incidência de doenças, muitas delas causadas ou agravadas pelo estresse oxidativo. Desta forma torna-se necessário o desenvolvimento de novas tecnologias que permitam incrementar a produção de organismos aquáticos. A utilização de antioxidantes não enzimáticos tem sido empregada como uma das formas de prevenir e/ou minimizar este problema (Sies 1997, Hamre 2011, Gao *et al* 2012).

Situação da aquicultura mundial e da piscicultura marinha no Brasil

A produção de alimentos em larga escala, facilitada pela tecnologia vem garantindo a oferta de alimento a população humana. O pescado apresenta papel de destaque neste quesito representando aproximadamente 20% do consumo per capta de proteína de origem animal, consumida por mais de 1,5 bilhões de pessoas no mundo (FAO 2010). O consumo per capta mundial de pescado vem aumentando nos últimos anos passando de 16,2 kg em 2004 para 17,1 kg em 2008 (FAO 2010). No Brasil o consumo per capta também aumentou passando de 6,6 kg em 2004 para 9,0 kg em 2009 (MPA 2012).

A contribuição da aquicultura mundial com o pescado destinado para alimentação humana chegou a 46% da produção de pescado no ano de 2008 (FAO 2010). Nas últimas décadas a aquicultura tem se destacado como o setor de produção animal com o maior crescimento. A produção mundial da aquicultura incluindo as plantas aquáticas era de 32,4 milhões de toneladas no ano 2000 e passou para 68,3 milhões de toneladas em 2008 (FAO 2010).

Dentre as regiões que vem demonstrando aumento da produção pode-se destacar a América Latina e o Caribe, que apresentaram o maior crescimento anual na aquicultura (21,1 %/ano) no período de 1970 à 2008 (FAO 2010). No Brasil a taxa de crescimento médio do setor foi de 11,5 % ano (2005-2010). Este desempenho positivo da aquicultura brasileira ocorreu devido ao aumento da piscicultura de água doce, que aumentou sua produção de 176.397 t (2005) para 388.513 t (2010), taxa de crescimento media de 14,3%/ano (FAO 2010). Entretanto, a produção da aquicultura marinha apresentou redução nos últimos anos (2008-2009) passando de 83.359 t para 78.296 t (MPA 2012). Esta queda ocorreu principalmente pela incidência de patologias: mancha branca e mionecrose infecciosa viral; reforçada pelo grande volume de chuva que causou enchentes na região nordeste onde esta concentrada grande parte dos produtores de camarão marinho (MPA 2012).

A aquicultura marinha no Brasil esta representada basicamente pela criação de moluscos e crustáceos, sendo que a piscicultura marinha encontra-se em fase experimental, tendo sido produzido em 2009, 49 t de beijupirá, *Rachycentron canadum* (MPA, 2012). No entanto, o país apresenta um grande potencial de crescimento, tendo em vista a grande extensão do litoral (8.400 Km), condições climáticas e hidrográficas adequadas além da elevada diversidade de espécies com potencial para sua utilização na

aquicultura (Assad & Bursztyn 2000). A ictiofauna marinha brasileira conta com diversas espécies com potencial para esta utilização: o robalo-peva (*Centropomus parallelus*), o robalo-flecha (*Centropomus undecimalis*), a cioba (*Lutjanus analis*), o ariocó (*Lutjanus synagris*), a garoupa (*Epinephelus marginatus*), o pargo rosa (*Pagrus pagrus*), o peixe-rei (*Odonthestes argentinensis*), a arabaiana (*Seriola dumerili*), o dourado (*Coryphaena hippurus*), a carapeba (*Eugerres brasilianus*), o mero (*Epinephelus itajara*), o badejo (*Mycteroperca bonaci*), o beijupirá (*Rachycentron canadum*), o linguado (*Paralichthys orbignyanus*) e o pampo (*Trachinotus marginatus*) (Baldisserotto & Gomes 2010, Cavalli *et al*. 2011).

Em relação à aquicultura mundial as espécies marinhas contribuem com 32,3% da produção, sendo que os peixes marinhos representam 3,4% deste montante, arrecadando 6,7% do valor total da aquicultura mundial (FAO 2010). Destes podemos destacar espécies de peixes Pleuronectiformes e o bacalhau *Gadus morhua*, que apresentam elevado valor de mercado tendo grande parte de sua produção direcionada para exportação.

Embora o Brasil apresente um elevado número de candidatos para piscicultura marinha, à lucratividade deste setor que é encontrada em outras partes do mundo não tem despertado interesse de investimentos nesta área no país. Esta falta de investimento na piscicultura marinha faz com que o país apresente um déficit na balança comercial em relação à comercialização do pescado, uma vez que, os principais produtos pesqueiros importados são referentes aos peixes marinhos: bacalhau, salmões, filé de merluza, sardinha e filé de outros peixes que correspondem a 81,81% das compras feitas no mercado externo (IBAMA, 2007).

Alguns fatores têm sito citados como obstáculos para o desenvolvimento da piscicultura marinha no Brasil como: falta de tecnologia, fatores econômicos de mercado, falta de mão de obra e serviços especializados, ausência de legislação específica e qualidade da ração (Cavalli *et al*. 2011).

Dentre os fatores mencionados acima a qualidade da ração destaca-se como principal desafio da piscicultura marinha mundial, por apresentar dependência de subprodutos da pesca para produção de farinha e óleo de peixe (Tacon & Metian 2008). Além disso, na produção de pescado a dieta representa entre 40-50% do custo de produção (Craig & Helfrich 2002). Desta forma, na formulação da ração devem ser selecionados ingredientes que não aumentem o custo ou diminuam a eficiência da dieta (Hardy & Barrows 2002). Além disso, compreender as exigências nutricionais é essencial para produção de pescado saudável e de alta qualidade.

A adição de suplementos, vitamínicos e minerais e de compostos antioxidantes nas rações vem crescendo nas ultimas décadas. Estudos sobre a suplementação da dieta com antioxidantes naturais em organismos aquáticos tem demonstrado que estes compostos melhoram o crescimento, sobrevivência e resposta imune dos animais além de atuarem na qualidade nutricional do pescado (Waagbø *et al*. 1993, Cristiansen *et al*. 1995, Sant'Ana & Mancini-Filho 2000, Hamre *et al*. 2004, Qinghui *et al*. 2004, Wang *et al*. 2006). No entanto, embora tenham sido testados diversos compostos apenas um pequeno número apresentou as qualidades necessárias para serem utilizados na prevenção da oxidação do alimento e melhoria da capacidade antioxidante dos animais (Rumsey 1980, Ajiboye et al 2012).

O pampo *Trachinotus marginatus* (Cuvier, 1832)

O aumento de espécies utilizadas na aquicultura é um fenômeno contemporâneo. Atualmente são utilizadas na aquicultura mais de 430 espécies de organismos incluindo, plantas, equinodermos, cnidários, anelídeos, crustáceos, moluscos, peixes e outros vertebrados. Destas, estima-se que aproximadamente 106 espécies foram domesticadas na ultima década (Duarte *et al*. 2007). Diferentemente das espécies terrestres que tiveram grande parte de suas espécies domesticadas entre 11 e nove mil anos, a maioria das espécies aquáticas foi domesticada nos últimos 100 anos. Dentre os fatores que contribuiram para este elevado número de espécies aquáticas, particularmente as marinhas, sendo domesticadas recentemente, é que em comparação com as terrestres, as espécies aquáticas apresentam uma maior diversidade sendo utilizada para alimentação o que gera um maior potencial para domesticação.

Dentre os peixes marinhos as espécies do gênero *Trachinotus* têm despertado interesse no desenvolvimento de técnicas para criação comercial (Stickney 2009). A sua rápida adaptação ao cativeiro, a boa tolerância às condições ambientais extremas e altas taxas de crescimento (Jory *et al*. 1985), são apontadas como características desejáveis na aquicultura. Na China e nos EUA, as espécies *T. ovatus* e *T. carolinus* já são criadas comercialmente. Nestes países estas espécies são produzidas em pequena escala, no entanto o pescado produzido apresenta elevado valor de mercado (Hong & Zhang 2003, MacMaster *et al*. 2004).

O pampo *Trachinotus marginatus* Cuvier 1832 é uma espécie endêmica do Oceano Atlântico Sul Ocidental habitando águas costeiras e estuarinas (Fisher *et al*. 2004) (Fig. 1). Os jovens são encontrados em grande quantidade na zona de arrebentação das praias do litoral do Rio Grande do Sul durante todo período do ano, sendo menos comum sua ocorrência em água mais quente do Sudeste do Brasil,

enquanto que os adultos são encontrados em águas mais profundas desde o Rio de Janeiro até o Uruguai (Menezes & Figueiredo 1980, Lima & Vieira 2009).

Figura 1. Juvenil de *Trachinotus marginatus,* 500 mm de comprimento total, coletado na praia do Cassino, RS, Brasil (Kütter M.T. 2012).

Os juvenis alimentam-se principalmente de crustáceos e à medida que crescem passam a consumir com maior frequência moluscos e poliquetas (Monteiro-Neto & Cunha 1990). Na costa do Rio Grande do Sul o período reprodutivo desta espécie ocorre durante a primavera e o verão, sendo o tamanho dos exemplares na primeira maturação sexual estimado em 187,2 mm para os machos e 254,9 mm para as fêmeas que apresentam duas modas de diâmetro oocitário o que sugere uma desova total (Lemos *et al*. 2011).

A pesca de peixes do gênero *Trachinotus*, ocorre principalmente nos estados de Santa Catarina e do Rio Grande do Sul que tem contribuido com 94,3% (904,8 t) do total de capturas no Brasil, (IBAMA 2007). Onde foram capturadas no ano de 2005 959,5 toneladas no território nacional, sendo toda captura proveniente da pesca artesanal o que demonstra a importância deste gênero na pesca artesanal dos estados do sul do Brasil (IBAMA 2007).

A disponibilidade de conhecimento sobre o pampo *T. marginatus* são limitados. Nos últimos anos, estudos investigando esta espécie têm sido realizados pelo grupo de pesquisa em Piscicultura Estuarina e Marinha da Universidade Federal do Rio Grande (FURG) com intuito de gerar informações que possibilitem a criação desta espécie. De acordo com os estudos produzidos por este grupo, esta espécie é eurialina tolerando uma ampla faixa de salinidade (7-58) quando submetidos a choque agudo de salinidade (Sampaio *et al*. 2003). Também foi constatado que o pampo apresenta baixa sensibilidade a amônia e ao nitrito (LC_{50}/96 h de 1,87 mg/L e 116,68 mg/L respectivamente) quando criado em ambiente isosmótico (Costa *et al*. 2008). Em estudo sobre a resposta imunológica desta espécie durante infestação parasitária por *Bicotylophora trachinoti* demonstrou-se uma resposta não específica do mecanismo defesa (Chaves *et al*. 2006). Foi ainda demonstrado que o pampo retorna ao seu consumo de oxigênio basal 2,5 h após alimentação, podendo ser alimentado com frequência de aproximadamente oito vezes por dia (Cunha *et al*. 2009). Em estudo investigando a concentração de anestésicos, foi determinado que a melhor concentração de benzocaína e eugenol, foram de 50 ppm (Okamoto *et al*. 2009). O ponto isosmótico do pampo foi investigado por Abou Anni (2011), sendo determinado em 357,5 mOsmoles/kg H_2O^{-1}, o que equivale à salinidade 13. Neste trabalho também foi constatado que os juvenis de pampo apresentam maior crescimento na salinidade de 3 a 12. Recentemente foi diagnosticado o desenvolvimento de enterite da boca vermelha (yersiniose) em uma criação de *T. marginatus* em que os animais foram submetidos a estresse luminoso (Romano *et al*. 2012).

Estresse oxidativo

Ao se intensificar a criação de animais, as doenças que normalmente apresentam baixa incidência nas populações selvagens podem ocorrer em grande número de indivíduos em ambiente confinado. O estresse causado pelo manejo combinado com a rápida transmissão de doenças em altas densidades de estocagem e problemas nutricionais são problemas determinantes para o sucesso de qualquer piscicultura (Alderman 1988). Em animais de criação o estresse oxidativo está envolvido em diversas condições patológicas incluindo condições que são relevantes para produção animal e bem estar geral dos indivíduos (Lykkesfeldt & Svendsen 2006).

O estresse oxidativo é caracterizado por uma sobrecarga oxidativa decorrente do desequilíbrio entre oxidantes e anti-oxidantes em favor dos primeiros (Sies 1985). Os principais compostos responsáveis por este desequilíbrio são espécies ativas de nitrogênio (EAN) e espécies ativas de oxigênio (EAO) (Halliwell & Gutteridge 2007). A produção das EAO ocorre, entre outros processos, pela redução do oxigênio O_2, durante o metabolismo e produção de energia na mitocôndria, resultando em diversos compostos intermediários altamente reativos e com alto potencial tóxico como o radical ânion superoxido ($O_2^{\cdot -}$), o peróxido de hidrogênio (H_2O_2), o radical hidroxila ($^{\cdot}OH$) e o oxigênio singlet (O_2^1) (Sies 1985).

A primeira etapa de redução do O_2 em $O_2^{\cdot -}$ utiliza um doador de elétrons e ocorre principalmente no complexo I (NADH-ubiquinona oxiredutase) e complexo III (ubiquinol citocromo *c* oxiredutase) da cadeia transportadora de elétrons (Kussmaul & Hirst 2006). No entanto, a fase seguinte de redução desta reação pode ocorrer espontaneamente ou requerer um doador de e^-/H^+. Esta etapa, que é crucial para cascata de formação das espécies secundárias de EAO, é a reação do $O_2^{\cdot -}$ com íons de metais de

transição (Fe^{2+}, Cu^{+}) que atuam como doadores de elétrons, promovendo a geração da EAO mais reativa, o radical hidroxila $HO^{\cdot}$ (Blokhina *et al*. 2003).

Além disso, as EAO estão envolvidas na regulação da sinalização celular e expressão gênica, atuando também na ação antimicrobiana e citotóxica das células do sistema imunológico entre outros (Butler *et al*. 2009, Cui *et al*. 2009). No entanto, a produção excessiva destes compostos pode levar a oxidação de lipídios, proteínas e do DNA, desencadeando processos patológicos (Bergamini *et al*. 2004). Para proteger as células e órgãos contra o excesso de produção de EAO existe uma variedade de componentes envolvidos, endógenos e exógenos, que funcionam interativamente e sinergicamente para neutralizar as EAO. Neste grupo estão incluídas enzimas antioxidantes que catalisam a extinção de radicais livres, proteínas que ligam-se a metais, e que podem gerar EAO, como Fe^{2+}, Cu, Mn^{2+} e anti-oxidantes derivados da dieta como ácido ascórbico (vitamina C), alfa tocoferol (vitamina E), carotenóides, polifenóis e outros compostos como o ácido lipóico (Block 1991, Wang & Luo 2007, Jiang *et al*. 2009).

O antioxidante α-ácido lipóico

A utilização de antioxidantes na alimentação esta relacionada à conservação das propriedades nutricionais destes, incluindo a inibição ou retardo da oxidação de lipídios (Ramalho & Jorge 2006). Por outro lado, a ingestão de antioxidantes pode também auxiliar no controle do estresse oxidativo no organismo.

O ácido lipóico (AL) e sua forma reduzida o ácido dihidro-lipóico (ADHL) são dissulfitos que ocorrem naturalmente em micro organismos, plantas e animais (Navari-Izzo *et al*. 2002) (Fig.2). O AL apresenta dois isômeros: o R que ocorre naturalmente, e o S que é a forma sintética. Esta molécula foi inicialmente classificada como vitamina,

mas posteriormente foi constatada sua síntese em células animais (Carreau 1979), onde atua como co-fator em complexos multi-enzimáticos mitocondriais que catalisam a descarboxilação oxidativa no ciclo de Krebs (Reed 1974). A síntese do AL ainda não é bem compreendida, no entanto, Navari-Izzo *et al*. (2002) sugerem que em eucariontes o AL é sintetizado nas mitocôndrias a partir do malonato. As fontes de AL na dieta são principalmente provenientes da carne vermelha, fígado, coração e rins e em menor concentração nos vegetais, sendo que se apresenta em maior concentração no espinafre, brócolis, tomate, batata e ervilha (Goraca *et al*. 2011).

O AL e ADHL possuem um conjunto de características que os colocam como antioxidantes ideais para utilização terapêutica incluindo: especificidade na extinção de EAO, capacidade de quelar metais, regenerar outros antioxidantes, capacidade de atuar em ambos os domínios lipídico e aquoso, além de regular a ação de fatores de transcrição que regulam a expressão de genes envolvidos no sistema antioxidante e de detoxificação (Packer & Tritschler 1995). O AL ao entrar na célula é reduzido a ADHL (Handelman *et al*.1994), o qual pode atuar regenerando a vitamina E, o ascorbato e a glutationa (Packer *et al*. 2001). O AL é capaz de atravessar a membrana capacitando-o a agir na interfase entre lipídio e água da célula. Seus efeitos antioxidantes incluem sua interação com radicais hidroxil, que são essências para o início da peroxidação lipídica.

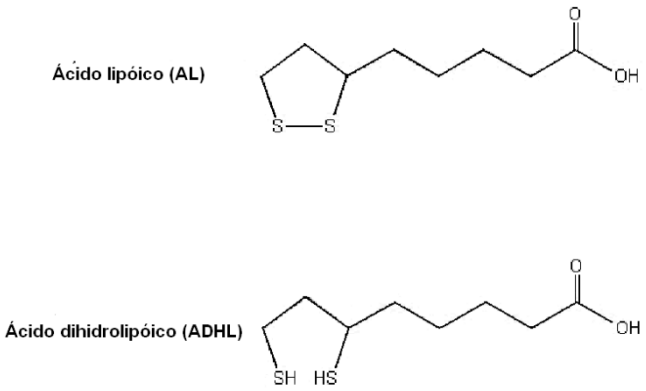

Figura 2. Estrutura molecular do ácido lipóico (AL- forma oxidada) e do ácido dihidrolipóioco (ADHL- forma reduzida). Fonte: Longaray (2012).

Nas últimas décadas tanto o AL quanto o ADHL tem recebido atenção intensiva das pesquisas, devido a sua função antioxidante que tem demonstrado benefícios no uso terapêutico (Biewenga *et al*. 1997). O AL é utilizado no tratamento de uma variedade de doenças em humanos e animais, incluindo doenças hepáticas (Bustamante *et al*. 1998), doenças neuro-degenerativas (Bergamini *et al*. 2004), neuropatia diabética (Simbula *et al*. 2007) e contaminação por metais pesados (Bahtt & Flora 2009).

Nos sistemas de criação, algumas vezes os organismos aquáticos estão suscetíveis a compostos tóxicos como microcistina, mudanças ambientais, como pH, e à infestação por parasitas, todos os quais podem induzir situações de estresse oxidativo (Leão *et al*. 2008, Zhou *et al*. 2009, Amado & Monserrat 2010, Garcia *et al*. 2011). Os peixes estuarinos apresentam uma substancial defesa contra os danos causados pelas espécies ativas de oxigênio, que é um dos vários mecanismos fisiológicos utilizados para enfrentar condições de estresse neste ambiente onde existe grande variação temporal e espacial dos parâmetros abióticos (Ross *et al*. 2001). No entanto, nos peixes

as membranas ricas em ω-3 de ácidos graxos polinsaturados (PUFA) são altamente susceptíveis a oxidação por EAO (Sargent *et al*. 1999).

Os efeitos antioxidante da suplementação alimentar com ácido lipóico, foram estudados em duas espécies de peixes tropicais. A suplementação da dieta por oito semana com 1000 mg AL/kg ração, afetou os níveis de aminoácidos livres no plasma sanguíneo, preveniu a deficiência de ácido ascórbico e aumentou a concentração de ácido eicosapentaenóico no músculo de pacu (*Piaractus mesopotamicus*) (Terjesen *et al*. 2004, Park *et al*. 2006, Trattner *et al*. 2007), enquanto que em coridora (*Corydoras paleatus*) alimentadas com dieta contendo 70 mg AL/ kg massa corporal por quatro semana ocorreu neuroproteção (Monserrat *et al*. 2008). Entretanto, os resultados obtidos por Park *et al*. (2006) demonstraram que os peixes alimentados com a dieta de 1000 mg AL/kg ração, apresentaram um retardo no ganho de peso. A utilização do AL também foi estudada em outros grupos de organismos aquáticos empregados na aquicultura. No abalone (*Haliotis discus hannai*) a suplementação da dieta com AL pelo período de 16 semanas, apresentou aumento da capacidade antioxidante e crescimento nas concentrações de 200 e 800 mg AL/kg ração (Zhang *et al*. 2010). Para o camarão do Pacífico (*Litopenaeus vannamei*) a suplementação da dieta com AL apresentou aumento no crescimento na concentração de 70 mg AL/kg ração e melhorou a capacidade antioxidante após período de anoxia e re-oxigenação (Martins 2011). Outra forma de intervenção de AL, por meio de injeção, foi estudada em carpa (*Cyprinus carpio*) por Longaray (2012), sendo observado neste estudo que a utilização de AL livre ou nanocapsulado aumenta a capacidade antioxidante do organismo.

Desta forma, e pelo exposto acima, a utilização de AL para minimizar os danos causados pelo estresse oxidativo apresentam um potencial emprego na aquicultura. No entanto, a literatura farmacêutica está repleta de relatos de compostos que causam

efeitos adversos em diferentes espécies. O AL apresenta toxicidade diferente dependendo da espécie de animal estudada, sendo dez vezes mais tóxico para gatos que para cães e ratos (Hill *et al*. 2003). Estudos com camundongo e rato demonstraram que a suplementação com AL reduz o consumo alimentar, sugerindo um efeito anoréxico deste composto (Kim *et al*. 2004, Shen *et al*. 2005, Huong & Ide 2008). Além disso, foi relatado que o AL apresenta efeito pró-oxidante dependendo da dose administrada e da circunstância fisiológica do animal (Moini *et al*. 2002, Çakatai *et al*. 2005, Çakatai *et al*. 2006, Atukeren *et al*. 2010). Devido à grande variedade de espécies criadas na aquicultura aliada ao efeito dualístico do AL, torna-se necessário à descrição da forma de ação e efeitos colaterais deste composto na espécie alvo quando administrados de forma aguda e crônica. Portanto, ambas as rotas de administração, via intraperitoneal (aguda) e suplementação alimentar (crônica), são comumente utilizadas na administração de medicamentos na aquicultura (Zhang *et al* 2010, Amado *et al* 2011).

Desta forma o presente estudo analisou doses respostas do ácido lipóico, quando administrado de forma aguda e crônica, a fim de avaliar a concentração que apresenta efeito de quimioproteção para o pampo *Trachinotus marginatus* (Cuvier, 1832) (Carangidae).

Objetivo

Geral

Investigar a dose necessária de ácido lipóico (AL) administrada de forma aguda (injeção) e crônica (alimentação) para aumentar a competência antioxidante e de detoxificação no peixe *Trachinotus marginatus*.

Específicos

1. Avaliar o efeito de quimioproteção do AL administrado por meio de injeção e na alimentação em diferentes órgãos de *Trachinotus marginatus*;

2. Verificar o efeito da administração de AL por meio de injeção e na alimentação na atividade de uma enzima envolvida em processos de desintoxicação glutationa S-transferase (GST) em diferentes órgãos de *Trachinotus marginatus*;
3. Avaliar o efeito da administração de AL na concentração de produtos de peroxidação lipídica (TBAR) em diferentes órgãos de *Trachinotus marginatus*;
4. Analisar o efeito da suplementação da dieta com AL na composição corporal de *Trachinotus marginatus*;
5. Determinar a concentração ideal de AL na dieta para o crescimento de *Trachinotus marginatus*;
6. Analisar o período necessário para ocorrer a quimioproteção do AL nos órgãos do peixe *Trachinotus marginatus* alimentado com dieta suplementada com AL.

Referencias bibliográficas

ABOU ANNI, I. 2011. Efeito da salinidade sobre o comportamento iono-osmorregulatório e crescimento de juvenis de pampo *Trachinotus marginatus*. Dissertação de mestrado. Universidade Federal do Rio Grande, Rio Grande,RS

ALDERMAN, D.J. 1988. Fisheries Chemotherapy: A review. In: Muir JM and Roberts RJ. Recent advances in aquaculture Vol.3, Ed.Timber Press, Portland Oregon 420p

AMADO, L.L. & MONSERRAT, J.M. 2010. Oxidative stress generation by microcystin in aquatic animals: Why and how. *Environ. Int.* 36, 226-235.

ASSAD, T. L., BURSZTYN, M. 2000. Aquicultura Sustentável. *In:* Aqüicultura no Brasil: bases para um desenvolvimento sustentável. Editado por Valenti, W.C; Poli, C.R; Pereira, J.A & Borghetti, J.R. Brasília: CNPq. Ministério da Ciência e Tecnologia. 399p.

ATUKEREN, P., AYDIN, S., USLU, E., GUMUSTAS, M.K., ÇAKATAY U.2010. Redox homeostasis of albumin in relation to alpha-lipoic acid and dihydrolipic acid. *Oxid. Med. Cell. Long.* 3, 206-213.

BAHTT, K. & FLORA, S.J.S. 2009. Oral co-administration of α-lipoic acid, quercetin and captopril prevents gallium arsenide toxicity in rats. *Environ.Toxicol. Pharmacol.* 28, 140-146.

BALDISSEROTO, B. & GOMES, L.C. 2010. Espécies nativas para piscicultura no Brasil. Editora UFSM, Santa Maria 2ª Ed. 606p.

BERGAMINI, C.M., GAMBETTI, S., DONDI, A., CERVELLATI, C. 2004. Oxygen, reactive oxygen species and tissue damage, *Curr. Pharm. Des.* 10, 1611-1626.

BIEWENGA, G.P.H., HAENEN, G.R.M.M., BAST, A. 1997. The pharmacology of the antioxidant lipoic acid. *Gen Pharmacol*., 29, 315-331

BLOCK, G.1991. Vitamin C and cancer prevention: The epidemiologic evidence. *Am. J. Clin. Nutr.* 53, 270S-282S.

BLOKHINA, O., VIROLAINEN E., FAGERSTEDT K.V. 2003. Antioxidant, oxidative damage and oxygen deprivation stress: a review. *An. Botany.* 91, 179-194.

BUSTAMANTE J., LODGE J.K., MARCOCCI, L., TRITSCHLER, H.J., PACKER, L., RHIN, B.H.1998. α-lipoic acid in liver metabolism and disease. *Free Rad. Biol. Med*. 24, 1023-1039.

BUTLER, J.A, HAGEN, T.M., MOREAU, R. 2009. Lipoic acid improves hypertriglyceridemia by stimulating tryacilglycerol clearance and downregulating liver triacylglycerol secretion. *Arch. Bioch. Biophy*s. 485, 63-71.

ÇAKATAY, U., KAYALI, R., SIVAS, A., TEKELI, F. 2005. Prooxidant activities of alpha-lipoic acid on oxidative protein damage in the aging rat heart muscle.*Arch. Geront. Ger*. 40, 231-240.

ÇAKATAY, U. 2006. Prooxidant actions of α-lipoic acid and dihydrolipoic acid. *Med. Hypoth*. 66, 110-117.

CARREAU, J.P. 1979. Biosynthesis of lipoic acid via unsaturated fatty acids. *Methods Enzymol.* 62, 152-158.

CAVALLI, R.O., DOMINGUES, E.C., HAMILTON, S. 2011. Desenvolvimento da produção de peixes em mar aberto no Brasil: possibilidades e desafios. *Rev. Bras. Zootec*. 40, 155-164.

CHAVES, I.S., LUVIZZOTTO-SANTOS R., SAMPAIO, L.A.N., BIANCHINI, A., MARTÍNEZ, P.E. 2006. Immune adaptative response induced by *Bicotylophora trachinoti* (Monogenea: Diclidophoridae) infestation in pompano *Trachinotus marginatus* (Perciformes: Carangidae). *Fish and Shellfish Immunol*. 21, 242-250.

COSTA, L.F., MIRANDA-FILHO, K.C., SEVERO, M.P., SAMPAIO, L.A. 2008. Tolerance of juvenile pompano *Trachinotus marginatus* to acute ammonia and nitrite exposure at different salinity levels. *Aquaculture*. 285, 270-272.

CHRISTIANSEN, R., GLETTE, J,.LIE, Ø,.TORRISSEN, O. J,.WAAGBø, R. 1995. Antioxidant status and immunity in Atlantic salmon, *Salmo salar* L., fed semi-purified diets with and without astaxanthin supplementation. *J. Fish Dis*.18, 317-328.

CUI, J., GUOWEI, L., YANG, R., SHI, Y. 2009. Lipoic acid attenuates high fat diet-induced chronic oxidative stress and immunosuppression in mice jejunum: A microarray analysis. *Cell. Immunol.* 260, 44-50.

CUNHA, V.L., RODRIGUES, R.V., OKAMOTO, M.H., SAMPAIO, L.A. 2009. Consumo de oxigênio pós-pandrial de juvenis do pampo *Trachinotus marginatus*. *Ciência Rural.* 39, 1257-1259.

DIAMOND JARED M. 2001. Armas, germes e aço. Record Ed. Rio de Janeiro, p 472.

DUARTE C.M., MARBÁ, N., HOLMER, M. 2007. Rapid domestication of marine species. *Science* 316, 382-383.

FAO. 2010. The State of World Fisheries and Aquaculture. Organización de las Naciones Unidas Para la Agricultura y la Alimentación. Fisheries Department. Roma. 197p.

FAO. 2012. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of United Nations. Fisheries and Aquaculture Department. Roma. 209p.

FISHER, L. G., PEREIRA, L.E.D., VIEIRA, J.P. 2004. Peixes estuarinos e costeiros. 2 Ed. Rio Grande, 130p.

GARCIA, L.O., BECKER, A.G., BERTUZZI, T., CUNHA, M.A., KOCHHANN, D., FINAMOR, I.A., RIFFEL, A.P.K., LLESUY, S., PAVANATO, M.A., BALDISSEROTTO, B. 2011. Oxidative stress parameters in silver catfish (*Rhamdia quelen*) juveniles infected with *Ichthyophthirius multifiliis* and maintained at different levels of water pH. *Vet. Parasitol*. 178 (1-2), 15-21.

GAO, J., KOSHIO, S., ISHIKAWA, M., YOKOYAMA, S., NGUYEN, B.T., MAMAUAG, R.E. 2013. Effect of oxidized fish oil and vitamin C supplementation on growth performance and reduction of oxidative stress in red sea bream Pagrus major. *Aquacult. Nutr.* 19, 35-44.

GORACA, A., HUK-KOLEGA, H., PIECHOTA, A., KLENIEWSKA, P., CIEJKA, E. SKIBSKA, B. 2011. Review: Lipoic acid- biological activity and therapeutic potential. *Pharmacol. Rep.* 63, 849-858.

HALLIWEL, B., GUTTERIDGE, J.M.C.2007. 4 Ed. Oxford, USA, 704p.

HAMRE, K., CHRISTIANSEN, R., WAAGBØ, R., MAAGE, A., TORSTENSEN, B.E., LYGREN, B.,LIE, Ø.,WATHNE, ALBREKTSEN, S. 2004. Antioxidant vitamins, minerals and lipid levels in diets for Atlantic salmon (*Salmo salar*, L.): effects on growth performance and fillet quality. *Aquacult. Nutr.* 10, 113-123.

HAMRE, K. 2011. Metabolism, interactions, requirements and functions of vitamin E in fish. *Aquacult. Nutr*. 17, 98-115.

HANDELMAN, G.J., HAN, D., TRITSCHLER, H., PACKER, L. 1994. Alpha-lipoic acid reduction by mammalian cells to the dithiol form, and release into the culture medium. Biochem. *Pharmacol.* 18, 1725-1730.

HARDY, R.W. & BARROWS, F.T. 2002. Diet formulation and manufacture. In: *Fish nutrition* . J.E. Halver and R.W. Hardy (eds) (3rd Ed.). London Academic Press. Chap 9: 505-600.

HILL, A.S., WERNER, J.A., ROGERS, Q.R., O'NEIL, S.L., CHRISTOPHER, M.M. 2004. Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats. *J. Animal Physio. Animal Nutr.* 88, 150-156.

HONG, W. & ZHANG, Q. 2003. Review of captive bred species and fry production of marine fish in China. *Aquaculture*. 227, 305-318.

HUONG, D.T.T., & IDE, T. 2008. Dietary lipoic acid-dependent changes in the activity and mRNA levels of hepatic lipogenic enzymes in rats. *British J. Nutr.*, 100, 79-87.

IBAMA 2007. Estatística da pesca 2007 Brasil: grandes regiões e unidades da federação. Brasília 113p.

JIANG, J., ZHENG,T., ZHOU, X-Q., LIU,Y. & FENG, L. 2009. Influence of glutamine and vitamin E on growth and antioxidant capacity of fish enterocytes. *Aquaculture Nutr.* 15, 409-414.

JORY, E.D., IVERSEN E.S., LEWIS, R.H. 1985. Culture of the fishes of the genus Trachinotus (Carangidae) in the Western Atlantic: prospects and problems. *J. World Mar. Soc.*16, 87-94.

KIM, M. S., PARK, J. Y., NAMKOONG, C., JANG, P. G. RYU J. W, SONG, H. S, YUN, J. Y., NAMGOONG, I. S., HA, J., PARK, I. S., LEE, I. K., VIOLLET, B., YOUN, J. H., LEE, H. K, LEE, K. U., 2004. Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nat. Med*. 10, 727-733.

KUSSMAUL, L., HIRST, J. 2006. The mechanism of superoxide production by NADH: ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. PNAS 103, 7607-7612.

LEÃO, J.C., GERACITANO, L.A., MONSERRAT, J.M., AMADO, L.L., YUNES, J.S. 2008. Microcystin-induced oxidative stress in *Laeonereis acuta* (Polychaeta, Nereididae). *Mar. Environ. Res.* 66, 92-94.

LEMOS V.M, VARELA JUNIOR A.S., VELASCO G., VIEIRA J.P.2011. The reproductive biology of plata pompano, *Trachinotus marginatus* (Teleostei: Carangidae), in southern Brazil. *Zoologia* 28, 603-609.

LYKKESFELDT, J. & SVENDSEN, O. 2007. Oxidants and antioxidant in diseases: oxidative stress in farm animals. *Vet. Journal* 173, 502-511.

LIMA, M.S.P., VIEIRA, J.P. 2009. Variação espaço-temporal da ictiofauna da zona de arrebentação da praia do Cassino, Rio Grande do Sul, Brasil. *Zoologia* 26, 499-510.

LONGARAY, M. 2012. Modulação da capacidade antioxidante e de detoxificação no peixe *Cyprinus carpio* (Ciprinidae) após tratamento com nanoecápsulas de ácido lipóico. Dissertação de mestrado, Universidade Federal do Rio Grande, Rio Grande. 54p

MACMASTER M.F., KLOTY, T.C., COBURN, J.F. 2004. Pompano mariculture - 2004. *Aquaculture Magazine* 30: 25-29.

MARTINS, A.C.S. 2011. Potencial antioxidante do ácido lipóico na ração do camarão *Litopenaeus vannamei* (Bonne, 1931). Dissertação de mestrado, Universidade Federal do Rio Grande, Rio Grande.62p

MENEZES, N.A. & FIGUEIREDO, J.L. 1980. Manual de peixes marinhos do sudeste do Brasil. Teleostei IV. Museu de Zoologia USP, São Paulo. 96 p

MOINI, H., PACKER, L., SARIS, N.-E.L. 2002. Review: Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid. *Toxicol. Appl. Pharmacol*. 182, 84-90.

MONSERRAT, J.M., VENTURA,J.L., FERREIRA, J.L.R., ACOSTA, D., GARCIA, M.L., RAMOS, P.B., MORAES, T.B., DOS SANTOS,L.C., AMADO, L.L. 2008. Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras paleatus* (Callychthyidae). *Comp. Biochem Physiol.* 148, 287-292.

MONTEIRO-NETO, C. & CUNHA, L.P.R.1990. Seasonal and ontogenetic variations in food habits of juvenile Trachinotus marginatus Cuvier, 1832 (Teleostei, Carangidae) in the surf zone of Cassino Beach, RS, Brazil. *Atlântica*, 12, 45-54.

MPA. 2012. Ministério da pesca e aquicultura. Boletim estatístico da pesca e aquicultura Brasil 2008-2009. Brasília, 82p.

NAVARI-IZZO, F., QUARTACCI, M., SGHERRI, C. 2002. Lipoic acid: a unique antioxidant in the detoxification of activated oxygen species. *Plant Physiol. Biochem*. 40, 463-470.

OKAMOTO, M. H., TESSER, M. B., LOUZADA, L. R., SANTOS, R. A., SAMPAIO, L. A. 2009. Benzocaína e eugenol como anestésicos para juvenis do pampo *Trachinotus marginatus*. *Ciência Rural.* 39, 866-870.

PACKER, L., WITT, E.H., TRITSCHLER, H.J.1995. Alpha-lipoic acid as a biological antioxidant. *Free Rad. Biol. Med.* 19, 227-250.

PACKER, L., KRAEMER, K., RIMBACH, G. 2001. Molecular Aspects of Lipoic Acid in the prevention of diabetes complications. *Nutrition* 17, 888-895.

PARK, K.H., TERJESEN, B.F., TESSER, M.B., PORTELLA, M.C., DABROWSKI, K. 2006. α-Lipoic acidenrichment partially reverses tissue ascorbic acid depletion in pacu (Piaractus mesopotamicus) fed vitamin C-devoid diets. *Fish Physiol. Biochem*. 32, 329-338.

QINGHUI, A., KANGSEN, M., ZHANG, C., XU, W., DUAN, Q., TAN, B., LIUFU, Z. 2004. Effects of dietary vitamin C on growth and immune response of Japanese seabass, *Lateolabrax japonicus*, *Aquaculture*. 242, 489-500.

RAMALHO, V.C. & JORGE, N. 2006. Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos. *Quim. Nova* 4, 755-760.

REED, L.J., FLORKIN, M., STOTZ, E.H. 1974. Multienzyme complex. *Acc. Chem. Res.* 7, 40-46.

ROMANO, L., TESSER, M.B., SAMAPIO, L.A., ABREU, P.C. 2012. Yersiniose em *Trachinotus marginatus* (pampo). Diagnóstico histopatológico e imuno-hitoquímico. *Arq.Bras. Med. Vet. e Zootec*. 64, 909-915.

ROOS, S.W., DALTON, D.A., KRAMER, S., CHRISTENSEN, B.L.2001. Physiological (antioxidant) responses of estuarine fishes to variability in dissolved oxygen. *Comp. Biochem. Physiol.* 130, 289-303.

SAMPAIO, L.A., TESSER, M.B., BURKERT, D. 2003. Tolerância de Juvenis do pampo *Trachinotus marginatus* (Teleostei, Carangidae) ao choque agudo de salinidade em laboratório. *Ciência Rural.* 33,757-761.

SANT'ANA, S., MANCINI-FILHO, J. 2000. Influence of the addition of antioxidants in vivo on the fatty acid composition of fish fillets, *Food Chem.* 68, 175-178.

SHEN, Q. W., JONES, C. S., KALCHAYANAND, N., ZHU, M. J., DU, M. 2005. Effect of dietary α-lipoic acid on growth, body composition, muscle pH, and AMP-actived protein kinase phosphorylation in mice. *J. Animal Sci.* 83, 2611-2617.

SIES, H. 1985. Oxidative stress: introductory remarks. In *Oxidative Stress*, ed. SIES, H., pp. 1-8. Academic Press, London.1985.

SIES, H. 1997. Oxidative stress: oxidant and antioxidant. *Exp. Physiol.* 82, 291-295.

SARGENT, J.R., BELL, J.G., BELL, M.V., HENDERSON, R.J., TOCHER, D.R. 1999. The metabolism of phospholipids and polyunsaturated fatty acids in fish. *In*: Lahlou, B., Vitello, P. (Eds.), Aquaculture: Fundamental and Applied Research, Coastal and Estuarine Studies, vol. 43. *American Geophysical Union, Washington*, pp. 103- 124.

SIMBULA G., COLUMBANO, A., LEDDA-COLUMBANO, G.M, SANNA, L., DEIDDA, M., DIANA, A., PIBIRI, M. 2007. Increased ROS generation and p53 activation in α-lipoic acid induced apoptosis of hepatoma cells. *Apoptosis*. 12,113-123.

STICKNEY, R.R.2009. Aquaculture: and introductory text. 2nd ed.CABI,Wallingford, U.K. 320pp.

TACON, A.G. J., METIAN, M. 2008. "Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects". *Aquaculture*. 285,146‐158.

TERJESEN B.F., PARK, K., TESSER, M.B., PORTELLA, M.C., ZHANG, Y., DABROWSKI, K. 2004. Lipoic acid and ascorbic acid affect plasma free amino acids selectively in the teleost fish pacu (*Piaractus mesopotamicus*). *J.Nutr*. 134, 2930-2934.

TRATTNER, S., PICKOVA, J., PARK, K.H., RINCHARD, J., DABROWSKI, K. 2007. Effects of α-lipoic and ascorbic acid on the muscle and brain fatty acids and antioxidant profile of the South American pacu *Piaractus mesopotamicus*. *Aquaculture.* 273, 158-164.

UNITED NATIONS. 2011. World population prospects: The 2010 revision, vol.1, Comprehensive Tables. Department of economic and social affairs. New York ST/ESA/SER.A/313.

WAAGBØ, R., GLETTE, J., RAA-NILSEN, E., SANDNES, K. 1993. Dietary vitamin C, immunity and disease resistance in Atlantic salmon (Salmo salar) *Fish Physiol. Biochem.* 12, 61-73

WANG, Y-J., CHIEN, Y-H., PAN, C-H. 2006. Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, Hyphessobrycon callistus, *Aquaculture.* 261, 641-648.

WANG, Z.J. & LUO, D.H. 2007. Antioxidant activities of different fractions of polysaccharide purified from Gynostemma pentaphyllum Makino. *Carbohydrate Polymers*, 68, 54-58.

ZHANG, W., CHEN, Q., MAI, K., XU, W., WANG, X., LIUFU, Z. 2010. Effects of dietary α-lipoic acid on the growth and antioxidative responses of juvenile abalone *Haliotis discus hannai* Ino. *Aquacul. Res*. 41,781-787.

ZHOU, J., WANG, W., WANG, A., HE, W., ZHOU, Q., LIU, Y., XU, J. 2009. Glutathione S-transferase in the white shrimp *Litopenaeus vannamei*: Characterization and regulation under pH stress. *Comp. Biochem. Physiol.* 150, 224-230.

1. Efeito da dose-resposta do antioxidante α-ácido lipóico no fígado e cérebro do pampo, *Trachinotus marginatus* (Pisces, Carangidae).

Dose-response effects of the antioxidant α-lipoic acid in the liver and brain of pompano Trachinotus marginatus (Pisces, Carangidae).

M.T. Kütter[1, 4], J.M. Monserrat[1, 3], R. A. Santos[2,3], M. B. Tesser[1, 4*]

[1] Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande- FURG, Av. Itália km 8, Rio Grande, RS, Cx. P. 474, CEP 96.200-970, Brazil. [2] Programa de Pós-Graduação em Ciências Fisiológicas- Fisiologia Animal Comparada, Universidade Federal do Rio Grande- FURG, Av. Itália km 8, Rio Grande, RS, Cx. P. 474, CEP 96.200-970, Brazil. [3] Instituto de Ciências Biológicas (ICB) FURG, Brazil. [4] Instituto de Oceanografia (IO-FURG), Laboratório de Nutrição de Organismos Aquáticos, FURG, Brazil.

Trabalho aceito para publicação no Journal of Applied Ichthyology em 18 outubro 2012, doi:10.1111/jai.12137

Resumo

O presente estudo avaliou o efeito de diferentes concentrações do anti-oxidante α-ácido lipóico administrado através de injeção intraperitoneal na capacidade de desintoxicação (atividade glutationa-S-transferase) e dano oxidativo (lipídios e proteínas) no peixe *Trachinotus marginatus*. O índice hepatossomatico e glicose plasmática não apresentaram diferença entre os tratamentos ($p>0,05$). No cérebro, Atividade GST foi significativamente maior ($p<0,05$) nos peixes injetados com 40 mg AL/kg em comparação ao controle. No músculo, a atividade da GST não foi influenciada pelo tratamento com AL ($p>0,05$); no fígado peixes tratados com 20 mg AL/kg apresentaram aumento significativo na atividade da GST. Entretanto, as maiores concentrações (40 e 60 mg AL/kg) apresentaram redução da atividade da GST no fígado quando comparadas ao grupo controle ($p>0,05$). As duas maiores concentrações de AL (40 e 60 mg AL/kg) apresentaram efeito oposto dependendo do órgão analisado: o AL foi antioxidante no cérebro, reduzindo a peroxidação lipídica ($p<0,05$), e pro-oxidante no fígado, aumentando a oxidação lipídica ($p<0,05$). Este último efeito foi acompanhado do aumento da concentração de ferro livre no fígado nas maiores concentrações. Estes resultados indicam a necessidade de avaliar com cautela o efeito dos antioxidantes nos organismos aquáticos, pois algumas concentrações e/ou órgãos podem não apresentar benefícios antioxidantes.

SUMMARY:

This study evaluated the effect of different doses of the antioxidant α-lipoic acid (LA) administered by intraperitoneal injection on the detoxifying capacity (activity of glutathione-S-transferase, GST) and oxidative damage (lipids and proteins) in the fish

Trachinotus marginatus. The plasma glucose levels showed that there were no differences between the treatments ($p>0.05$). In the brain, GST activity was significantly higher ($p<0.05$) in fish injected with 40 mg LA/kg when compared with the control group. In muscle, GST activity was not influenced by LA treatment ($p>0.05$); in liver, fish injected with 20 mg LA kg^{-1} showed higher GST activity than the control group ($p<0.05$). However, higher doses (40 and 60 mg LA kg^{-1}) led to a reduction of GST activity in the liver, which was comparable to that observed in the control group ($p>0.05$). The two highest LA doses (40 and 60 mg kg^{-1}) had opposite effects depending on the tissue examined: LA was antioxidant in the brain, reducing lipid peroxidation ($p<0.05$), and pro-oxidant in the liver, augmenting oxidative lipid damage ($p<0.05$). The latter effect was accompanied by an increase in the free iron concentration in the liver at higher LA doses. These results indicate the need to thoroughly evaluate the antioxidant effects on aquatic organisms because at some doses and/or in some organs, their beneficial effects can be lost.

INTRODUCTION

Oxidative stress is a biochemical condition that occurs when the concentration of reactive oxygen species (ROS) become greater than the concentration of antioxidants. This condition has potential deleterious effects if important biomolecules are oxidised (Sies, 1991). ROS are formed in several biochemical processes, including oxidative phosphorylation; ROS are also involved in the regulation of cellular signalling and gene expression and in the antimicrobial defence elicited by the immune system (Butler et al., 2009; Cui et al., 2009).

To protect cells and organs from oxidative damage caused by ROS, there are a variety of endogenous and exogenous antioxidant molecules that neutralise and/or intercept ROS. This group includes antioxidant enzymes that promote ROS degradation, proteins that bind metals (i.e., iron and copper) that catalyse ROS generation and non-enzymatic antioxidants derived from the diet and/or sinthetized like ascorbic acid, α-tocopherol, carotenoids, polyphenols and other compounds such as lipoic acid (LA) (Block, 1991; Wang and Luo, 2007; Jiang et al., 2009).

LA is a disulphate derived from octanoic acid, which occurs naturally in microorganisms, plants and animals (Navari-Izzo et al., 2002). This molecule is a cofactor of mitochondrial enzymes that catalyses oxidative decarboxylation in the Krebs cycle (Reed, 1974; Christensen, 1983). In the last decade, LA has been intensively investigated because of several therapeutic benefits associated with its antioxidant action (Biewenga et al., 1997). LA is utilised in the treatment of a variety of diseases: ascites (Diaz-Cruz et al., 2003), neurodegenerative diseases (Bergamini et al., 2004), diabetic polyneuropathy (Simbula et al., 2007), and metal contamination (Bahtt and Flora, 2009). LA is hydro- and lipophilic; this capability permits LA to act as an antioxidant at the lipid-water interface. Some of the antioxidant properties of LA include its interaction with the hydroxyl radical ($HO^{\cdot}$), an ROS responsible for induction of lipid peroxidation (LPO). The reduced form of LA, dihydrolipoic acid (DHLA), can act in the regeneration of other antioxidants, such as α-tocopherol, ascorbate and glutathione (Packer et al., 2001). Because of these characteristics, LA can be used to improve the antioxidant status of cells, allowing important applications for several purposes. For example, treatment with LA has been proposed as a preventive procedure to reduce the toxic effects of cyanotoxins in fish species that are important for aquaculture, such as carp *Cyprinus carpio* L. 1758 (Amado et al., 2011).

To our knowledge, published information regarding the effects of LA on fish is limited to a few studies (Otto et al., 1997; Terjesen et al., 2004; Park et al., 2006; Trattner et al., 2007; Monserrat et al., 2008; Amado et al., 2011), and none of these studies have analysed the relationship between the dose and the antioxidant capacity of LA. Only one study (Zhang et al., 2010) has studied this relationship in the abalone, *Haliotis discus hannai,* fed with an LA-enriched diet. These authors observed a positive correlation between the LA concentration in the diet and antioxidant capacity and growth, with LA doses ranging from 200 to 800 mg kg^{-1}. However, at higher doses (1,600 and 3,200 mg kg^{-1}), they observed reduced weight gain and a decrease in the total antioxidant capacity. Other authors (Hill et al., 2004) have also reported that high doses of LA can induce hepatocellular toxicity.

The Plata pompano *Trachinotus marginatus* Cuvier 1832 possesses biological characteristics that make this species a candidate to be used in aquaculture. It is an euryhaline species that tolerates a wide range of salinity (7-58) (Sampaio et al., 2003); it also demonstrates a tolerance to ammonia and nitrite when reared in isosmotic environment (Costa et al., 2008). Moreover, this species had a high metabolism (Cunha et al., 2009) making an interesting model to analyze the antioxidant responses in marine organisms. Thus, the aim of the present study was to investigate the effects elicited by different doses of dietary LA administered by intraperitoneal injection on antioxidant status of *T. marginatus*. Furthermore, the second objective of this study is evaluate if high doses of LA generate pro-oxidant effects in different organs of Plata pompano, likewise other animal groups (Çakatay, et al., 2005; Zhang et al., 2010)

MATERIAL AND METHODS

Fish and experimental conditions

T. marginatus of both sexes were captured at Cassino Beach (32 ^{o} 12'S; 52 ^{o} 10'W, Rio Grande, RS, Southern Brazil) by fisherman. The fish were transferred to the Aquaculture Marine Station at the Federal University of Rio Grande - FURG.

Total of 45 fish (mean weight 167.8±33.7 g) were acclimated for seven days prior to experimentation. They were stocked in five plastic tanks (9 fish per tank) with 200 L of seawater in a static system with a daily water exchange of 90% before the first meal. Each tank was cleaned daily by siphoning the bottom to remove deposited debris. Water parameters were measured daily. The salinity, temperature and dissolved oxygen were monitored using multi-parameter electrode (550 A, YSI, Yellow Springs, OH, USA). The pH was measured using electrode (FE20-FiveEasy ^{TM}, Mettler Toledo, Schwerzenbach, Switzerland). The ammonium was determined according to the methods presented by UNESCO (1983). Mean water salinity, temperature, oxygen saturation, pH and ammonium during the entire period (9 days) were 26.2 ± 0.6, 22.3 ± 0.8 ^{o}C, 83.9 ± 6.3%, 8.2 ± 0.2, 1.9 ± 0.5 mg L^{-1}, respectively. The photoperiod was fixed at 12 L:12 D. The fish were fed at 2 % of body mass for seven days, two times a day (0800 and 1600 hours), with prepared food containing: 42 % protein, 28% carbohydrate, 9% lipid, with 3,600 Kcal (Aqualine, Supra). The fish were not fed 24 h prior to LA infusion.

Lipoic acid was administered through a single intraperitoneal injection following

the methods of Amado et al. (2011). Briefly, each fish was anaesthetised with benzocaine (50 ppm) (Okamoto et al., 2009) and weighed prior to the intraperitoneal injection and the volume injected in each animal was adjusted to achieve the desired dose according to fish body weight. Each dose was measured using micropipette (1000 µL LABMATE^{+}, PZ HTL S.A, Danieszewka, Poland) than this volume was transferred to syringe. The fish were injected with one of the following doses of LA: saline solution (control), or 10, 20, 40, or 60 mg LA kg^{-1} body weight. Doses were selected ranging concentrations above and below the 40 mg LA kg^{-1} body weight tested in *Cyprinus carpio* by Amado et al. (2011). Twenty-four hour after the infusion, the fish were anaesthetised with the same procedure described above, and blood was sampled from the caudal vein using heparinized syringes. The animals were euthanized by cervical dislocation, and the brain, liver and muscle were dissected. These organs were immediately frozen in liquid nitrogen and stored at -80 °C until biochemical analysis.

The infusion was prepared according to Suh et al. (2004) by dissolving DL-**α**-lipoic acid (Sigma-Aldrich) in a solution containing 2 M 6 NaOH and 154 mM NaCl. After dissolution, the pH was adjusted to 7.40, and the final volume of the solution was adjusted with 2.154 M NaCl.

Organ homogenisation and blood samples preparation

Organs were homogenised (1:5 w/v) in a Tris–HCl (100 mM, pH 7.75) buffer with EDTA (2 mM) and Mg^{2+} (5 mM) (Amado *et al*., 2011). The homogenates were centrifuged at 10,000 x g for 20 min at 4 °C, and the supernatants resulting from this centrifugation were employed for all of the measurements described here. The total protein content was determined in triplicate with a microplate reader (BioTek LX 800),

using a commercially available kit based on the Biuret assay (550 nm). The protein content was expressed as mg proteins g tissue^{-1}.

Blood samples were centrifuged at 10,000 x g for 15 min at 4 °C; the resulting plasma was used for glucose determination, which was measured using a commercial reagent kit based on oxidation of 4-aminoantipirine (510 nm), using the same microplate reader cited above.

Activity of glutathione-S-transferase (GST)

The activity of the enzyme glutathione-S-transferase (GST) was determined following the conjugation of 1 mM glutathione (GSH) with 1 mM 1-chloro-2, 4-dinitrobenzene (CDNB), as described by Habig et al. (1974) and Habig and Jakoby (1981). Absorbance was measured at 340 nm in a spectrofluorometer with a microplate reader (Victor 2, Perkin Elmer, Waltham, MA, USA) at 25 °C. GST activity, which was normalised to the protein concentration in the supernatant, was expressed in nanomoles of conjugated GSH-CDNB mg proteins^{-1} min^{-1}.

Measurement of lipid peroxide concentration

Lipid peroxidation was determined according Oakes and Van der Kraak (2003). The method includes the derivatisation of a by-product of lipid peroxidation, malondyaldehyde, with thiobarbituric acid, followed by fluorescence measurement. Homogenised extract (40 µL) was added to a reaction mixture made with 150 µL of 20 % acetic acid, 150 µL of thiobarbituric acid (0.8 %), 50 µL of Milli Q water and 20 µL of sodium dodecyl sulphate (SDS, 8.1 %). The samples were heated at 95 °C for 30

min, and after cooling for 10 min, 100 µL of Milli Q water and 500 µL of n-butanol were added. After centrifugation (3,000 x g for 10 min at 15 °C), the organic phase (150 µL) was placed in a microplate reader, and the fluorescence was measured after excitation at 520 nm and emission at 580 nm. The concentration of thiobarbituric acid reactive substances (TBARS) (nmoles mg of wet tissue^{-1}) was determined using tetramethoxypropane (TMP, Across Organics) as a standard.

Protein oxidation

Measurement of the protein modified by oxidation (carbonyl groups) was performed in muscle samples using a commercial immunoassay kit (OxySelect^{TM}, Cell Biolabs Inc.) according to the manufacturer's instructions. Aliquots of muscle homogenate were added to a 96-well microtitre plate and incubated overnight at 4 °C to adsorb the protein to the bottom. The plate was then washed, and a solution of 2, 4-dinitrophenylhydrazine (DNP) was added to derivatise (45 minutes at room temperature in the dark) the carbonyl groups of the oxidised proteins side chains, forming 2, 4-dinitrophenylhydrazone (DNP-hydrazone). The DNP-derivatised proteins were then incubated with a mouse monoclonal antibody specific to the DNP moiety for 1 h at room temperature. Afterwards, a secondary antibody conjugated to horseradish peroxidise (HRP) was added, the samples were incubated with an HRP substrate, and the absorbance was read at 450 nm in a microplate reader spectrophotometer (BioTek LX 800). Readings were expressed as nmoles of carbonyl groups/mg of protein; a calibration curve was obtained by mixing different proportions of fully reduced and fully oxidised bovine serum albumin.

Free iron concentration

Free liver iron content was determined by the method described by Oakes and Van der Kraak (2003). Livers were homogenised in 10 % (w/v) 1.15% KCl. After measurement of the protein concentration, the samples were adjusted to 2 mg/ml. The free iron present in the samples reacted with 2, 2-bipyridyl to produce a complex that absorbs maximally at 520 nm. A standard of $FeCl_3.6\ H_2O$ was employed to express the data as µg Fe/mg of protein.

Data and statistical analyses

The data were expressed as the mean ± the standard error. Differences between treatments were evaluated by a one-way analysis of variances (ANOVA), followed by the Newman-Keuls post-hoc test or orthogonal contrast. The significance level adopted was 5% (α=0.05). ANOVA assumptions (normally distribution and variance homogeneity) were checked previously (Zar, 1984).

RESULTS

The plasma glucose exhibited no significant differences between treatments ($p>0.05$). Fish injected with a dose of 60 mg LA kg^{-1} showed a concentration of 84.9 ± 19.5 mg dl^{-1}, followed by fish injected with 10 mg LA kg^{-1} (76.3 ± 17.7 mg dl^{-1}) and finally by fish injected with 40 mg LA kg^{-1} (74.8 ± 11.0 mg dl^{-1}) and control fish (74.8 ± 16.1 mg dl^{-1}).

Fish that were administered an intraperitoneal injection with LA presented differences in GST activity in the organs analysed. Activity in the muscle was 10 times lower than in the brain and the liver (compare the scales in Fig. 1). In the brain, GST activity was significantly higher in the fish injected with 40 mg LA kg^{-1} when compared with the other treatments ($p<0.05$; Fig. 1a). GST activity in the liver was higher ($p<0.05$) in fish that were injected with 10 mg LA kg^{-1} and 20 mg LA kg^{-1}, while fish exposed to the highest dose (60 mg LA kg^{-1}) presented the lowest GST activity ($p<0.05$; Fig. 1b). In muscle, GST activity was not influenced by LA treatment ($p>0.05$; Fig. 1c).

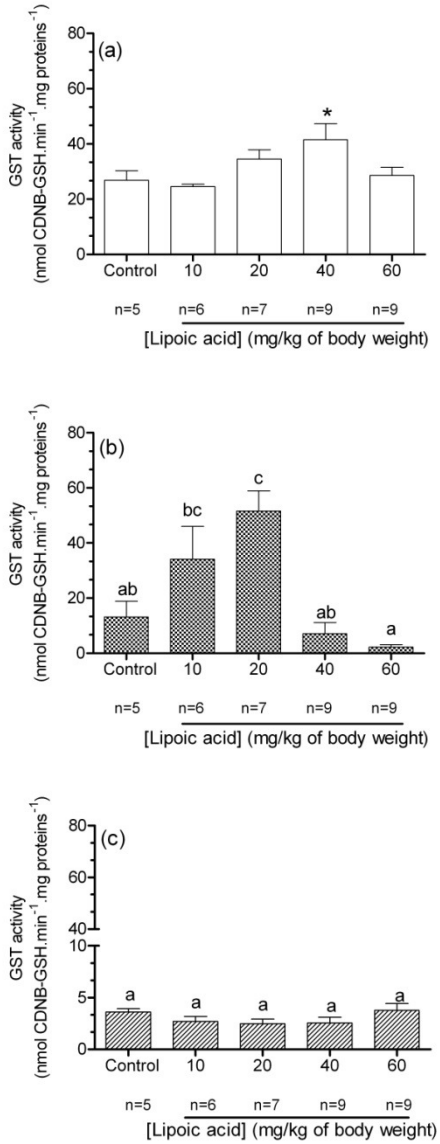

Figure 1. Activity of glutathione-S-transferase (GST) (nanomoles of conjugated GSH-CDNB mg of proteins^{-1} min^{-1}) in *Trachinotus marginatus*, 24h after injected intraperitoneally with lipoic acid (LA). **Control**: fish injected with saline solution. The data are expressed as the mean ± the standard error (n=5-9). Asterisks indicate significant difference ($p<0.05$), after comparison with orthogonal contrasts. Similar letters indicate the absence of significant differences ($p>0.05$) after Newman-Keuls post-hoc test. Analysed organs: **(a)** brain, **(b)** liver, **(c)** muscle.

Lipid peroxidation was also different in the organs analysed. The brains of fish injected with either 40 mg LA kg^{-1} or 60 mg LA kg^{-1} showed lower TBARS levels ($p<0.05$; Fig. 2a). In liver, fish from the control group and those injected with 10 mg LA kg^{-1} or 20 mg LA kg^{-1} presented similar TBARS levels ($p>0.05$), whereas the fish injected with either 40 mg LA kg^{-1} or 60 mg LA kg^{-1} showed TBARS levels that were four times higher than those of the control group ($p<0.05$; Fig. 2b). In muscle, no differences between the treatments were detected ($p>0.05$; Fig. 2c).

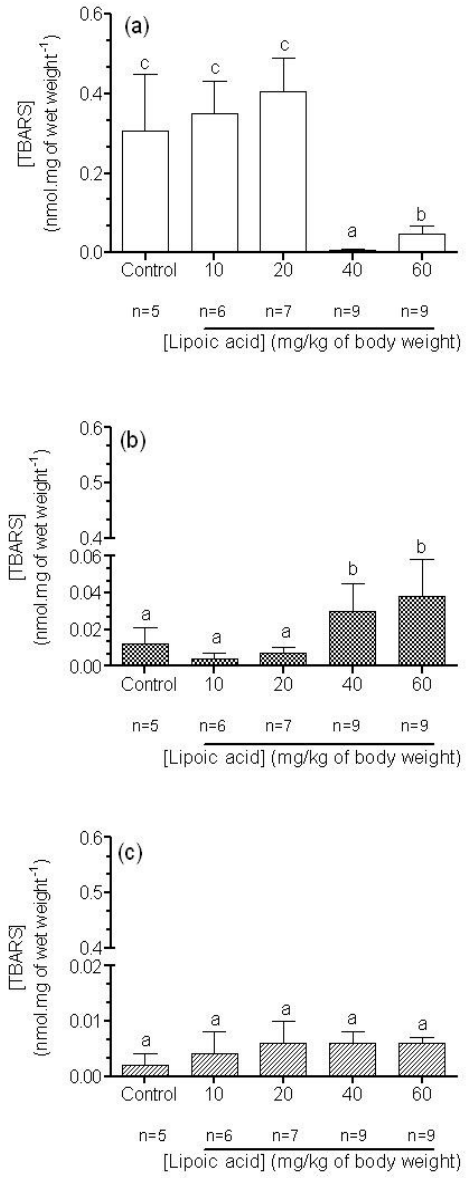

Figure 2. Lipid peroxidation (TBARS) in *Trachinotus marginatus*, 24h after injected intraperitoneally with lipoic acid (LA). **Control**: fish injected with saline solution. The data are expressed as the mean ± the standard error (n=5-9). Similar letters indicate the absence of significant differences ($p>0.05$) after Newman-Keuls post-hoc test. Analysed organs: **(a)** brain, **(b)** liver, **(c)** muscle.

Because muscle was the only organ that did not show any changes after LA injection, the protein carbonyl group concentration was determined. Again, no

differences between the groups were detected (p>0.05; Fig.3a). DHLA is the reduced form of LA; thus, DHLA can increase free iron levels (Çakatay et al., 2005). Moreover, the liver showed increased lipid peroxidation at the highest doses of LA (40 and 60 mg LA kg^{-1}). Therefore, we measured the free iron concentration in the liver. Indeed, free iron levels were higher in the livers of fish injected with 40 or 60 mg LA kg^{-1} (Fig. 3b).

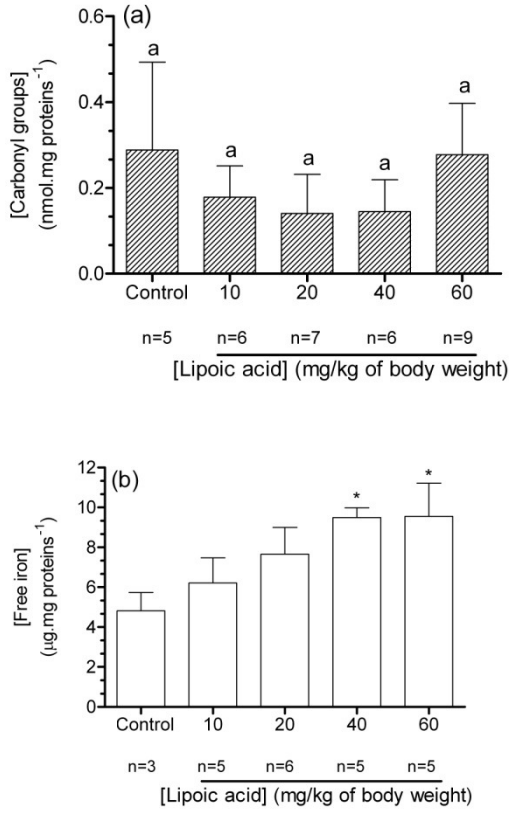

Figure 3. (a) Concentration of carbonyl groups in proteins in the muscle of *Trachinotus marginatus*, 24h after injected intraperitoneally with lipoic acid (LA). **Control**: fish injected with saline solution. The data are expressed as the mean ± the standard error (n=5-9). Similar letters indicate the absence of significant difference (p>0.05) after Newman-Keuls post-hoc test. **(b)** Same as **(a)** for the liver free iron concentration. The

data are expressed as the mean ± the standard error (n=3-5). Asterisks indicate significant difference ($p<0.05$), after comparison with orthogonal contrasts.

DISCUSSION

The treatment with LA via intaperitoneal injection has been shown to be an alternative route of antioxidant administration in fish species (Amado et al., 2011). In culture systems, fish may be susceptible to contamination and environmental variations, such as pH changes, which can cause oxidative stress (Zhou et al., 2009). According to Arivazhagan et al. (2002), LPO is the primary source of damage brought about by oxidative stress due to its propagative nature and the cytotoxicity of its metabolic by-products. In several organisms, including aquatic organisms, GST is one of the major xenobiotic detoxifying enzymes (Blanchette et al., 2007). Furthermore, the increase in glutathione GST activity should reduce the levels of LPO by-products because this enzyme has been reported to catalyse the conjugation of 4-hydroxynonenal with GSH in fish (Pharm et al., 2002).

In the present study the analysed biochemical variables these parameters showed different effects depending on the concentration of LA and the organs analysed. In the brain, LA reduced LPO and increased GST activity in fish injected with 40 mg LA kg^{-1}, suggesting that antioxidant treatment not only ameliorates lipid oxidative damage but also augments the ability to perform detoxification reactions. Moreover, many studies have indicated that LA can cross the blood-brain barrier (Arivazhagan et al., 2002; Chng et al., 2009; Freitas, 2009; Ferreira et al., 2009), which is very important for the neuroprotective ability of LA. The brain and neural system contain a source of ROS,

which is unique to these tissues, including excitatory amino acids and neurotransmitters whose metabolism produces ROS (Packer et al., 1997).

The livers from fish treated with doses of either 40 or 60 mg LA kg^{-1} showed a pro-oxidant effect. This pro-oxidant effect induced by LA was also found when the mollusc *Haliotis discus hannai ino* was fed with different concentrations of LA (Zhang et al., 2010). The antioxidant/pro-oxidant properties of LA, in different circumstances, are dependent on its concentration (Çakatay et al., 2005; Atukeren et al., 2010; Valdecantos et al. 2010). The pro-oxidant effect of LA could be due to its ability to reduce Fe^{3+} to Fe^{2+}, resulting in the generation of a hydroxyl radical via Fenton chemistry that may propagate chain reactions including lipid peroxidation (Goralska et al., 2003). DHLA also showed the ability to remove Fe from ferritin, increasing the levels of free iron (Çakatay et al., 2005). Moreover, according to Haramaki et al. (1997), mitochondrial dihydrolipoamide dehydrogenase reduces LA to dihydrolipoic acid (DHLA) at the expense of NADH and has preference for the R-enantiomer. In contrast, cytosolic glutathione reductase also catalyses the reduction of LA, with preference for the S-enantiomer and at the expense of NADPH. This phenomenon may explain why the brain and the liver of *T. marginatus* showed different responses to the same LA dosage. For example, the different mitochondrial content in these tissues may have consequences for the relative concentrations of NADH or NADPH.

Muscle was the only organ that did not show any effect after exposure to LA in terms of GST activity, TBARS concentration and concentration of carbonyl groups. Similar results have previously been reported for GST activity by Monserrat et al. (2008) in the muscle of the fish *Corydoras paleatus* Jenyns 1842 fed with LA for four weeks. However, the authors did report a lower concentration of protein carbonyl groups (64.3 kDa) in the LA-treated group. Fish muscle possesses biochemical

characteristics that reduce oxidative damage because it contains fewer lipids and more α-tocopherol than mammals (Hamre and Lie, 1995). Under these conditions, treatment with lipoic acid should lead to a decreased effect in the muscle compared with other organs with a more pro-oxidant condition. It is likely that a persistent treatment with LA would be necessary to observe a protective effect of this antioxidant on muscle.

LA modulates glucose metabolism and is effective in lowering insulin resistance and type 2 diabetes (Henriksen, 2006). For this reason, it has been used in therapeutic treatment in humans. In the present study, plasma glucose levels did not show differences between the experimental groups. Fish in this study were injected with LA and were fasted for the 24 h until blood sampling was performed. Thus, if hypoglycaemia occurred due to the injection of LA, the fish may have re-established its glucose levels either through glycogen degradation (via glycogenolysis) or by de novo glucose synthesis (through gluconeogenesis) before blood sampling.

Considering that the effects of lipoic acid are dependent on the organ analysed, and taking into account that doses above 20 mg kg^{-1} may exert detrimental effects on the liver due to a pro-oxidant effect of LA, it is recommended that greater than 20 mg kg^{-1} LA should be administered when using intraperitoneal injection.

ACKNOWLEGMENTS

J.M. Monserrat receives a productivity research fellowship from the Brazilian agency CNPq. Part of this study was supported by a grant from the Brazilian Agency FAPERGS (PROCOREDES VI Program, process number 0905160), which was given

to J.M. Monserrat. A grant from the Brazilian Agency CNPQ (UNIVERSAL, process number 473680/2010) given to M.B. Tesser.

REFERENCES

Amado, L. L.; Garcia, M. L.; Pereira, T. C. B.; Yunes, J. S.; Bogo, M. R.; Monserrat, J. M., 2011: Chemoprotection of lipoic acid against microcystin-induced toxicosis in common carp (*Cyprinus carpio*, Cyprinidae). Comp. Biochem. Physiol. **154 C**, 146-153.

Arivazhagan, P.; Thilakavathy, T.; Ramanathan, K.; Kumaran, S.; Panneerselvam, C., 2002: Effect of DL-α-lipoic acid on the status of lipid peroxidation and protein oxidation in various brain regions of aged rats. J. Nutrit. Biochem. **13**, 619-624.

Atukeren, P.; Aydin, S.; Uslu, E.; Gumustas, K.M.; Çakatay, U., 2010: Redox homeostasis of albumin in relation to alfa-lipoic acid and dihydrolipoic acid. Oxidativy Med. Cell. Longev. **3**, 206-213.

Bahtt, K.; Flora, S. J. S., 2009: Oral co-administration of α-lipoic acid, quercetin and captopril prevents gallium arsenide toxicity in rats. Environ. Toxicol. Pharm. **28**, 140-146.

Bergamini, C. M.; Gambetti, S.; Dondi, A.; Cervellati, C., 2004: Oxygen, reactive oxygen species and tissue damage. Curr. Pharm. Design. **10**, 1611-1626.

Biewenga, G. P. H.; Haenen, G. R. M. M.; Bast, A., 1997: The pharmacology of the antioxidant lipoic acid. Gen. Pharm. **29**, 315-331.

Blanchette, B.; Feng X.; Singh B. R., 2007: Marine glutathione S-transferase. Mar. Biotech. **9**, 513-542.

Block, G. 1991: Vitamin C and cancer prevention: The epidemiologic evidence. Amer. J. Clin. Nutrit. **53**, 270S-282S.

Butler, J. A.; Hagen T. M.; Moreau, R., 2009: Lipoic acid improves hypertriglyceridemia by stimulating triacylglycerol clearance and down regulating liver triacylglycerol secretion. Arch. Biochem. Biophy. **485**, 63-71.

Çakatay, U.; Kayali, R.; Sivas, A., Tekeli, F., 2005: Prooxidant activities of alfa-lipoic acid on oxidative protein damage in the aging rat heart muscle. Arch. Geront. Geriat. **40**, 231-240.

Chng, H. T.; New, L. S.; Neo, A. H.; Goh, C. W.; Browne, E. R.; Chan, E. C. Y., 2009: Distribution study of orally administered lipoic acid in rat brain tissues. Brain Res. **1251**, 80-86.

Christensen K. 1983: Vitamins, pp. 215–272 In: *Dynamic biochemistry of animal production*. Ed: Riis P.M., Elsevier Science, Amsterdam, 501pp. ISBN0444420525

Costa, L. F.; Miranda-Filho, K. C.; Severo, M.P.; Sampaio, L. A., 2008: Tolerance of juvenile pompano *Trachinotus marginatus* to acute ammonia and nitrite exposure at different salinity levels. Aquaculture **285**, 270-272.

Cui, J.; Guowei, L.; Yang, R.; Shi, Y., 2009: Lipoic acid attenuates high fat diet-induced chronic oxidative stress and immunosuppression in mice jejunum: A microarray analysis. Cell. Immunol. **260**, 44-50.

Cunha, V. L.; Rodrigues, R. V.; Okamoto, M. H.; Sampaio, L.A., 2009: Consumo de oxigênio pós-pandrial de juvenis do pampo *Trachinotus marginatus*. Ciênc. Rural **39**, 1257-1259.

Diaz-Cruz, A.; Serret, M.; Ramirez, G.; Ávila, E.; Guinzberg, R.; Pina, E., 2003: Prophylactic action of lipoic acid on oxidative stress and growth performance in broilers at risk of developing ascites syndrome. Avian Pathol. **32**, 645-653.

Ferreira, P. M. P.; Militão, G. C. G.; Freitas, R. M., 2009: Lipoic acid effects on lipid peroxidation level, superoxide dismutase activity and monoamines concentration in rat hippocampus. Neurosci. Lett. **464**, 131-134.

Freitas, R. M. 2009: The evaluation of effects of lipoic acid on the lipid peroxidation, nitrite formation and antioxidant enzymes in the hippocampus of rats after pilocarpine-induced seizures. Neurosci. Lett. **455**, 140-144.

Goralska, M.; Dackor, R.; Holley, B.; McGahan, M. C., 2003: Alpha lipoic acid changes iron uptake and storage in lens epithelial cells. Experiment. Eye Res. **76**, 241-248.

Habig, W. H.; Pabst, M. J.; Jakoby, W. B., 1974: Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Haramaki, N.; Han, D.; Handelman, G. J.; Tritschler, H. J.; Packer, L., 1997: Cytosolic and mitochondrial systems for NADH- and NADPH dependent reduction of alpha-lipoic acid. Free Radic. Biol. Med. **22**, 535**–**542.

Habig, W. H.; Jakoby W. B., 1981: Assays for differentiation of glutathione S-transferases. Meth. Enzymol. **77**, 398-405.

Hamre, K.; Lie, O., 1995: α-Tocopherol levels in different organs of Atlantic salmon (*Salmo salar* L.): Effect of smoltification, dietary levels of n-3 polyunsaturated fatty acids and vitamin E. Comp. Biochem. Physiol. **4**, 547-554.

Henriksen, E. J., 2006: Exercise training and the antioxidant a-lipoic acid in the treatment of insulin resistance and type 2 diabetes. Free Radic. Biol. Med. **40**, 3-12.

Hill, A. S.; Werner, J. A.; Rogers, Q. R.; O'Neil, S. L.; Christopher M. M., 2004: Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats. J. Animal Physiol. Animal Nutrit. **88**, 150-156.

Jiang, J.; Zheng, T.; Zhou, X.; Liu, Y.; Feng, L., 2009: Influence of glutamine and vitamin E on growth and antioxidant capacity of fish enterocytes. Aquacult. Nutrit.**15**, 409-414.

Monserrat, J. M.; Ventura, J. L.; Ferreira, R.; Acosta, D.; Garcia, M. L.; Ramos, P. B.; Moraes, T. B.; Dos-Santos, L. C.; Amado, L. L., 2008: Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras paleatus* (Callychthyidae). Comp. Biochem. Physiol. **148 C**, 287-292.

Navari-Izzo, F.; Quartacci, M.; Sgherri, C., 2002: Lipoic acid: a unique antioxidant in the detoxification of activated oxygen species. Plant Physiol. Biochem. **40**, 463-470.

Oakes, K. D.; Van der Kraak, G. J., 2003: Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. Aquat. Toxicol. **63**, 447-463.

Okamoto, M. H.; Tesser, M. B.; Louzada, L. R.; Santos, R. A.; Sampaio, L. A., 2009: Benzocaína e eugenol como anestésicos para juvenis do pampo *Trachinotus marginatus*. Ciênc. Rural **39**, 866-870.

Otto, D. M. E.; Sen, C. K.; Casley, W. L.; Moon T. W., 1997: Regulation of 3,30,4,40-tetrachlorobiphenyl induced cytochrome P450 metabolism by thiols in tissues of rainbow trout. Comp. Physiol. Biochem. **117 C**, 299-309.

Packer, L.; Tritschler, H. J.; Wessel, K., 1997: Neuroprotection by the metabolic antioxidant α-lipoic acid. Free Radic. Biol. **22**, 359-378.

Packer, L.; Kraemer, K.; Rimbach, G. 2001: Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrit. **17**, 888-895.

Park, K. H.; Terjesen, B. F.; Tesser, M. B.; Portella, M. C.; Dabrowski, K. 2006: α-Lipoic acid enrichment partially reverses tissue ascorbic acid depletion in pacu (*Piaractus mesopotamicus*) fed vitamin C-devoid diets. Fish Physiol. Biochem. **32**, 329-338.

Pharm, R. T.; Gardner, J. L.; Gallagher, E.P., 2002: Conjugation of 4-hydroxynonenal by largemouth bass (*Micropterus salmoides*) glutathione S-transferases. Mar. Environ. Res. **54**, 291-295.

Reed, L.J.; Florkin, M.; Stotz, E. H., 1974: Multienzyme complexes. Acc. Chem. Res. **7**, 40-46.

Sampaio, L. A.; Tesser, M.B.; Burkert, D., 2003: Tolerância de juvenis do pampo *Trachinotus marginatus* (Teleostei, Carangidae) ao choque agudo de salinidade em laboratório. Ciênc. Rural **33**,757-761.

Sies, H., 1991. Oxidative stress: oxidants and antioxidants. Ed. Sies, H., Academic Press, London, 650pp. ISBN 0126427623.

Simbula, G.; Columbano, A.; Ledda-Columbano, G.M.; Sanna, L.; Deidda, M.; Adiana, A.; Pibiri, M., 2007: Increased ROS generation and p53 activation in a-lipoic acid induced apoptosis of hepatoma cells. Apoptosis**12**,113–123.

Suh, J. H.; Shenvi, S. V.; Dixon, B. M.; Liu, H.; Jaiswal, A. K.; Liu, R. -M.; Hagen, T. M., 2004: Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. Proceed. Nation. Acad. Sci. U. S. A. **101**, 3381-3386.

Terjesen, B. F.; Park, K.; Tesser, M. B.; Portella, M. C.; Zhang, Y.; Dabrowski, K., 2004: Lipoic acid and ascorbic acid affect plasma free amino acids selectively in the teleost fish Pacu (*Piaractus mesopotamicus*). J. Nutrit. **134**, 2930-2934.

Trattner, S.; Pickova, J.; Park, K. H.; Rinchard, J.; Dabrowski, K., 2007: Effects of α-lipoic and ascorbic acid on the muscle and brain fatty acids and antioxidant profile of the South American pacu *Piaractus mesopotamicus*. Aquaculture **273**, 158-164.

UNESCO, 1983.Chemical methods for use in marine environmental monitoring. Manual and Guides 12, Intergovernmental Oceanographic Commission. Paris, France.

Valdecantos, M. P.; Perez-Matute, P.; Quintero, P.; Martinez, J.A., 2010: Vitamin C, resveratrol and lipoic acid actions on isolated rat liver mitochondria: all antioxidants but different. Redox Report **15**, 207-216.

Wang, Z. J.; Luo, D. H., 2007: Antioxidant activities of different fractions of polysaccharide purified from *Gynostemma pentaphyllum* Makino. Carbohyd. Polym., **68**, 54-58.

Zar, J.H., 1984: Bioestatistical Analysis, 2nd. ed. Ed. Prentice Hall, New Jersey, pp-718

Zhang, W.; Chen, Q.; Mai, K.; Xu, W.; Wang, X.; Liufu, Z., 2010: Effects of dietary α-lipoic acid on the growth and antioxidative responses of juvenile abalone *Haliotis discus hannai* Ino. Aquacult. Res.**41**, 781-787.

Zhou, J.; Wang, W.; Wang, A.; He, W.; Zhou, Q.; Liu, Y.; Xu, J., 2009: Glutathione S-transferase in the white shrimp *Litopenaeus vannamei*: Characterization and regulation under pH stress. Comp. Biochem. Physiol. **150 C**, 224-230.

2. Efeito da suplementação de ácido lipóico no crescimento, composição corporal e status antioxidante em pampo *Trachinotus marginatus* (Pisces, Carangidae).

Effects of dietary α-lipoic acid on growth, body composition and antioxidant status in the Plata pompano *Trachinotus marginatus* (Pisces, Carangidae).

Kütter M.T[a], Monserrat J.M[a,b], Primel E.G.[c], Caldas S.S [c], Tesser M.B[a,d*]

[a]Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande (FURG), Av. Itália km 8, Rio Grande, RS, Cx.P. 474, CEP 96.200-970, Brazil.

[b] Instituto de Ciências Biológicas (ICB), FURG, Brazil.

[c] Escola de Química e Alimentos (EQA), FURG, Brazil

[d] Instituto de Oceanografia (IO), Laboratório de Nutrição de Organismos Aquáticos, FURG, Brazil.

Artigo publicado no Journal Aquaculture (368-369), 29-35, dx.doi.org/10.1016/j.aquaculture.2012.09.010. On line 16 setembro 2012.

Resumo

Este estudo avaliou o efeito de diferentes doses de ácido lipóico (AL) suplementados na dieta sobre o crescimento, composição corporal e status antioxidante no peixe *Trachinotus marginatus*. Os peixes foram alimentados por 42 dias com dieta contendo diferentes níveis de AL: 0 mg AL kg^{-1}de ração, 316,4 mg AL kg^{-1} de ração, 524 mg AL kg^{-1} de ração, 890,4 mg AL kg^{-1} de ração e 1.367,3 mg AL kg^{-1} de ração. As maiores concentrações (890,4 e 1.367,3 mg AL kg^{-1} de ração) reduziram significativamente o crescimento, taxa de crescimento específico e eficiência proteica ($p<0,05$). Quando suplementado em altas doses o AL reduziu o consumo alimentar ($p<0,05$) enquanto que a taxa de conversão alimentar aumentou nestes tratamentos ($p<0,05$). A composição corporal dos grupos controle, 316,4 e 524 mg AL kg^{-1} de ração apresentou maior porcentagem de lipídio ($p<0,05$). A suplementação da dieta com AL aumentou a atividade da glutationa-S-transferase no cérebro na concentração de 890,4 mg AL kg^{-1} de ração e reduziu a peroxidação lipídica no músculo (todas as concentrações com AL) ($p<0,05$). Estes resultados obtidos em relação ao crescimento, status antioxidante, metabolismo de lipídio e proteína indicam que o AL pode ser suplementado na dieta para *T. marginatus* em concentrações entre 316,4 a 524 mg AL kg^{-1} de ração.

Palavras-chave: antioxidante, composição corporal, peixe, crescimento, lipoato

Abstract

This study evaluated the effect of four doses of α-lipoic acid (LA) from dietary supplementation on growth performance, body composition and anti-oxidant status in the fish *Trachinotus marginatus*. The fish fed for 42 days with a diet containing different concentrations of LA: 0 mg LA kg^{-1} dry food, 316.4 mg LA kg^{-1} dry food, 524

mg LA kg^{-1} dry food, 890.4 mg LA kg^{-1} dry food and 1367.3 mg LA kg^{-1} dry food. High doses (890.4 and 1367.3 mg LA kg^{-1}) of dietary LA significantly reduced the growth ($p<0.05$), the specific growth rate and the protein efficiency ratio. When administered in high doses of LA, supplementation significantly decreased the feed intake ($p<0.05$), although the feed conversion ratio increased ($p<0.05$) in these groups. The carcass composition exhibited the highest lipid values in the control group and in the fish exposed to 316.4 and 524 mg LA kg^{-1} dry food ($p<0.05$). The LA dietary supplementation increased the brain activity of glutathione-S-transferase (GST) when administered at a dose of 890.4 mg LA kg^{-1} dry food, and the lipid peroxidation in muscle was reduced (at all LA doses) ($p<0.05$). The results obtained regarding growth, antioxidant status and lipid and protein metabolism indicated that LA could be supplemented in diets for T. marginatusat doses between 316.4 and 524 mg LA kg^{-1} dry food.

Key-words: antioxidant, body composition, fish, growth, lipoate

1.Introduction

Recently, studies concerning reactive oxygen (ROS) and nitrogen species (RNS) have correlated the accumulation of these species to a variety of disorders and diseases (Roberts & Sindhu, 2009). Disequilibrium between oxidants and antioxidants can lead to the oxidation of important molecules and, thus affect cellular functions. The production of ROS/RNS occurs during oxidative metabolism, and the accumulation of by-products can occur when antioxidants are depleted and/or when the rate of ROS/RNS production surpasses the antioxidant capacity.

In farm animals oxidative stress may be involved in several pathological conditions, including conditions that are relevant for animal production and the general

welfare of the individuals (Lykkesfeldt & Svendsen 2007). During the rearing process, fish are susceptible to abrupt alterations in water quality. Variations in salinity, oxygen and temperature can alter the ability of the fish to detoxify ROS (Da Rocha et al. 2009). Furthermore, in aquatic environments, toxins produced by cyanobacteria, such as microcystin, can also affect the antioxidant capacity of fish (Amado et al 2011).

A pro-oxidant condition can be prevented by the administration of exogenous dietary antioxidants (Linnane & Eastwood, 2006). Exogenous non-enzymatic antioxidants include ascorbic acid, α-tocopherol, carotenoids and, more recently, α-lipoic acid (LA), which has been extensively used as a dietary supplement for humans, farm animals and pets (Block 1991; Merchie et al. 1996; Moini et al. 2002; Tocher et al. 2002).

Lipoic acid (LA) and its reduced form, dihydrolipoic acid (DHLA), are powerful antioxidants, exhibiting the capacity to regenerate other antioxidants. These species also act as a co-factor in oxidative decarboxylation reactions in the Krebs cycle and quench several reactive species in both lipid and aqueous domains (Packer et al. 1995). Because of these characteristics, LA is considered to be a “universal antioxidant” or “ideal antioxidant” (Kagan et al. 1992, Packer et al. 1995). Thus, LA has been employed for the treatment or prevention of many diseases in humans such as: diabetes neuropathy, type II diabetes, assuagement of the aging process, AIDS, ischemia-reperfusion, and liver and cardiovascular diseases (Parcker 1995; Biewenga, et al. 1997; Bustamante et al. 1998; Ghibuet al. 2009).

In contrast, the majority of research related to the use of LA in pets and farm animals is restricted to experimental studies (Diaz-Cruz et al. 2003; Wollin et at. 2004; Hill et al. 2004; Hamano 2002, Ibrahim et al. 2008). Information regarding the effects

of LA in aquatic organisms of interest to aquaculture is also restricted to experimental studies and is also limited to only a few species (Otto et al. 1997; Terjesen et al. 2004; Park et al. 2006; Trattner et al. 2007; Monserrat et al. 2008; Zhang et al. 2010; Amado et al. 2011). Although LA supplementation in the diet was investigated in two fish species the pacu *Piaractus mesopotamicus* and the corydora *Corydoras paleatus* (Terjesen et al. 2004; Park et al. 2006; Trattner et al. 2007; Monserrat et al. 2008), none of these studies evaluated the effects of different LA doses. Moreover, one of these studies reported that fish fed with LA exhibited a growth delay (Park et al., 2006). Regarding abalone (*Haliotis discus hannai*), Zhang et al.(2010) reported a growth increase at intermediate LA doses (800mg kg^{-1}) and growth inhibition at the highest LA dose (1,600 and 3,200 mg kg^{-1}).

The importance of the Plata pompano, *Trachinotus marginatus* (Carangidae) for aquaculture in Brazil should be considered in this context: species of the *Tachinotus* genus are considered appropriate for fish rearing in virtue of their quick adaptation to captivity, good tolerance to extreme environmental conditions and because of their fast growth. Several investigations have been conducted in *T. marginatus* because of the interest in its commercial production (Sampaio et al. 2003, Costa et al. 2008, Cunha et al. 2009, Okamoto et al. 2009). Thus, the aim of the present study was to investigate the effects elicited by different doses of dietary LA supplementation on the growth, body composition and antioxidant status of *T. marginatus*.

2. Material and methods

2.1 Experimental Fish

Juvenile Plata pompano (*Trachinotus marginatus*) (Cuvier, 1832) were caught at the Cassino Beach (32°12'S; 52°10'W, Rio Grande, RS, Southern Brazil). The fish were

transferred to the Aquaculture Marine Station at the Federal University of Rio Grande (FURG) and stored in 300-L plastic tanks containing 200 L of seawater (salinity: 35±2 ‰) at 24.5±1.2°C, with continuous aeration. During this period, the fish were fed three times a day (08:00, 12:00 and 16:00 h) with commercial food (NRD 0.8/1.2, INVE).

2.2 Experimental design

After seven days of acclimation in the stock tanks, the fish were transferred to test tanks. A total of 150 juvenile *T. marginatus* fish (1.55±0.40 g) were randomly distributed into 15 cylinder tanks (80 L) containing 60 L of water at a stock density of 10 fish per tank. The experimental tanks were arranged in a unique recirculation water system containing a biofilter, a protein skimmer and a UV lamp. Three tanks were assayed per treatment. Each tank was aerated via an air stone. The salinity, temperature and dissolved oxygen were monitored daily using multi-parameter electrode (550 A, YSI, Yellow Springs, OH, USA). The temperature was controlled using submersed heater thermostat. The chemical parameters were measured twice week. The amounts of nitrogenous compounds (ammonium, nitrite and nitrate) were determined according to the methods presented by UNESCO (1983), Benderschneider & Robinson 1952; Strikcland & Parsons (1972) respectively. The alkalinity was measured according to the method of APHA (1989), and the pH was measured using electrode (FE20-FiveEasy ™, Mettler Toledo, Schwerzenbach, Switzerland). The photoperiod was adjusted to 14L:10D. The fish were fed four times a day (08:00, 12:00, 16:00 and 20:00 h) to apparent satiety. After acclimation period the fish were fed for three days with control food to test ingestion and satiety, prior to the beginning of the experiment.

2.3 Experimental diet

The diet was formulated with purified ingredients to contain 47% of protein casein and gelatin and 11% lipid from soybean oil and fish oil (Table 1). Five

experimental diets were supplemented to contain different concentrations of LA: 0 mg LA kg^{-1} (Control), 500 mg LA kg^{-1}, 1,000 mg LA kg^{-1}, 1,750 mg LA kg^{-1} and 3,000 mg LA kg^{-1}. The ingredients were mixed with fish oil and soybean oil (1:1), and warm water was added to produce stiff dough. The dough was pelleted and dried in an oven at 40°C for 24 h. The dry pellet was than crushed to a small diameter and stored at -20 °C until used.

Table 1- Formulation and proximate composition (% dry matter, DM) of basal diet feed for the juvenile Plata pompano *Trachinotus marginatus*.

Test ingredients	***(%) DM***
Fish meal	5.0
Casein	40.0
Gelatin	10.0
Dextrin	25.0
Fish oil	5.5
Soybean oil	5.5
Cellulose	8.0
Vitamin and mineral mix	1.0
Chemical analysis	**(% DM)**
Crude protein	48.4
Crude lipid	12.0
Ash	2.8
Crude fiber	5.8

2.4 Measurement of lipoic acid in the diet

To measure the effective dose of LA in diet, 0.25 g of dry food was dissolved in 2mL of methanol (HPLC grade) and extracted overnight at room temperature. The samples were filtered through 0.20 µm Millipore filters. The quantification of LA was performed using liquid chromatography with mass spectrometric detection. A Waters Alliance 2695 Separations Module (Waters, Milford, USA) fitted with an autosampler,

a membrane degasser and a quaternary pump was used. Mass spectrometry was performed using a Micromass Quattro Micro API (Waters, Milford, USA) with an ESI interface. The analytical instrument control, data acquisition, and treatment were performed using Masslynx version 4.1 2005 software (Micromass, Waters, Miliford, MA, USA). The chromatographic separation was achieved using a mobile phase consisting of acetonitrilite and 0.1 % acetic acid (55:45, v/v), with a flow rate of 0.2 mL min^{-1}. The analytical column was maintained at 25°C. The run time was 4 min. The sample injection volume was 10 µL. The column effluent was connected to an electrospray ionization MS interface. The negative-ion mode was used to detect the lipoic acid. Typical interface conditions were as follows: capillary voltage, 3.5 kV; nebulizer and desolvation (drying gas) flow, 550 and 50 L h^{-1}, respectively; and source block and desolvation temperatures, 100 and 250 °C, respectively. The transitions with m/z 205.19>171.13 using a cone voltage of 18 and collision energy of 10, and those with m/z 205.19>92.82 using a cone energy of 22 and a collision energy of 13 were selected for MRM mode due to their high stability and intensity. The LA content in the diets was quantified using analytical curves (r>0.99) that ranged from 0.001 to 1.0 mg L^{-1}.

2.5 Growth trial

Juvenile Plata pompano were weighed on different days (0 and 42) after being anesthetized with benzocaine (50ppm) according to the method of Okamoto et al. (2009). After 42 days, the fish were anesthetized following the protocol mentioned above and euthanized by cervical dislocation. Prior to sampling, the fish were fasted for 24 h. The brain, liver and muscle were dissected. These organs were immediately frozen in liquid nitrogen, and stored at -80°C until biochemical analyses.

The growth performance variables were calculated using the following formulae:

Specific growth rate (SGR) = $[(\ln Wt_2 - \ln Wt_1) / (t_2 - t_1)] \times 100$

where Wt_1 and Wt_2 are the initial and final wet weights (g), respectively, within the considered time period (t, in days)

Feed conversion ratio (FCR) = dry weight of feed consumed (g) / wet weight gain of fish (g)

Protein efficiency ratio (PER) = final body weight (g) – initial body weight (g) / protein consumed (g)

Feed intake (FI) = [mean daily consumption (g)/ mean fish mass] × 100 were mean fish mass (fish mass at t_2 + fish mass at t_1)/2 and fish mass = mean body weight × fish number. Here: t1 and t2 represent the initial and final dates of the weight measurements, respectively.

2.6 Analytical procedure

Samples of the fish carcasses and diet were collected and stored frozen (- 20 °C) until the posterior analyses of the proximate composition. The proximate composition analysis was performed using the standard methods of AOAC (1995). The amount of moisture was determined by drying the samples at 105 °C for 24h. The ash was analyzed by combustion at 550 °C for 12h. The protein content was determined by measuring the nitrogen content (Nx6.25) using the Kjeldahl method, the lipid content was determined by ether extraction using a Soxhlet extractor, and the crude fiber contend was determined using acid digestion followed by calcination.

2.7 Organs homogenization

The organs were homogenized (1:6 w/v) in a Tris–HCl (100 mM, pH 7.75) buffer with EDTA (2 mM) and Mg^{2+} (5 mM) (Amado et al., 2011). The homogenates were centrifuged at 10000 × g for 20 min at 4 °C, and the supernatants were employed for all of the measurements described here. The total protein content was determined in triplicate using a commercial kit based on the Biuret assay (550 nm), using a microplate reader (BioTek LX 800) (da Rocha et al., 2009). The protein content was expressed as mg protein/g wet tissue.

2.8Activity of glutathione-S-transferase (GST)

One of the major enzymes involved in antioxidant activity is glutathione-S-transferase (GST). The GST are an multi-gene family of enzymes involved in the detoxification and activation of a variety of chemicals (Eaton & Bammler 1999; Sheehan et al. 2001).

The activity of the enzyme glutathione-S-transferase (GST) was determined following the conjugation of 1 mM glutathione (GSH) and 1 mM1-chloro-2,4-dinitrobenzene (CDNB), as described by Habiget al. (1974) and Habig & Jakoby (1981). The absorbance was measured at 340 nm using a spectrofluorometer with a microplate reader (Víctor 2, Perkin Elmer, MA, USA). The GST activity was normalized based on the protein concentration in the samples, and is expressed in nanomoles of conjugated GSH-CDNB/mg proteins/min.

2.9 Measurement of lipid peroxides concentration

Cellular macromolecules as polyunsaturated fatty acids are more susceptible to ROS oxidation, because of presence of double bonds with conjugated geometry (Bergamini et al. 2004). The degree of lipid peroxidation was determined according to

the method of Oakes & Kraak (2003). The method includes the derivatization of a by-product of lipid peroxidation, malondialdehyde (MDA), with thiobarbituric acid, which is followed by determination of the fluorescence. The homogenized extract (10 μL) was added to a reaction mixture containing 150 μL of 20 % acetic acid, 150 μL of thiobarbituric acid (0.8%), 50 μL of Milli Q water and 20 μL of sodium dodecyl sulfate (SDS, 8.1 %). The samples were heated to 95 °C for 30 min, and after cooling for 10 min, 100 μL of Milli Q water and 500 μL of n-butanol were added. After centrifugation (3,000 × g for 10 min at 15 °C), the organic phase (150 μL) was placed in a microplate reader and the fluorescence was registered after excitation at 520 nm and emission at 580 nm. The concentration of thiobarbituric acid-reactive substances (TBARS) (nmol/mg of wet tissue) was determined using tetramethoxypropane (TMP, Acros Organics) as a standard.

2.10 Data and statistical analyses

The data were expressed as the means ± standard deviation. The differences between the treatments were evaluated using a one-way (factor: LA doses) analysis of variances (ANOVA) followed by the Newman-Keuls *post-hoc* test or orthogonal contrast. The significance level adopted was 5% (α=0.05). The ANOVA assumptions (normality by Shapiro-Wilks and variance homogeneity by Levene) were previously validated (Zar, 1984).

3. Results

The mean water salinity, temperature and oxygen saturation over the entire experiment (42 days) were 31.8±2.8, 25.4±1.2°C and 91.7±2.6 %, respectively. The concentrations of nitrogenous compounds (ammonium, nitrite and nitrate) were 0.13±0.06 mg L^{-1}, 0.16±0.26 mg L^{-1} and 7.90± 4.65 mg L^{-1}. The mean alkalinity was 94.46±25.45 mg L^{-1}.

In the diet without LA, a mean value of 8.4 mg LA kg^{-1} was estimated and subtracted from the diets that included LA. Thus, in the rest of text, the control diet will henceforth be referred to as 0 mg LA kg^{-1}. The LA was presented in concentration below nominal supplementation. The diets showed the respectively LA concentration: 500 mg LA kg^{-1} (316.4±8.49 mg LA kg^{-1}), 1000 mg LA kg^{-1} LA (524±38.2 mg LA kg^{-1}), 1,750 mg LA kg^{-1}(890.4±66.3 mg LA kg^{-1}) and 3,000 mg LA kg^{-1}(1,367.3±185.8 mg LA kg^{-1}).

The fish survival, body weight, SGR, FCR, PER, FI and carcass composition data after 42 days of LA supplementation are shown in Table 2. No significant differences were observed in survival rates between the treatments ($p>0.05$). The final body weight was significantly higher in the control group, and groups fed 316 and 524 mg LA kg^{-1}dry food when compared with 890 ($p= 0.00$) and 1,367mg LA kg^{-1} dry food ($p=0.00$). The SGR exhibited the same response pattern as that of the final body weight ($p<0.05$). Treatment with 1,367mg LA kg^{-1} dry food resulted in an increase in the FCR when compared with control treatment ($p=0.02$). LA supplementation decreased the PER at a dose of 1,367 mg LA kg^{-1}dry food compared with the control conditions

(p=0.04). A lower FI was observed in the treatments 524, 890 and 1,367 mg LA kg^{-1}dry food when compared with that of the control group ($p<0.05$).

The carcass composition responded differently to LA supplementation. The moisture content increased upon treatment with 890 mg LA kg^{-1}dry food (p=0.02) and 1,367 mg LA kg^{-1}dry food (p=0.00) compared with that of the control group. The highest lipid percentage was observed in the control group, 316 mg LA kg^{-1}dry food and 524mg LA kg^{-1}dry food ($p<0.05$). No changes were observed in the protein contents between the treatments ($p>0.05$). The ash content was a significantly different (p=0.00) only in the group treated with 1,367mg LA kg^{-1}dry food, which exhibited a higher ash content than that of the rest of experimental groups.

Table 2- Survival, initial body weight, final body weight, specific growth rate, feed conversion, protein efficiency ratio, feed intake and carcass composition of *Trachinotus marginatus* after 42 day of being fed containing different concentration of lipoic acid. Data are expressed as the means ± standard deviation. Lipoic acid doses: 0 mg kg^{-1}, 316.4±8.49 mg kg^{-1}, 524±38.2 mg kg^{-1}, 890.4±66.3 mg kg^{-1} and 1,367.3±185.8 mg kg^{-1}.

	Experimental group				
	Control	**316 mg LA kg^{-1} dry food**	**524 mg LA kg^{-1} dry food**	**890 mg LA kg^{-1} dry food**	**1,367 mg LA kg^{-1} dry food**
Growth					
S (%)	96.7±5.8	90±10.0	100±0.0	93.3±5.8	93.3±5.8
Initial BW (g)	1.55±0.40	1.54±0.41	1.56±0.41	1.54±0.38	1.57±0.41
Final BW (g)	4.33±0.50^{a}	3.62± 0.16^{b}	3.97± 0.24^{ab}	3.12±0.16^{c}	2.34±0.01^{d}
SGR (% day^{-1})	2.38±0.2^{a}	1.98±0.08^{b}	2.16±0.14^{ab}	1.65±0.12^{c}	0.92±0.04^{d}
FCR	2.12±0.4^{a}	2.47±0.34^{ab}	1.87±0.25^{a}	2.62±0.56^{ab}	3.47±0.61^{b}
PER	1.13±0.20^{a}	0.94± 0.13^{ab}	1.23± 0.16^{a}	0.90±0.18^{ab}	0.70±0.12^{b}
FI	5.09±0.20^{a}	4.77±0.00^{ab}	4.27±0.29^{b}	4.40±0.23^{b}	3.10±0.31^{c}
Carcass composition (in wet weight)					

Moisture	62.89±0.97[a]	63.58±0.93[ab]	64.35±0.70[ab]	65.83±1.02[bc]	66.85±1.49[c]
Crude lipid	14.46±0.27[a]	14.19±0.36[a]	13.58±0.92[a]	11.13±0.55[b]	7.98±0.45[c]
Crude protein	17.26±0.25	16.99±0.32	17.50±0.56	17.55±0.41	18.03±1.13
Ash	7.67±0.53[a]	8.53±1.47[a]	7.80±1.56[a]	7.76±0.87[a]	11.01±0.28[b]

S- survival

SGR- specific growth rate

BW- body weight

FCR-feed conversion ratio

PER- protein efficiency ratio

FI- feed intake

Three samples per treatment were analyzed and data expressed as means ± 1 standard deviation. The means in the same row with the same superscript letter are not significantly different ($p>0.05$).

The dietary supplementation of LA induced GST activity in the brains of the fish fed with 890 mg LA kg^{-1}dry food ($p<0.05$). In the liver was observed a reduction in GST activity in fish fed with 316 mg LA kg^{-1}dry food ($p=0.02$) and 524 mg LA kg^{-1}dry food ($p=0.04$) in comparison to control group. No change in GST activity was observed in the muscle as a result of the LA treatment (Fig.1a-c).

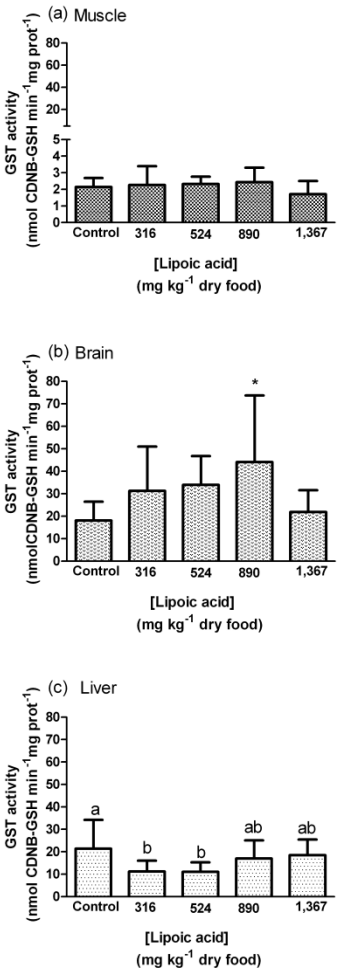

Figure 1. Activity of glutathione-S-transferase (GST) (nanomoles of conjugated GSH-CDNB/mg of proteins/min) in *Trachinotus marginatus* fed with lipoic acid. Lipoic acid doses: 0 mg kg^{-1}, 316.4±8.49 mg kg^{-1}, 524.0±38.2 mg kg^{-1}, 890.4±66.3 mg kg^{-1} and 1367.3±185.8 mg kg^{-1}. The data are expressed as the means ± standard deviation (n=6-9). The asterisks indicate significant differences ($p<0.05$) in respect to the control group

after orthogonal contrasts. Values accompanied by the same letters indicate the absence of statistical differences in the Newman-Keuls test ($p>0.05$). Analyzed organs: **(a)** muscle, **(b)** brain, **(c)** liver.

Lipid peroxidation was significantly reduced in the muscle ($p<0.05$) in the fish treated with 316, 890 or 1,367mg LA kg^{-1}dry food compared with that of the control group (Fig.2a). No significant effect ($p>0.05$) of LA on lipid peroxidation was observed in the brain and liver (Fig. 2b-c).

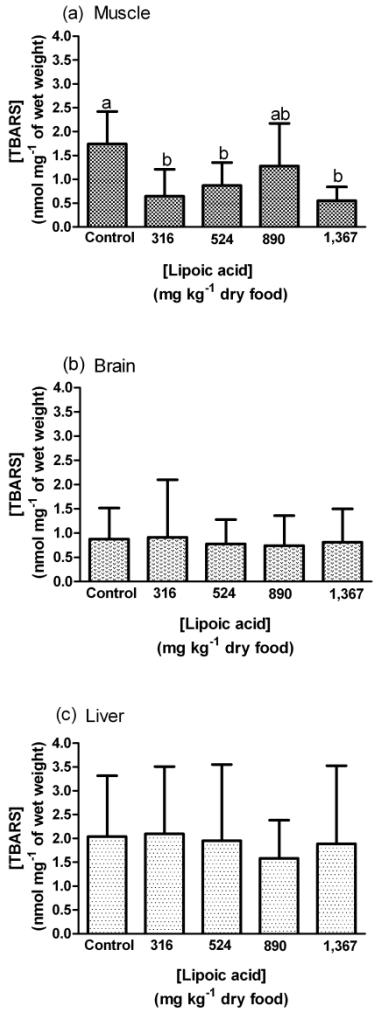

Figure 2. Lipid peroxidation (TBARS) in *Trachinotus marginatus* fed with lipoic acid. Lipoic acid doses: 0 mg kg^{-1}, 316.4±8.49 mg kg^{-1}, 524.0±38.2 mg kg^{-1}, 890.4±66.3 mg kg^{-1} and 1367.3±185.8 mg kg^{-1}. The data are expressed as the means ± standard deviation (n=6-9). Values accompanied by the same letters indicate the absence of statistical differences in the Newman-Keuls test ($p>0.05$). Analyzed organs: **(a)** muscle, **(b)** brain, **(c)** liver.

The protein concentration also exhibited different responses in the analyzed organs. No effects of LA ($p>0.05$) were observed in the muscle (Fig. 3a) or brain (Fig. 3c). In the liver, a reduction in the protein content ($p<0.05$) was observed in the fish treated with 890 mg LA kg^{-1}dry food and 1,367 mg LA kg^{-1}dry food compared with that of the fish treated with 316mg LA kg^{-1}dry food and 524mg LA kg^{-1}dry food, although none of LA doses resulted in a significant difference relative to the control group ($p>0.05$) (Fig. 3b).

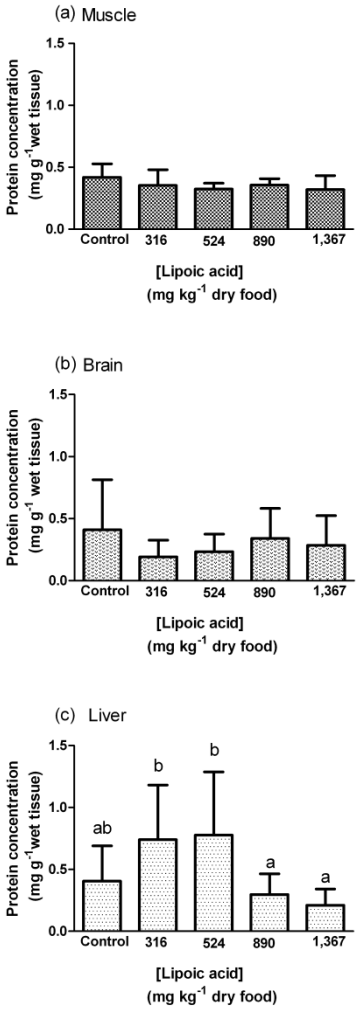

Figure 3- The total protein concentration of *Trachinotus marginatus* fed with lipoic acid. Lipoic acid doses: 0 mg kg^{-1}, 316.4±8.49 mg kg^{-1}, 524.0±38.2 mg kg^{-1}, 890.4±66.3 mg kg^{-1} and 1367.3±185.8 mg kg^{-1}. The data are expressed as mean ± standard deviation (n=6-9). The values accompanied by the same letters indicate the absence of significant differences in the Newman-Keuls test ($p>0.05$). Analyzed organs: **(a)** muscle, **(b)** brain, **(c)** liver.

4. Discussion

In the present study, we provide direct evidence that LA supplementation (measured effectively in the diet) affected the growth performance and anti-oxidant status of Plata pompano (*T. marginatus*). Previously, Terjesen et al. (2004) and Trattner et al. (2007) observed a decrease in weight that was caused by LA supplementation (1,000 mg LA kg^{-1}dry food, nominal concentration) in the fish *Piaractus mesopotamicus*. As mentioned above, Zhang et al. (2010) observed an increase in the growth of abalone (*Haliotis discus hannai*) following a dose of 800 mg LA kg^{-1}dry food (nominal concentration), whereas higher doses (1,600 and 3,200 mg LA kg^{-1}dry food, nominal concentration) resulted in a decrease in the growth. The FI and FCR founded in the present study suggest an anorexic effect of LA at high doses (890.4±66.3mg LA kg^{-1} and 1,367.3±185.8mg LA kg^{-1}dry food). A reduction in the feed intake was reported by Kim et al. (2004) and Shen et al. (2005) in rats and mice supplemented with LA. According to Kim et al. (2004), this decrease is related to reduction in the activity of the hypothalamic AMP-activated protein kinase that is responsible for signaling the cell to consume energy. In addition, some reports indicate that dietary LA enhances energy expenditure (Kim et al., 2004; Wang et al., 2010). Both of these factors can contribute to a reduction in the lipid content of the carcasses of fish in the groups fed with 890.4±66.3 mg LA kg^{-1} and 1,367.3±185.8 mg LA kg^{-1}dry food, and consequently, may influence the final fish weight. Similar results were previously reported by Shen et al. (2005) who observed a decrease in the lipid content in mouse carcasses after three weeks of LA supplementation in the diet. In addition, Zempleni et al. (1997) observed that rats treated chronically with LA exhibited decreased activity of biotin-dependent

carboxylase in the liver, which is an important enzyme in lipid synthesis. This fact also explains the decrease in the lipid percentage in the fish carcasses.

The protein concentration in the liver was higher in the groups treated with 316.4±8.49 mg LA kg^{-1} and 524±38.2 mg LA kg^{-1} dry food compared with that of the groups treated with 890.4±66.3 mg LA kg^{-1} and 1,367.3±185.8 mg LA kg^{-1} dry food. Gupte et al. (2009) demonstrated that LA increases the expression of heat shock protein in the skeletal muscle of rats. However, Terjesen et al. (2004) reported that fish feed with an LA supplementation diet exhibited a decrease in total free amino acid levels in the plasma. The authors have also suggested that protein utilization indices such as PER, apparent net protein utilization, and the expression of central enzymes of nitrogen metabolism should be monitored closely when doses of 1,000 mg LA kg^{-1} (nominal concentration) were included in the fish diet. Furthermore, a study conducted by Çakatai & Kayali (2005) show that LA can oxidize plasma proteins in aging rats. Considering the results describing the growth and PER obtained in this study, it can be infer that LA doses higher than 1,300 mg kg^{-1} may hamper pompano protein metabolism.

The brain is particularly susceptible to oxidative damage because of high levels of polyunsaturated fatty acid and iron coupled with low antioxidant activities, making it extremely susceptible to oxidative damage (O'Donnell & Lynch 1998). The increase in GST activity in the brain of Plata pompano fed diets supplemented with 890.4±66.3mg LA kg^{-1}dry food are in agreement with the results obtained by Monserrat et al. (2010), which indicated that the GST activity increased in fish brains after four weeks of LA supplementation in the diet (70 mg $kg^{-1}$of body mass, nominal concentration). Furthermore, Amado et al. (2011) showed that carp subjected to treatment with LA via intraperitoneal injection (40 mg kg^{-1} body mass) exhibited an increase in the gene

expression of GST isoforms. The increase in GST reported in the present study enhanced the detoxifying capacity of the brain. It has been show that GST catalyzes the conjugation of hydrophobic xenobiotic compounds that posses cytotoxic and/or genotoxic properties (Blanchette et al. 2007). Finally, many studies have indicated that LA can cross the blood-brain barrier (Arivazhagan, et al 2002; Freitas, 2009; Ferreira et al., 2009), which explains the effect of LA on the brain.

Overall, the findings reported in the present study indicate that LA exerts different effects on the analyzed organs regarding antioxidant status and metabolism. The increase in GST activity in the brain and the reduction in lipid peroxidation in the muscle should improve the antioxidant status of these organs. In fact, a reduction in the lipid levels in the carcass should include polyunsaturated fatty acids, thus impairing the occurrence of lipid peroxidation. Recently, Yasin et al. (2012) showed that α-lipoic acid and α-tocopherol exerts synergic effect on broiler's meat by reducing the lipid peroxidation and may have beneficial effects when included in the human diet.

To the best of our knowledge, the determination of LA in the diet of aquatic organisms was analyzed for the first time in this study, and the final LA concentration in the diet resulted in an average reduction of 52% compared with the nominal concentration. Thus, the effects of LA on the antioxidant status of an animal fed a nominal concentration should be used with caution because the present study demonstrated that doses below the nominal concentration can interfere with protein and lipid metabolism in fish. Based on the results, it is suggested that dietary LA can be supplemented in *T. marginatus* in doses between 316.4 and 524 mg LA kg^{-1} to reduce muscle lipid peroxidation without affecting growth performance.

5. Acknowledgments

Part of this study was supported by a grant from the Brazilian Agency FAPERGS (PROCOREDES VI Program, process number 0905160) given to J.M. Monserrat, and a grant from the Brazilian Agency CNPQ (UNIVERSAL, process number 473680/2010) given to M.B. Tesser. J.M. Monserrat and E.G. Primel received a productivity research fellowship from the Brazilian Agency CNPq (process PQ 306027/2009-7 and DT 311605/2009-5, respectively).

6. References

Amado, L. L, Garcia, M. L, Pereira, T. C. B., Yunes, J. S., Bogo, M. R., Monserrat, J. M., 2011.Chemoprotection of lipoic acid against microcystin-induced toxicosis in common carp (*Cyprinuscarpio*, Cyprinidae). *Comp. Biochem. Physiol.* C 154, 146-153.

Association of Official Analytical Chemists (1995) Official methods of analysis of AOAC International, vol I. Agriculture chemicals; contaminants, drugs, 16th edn. AOAC International, Arlington.

American Public Health Association (1985). Standard methods for the examination of water and waste water 13th ed. New-York.1193pp.

Arivazhagan, P., Thilakavathy, T., Ramanathan, K., Kumaran, S., Panneerselvam, C., 2002. Effect of DL-α-lipoic acid on the status of lipid peroxidation and protein oxidation in various brain regions of aged rats.*J. Nutrit. Biochem.* 13, 619-624.

Benderschneider, K., Robinson, R.J., 1952. A new spectrophotometric method for determination of nitrate in sea water, *J. Mar. Re*s. 1, 69-87.

Bergamini, C.M., Gambetti, S., Dondi, A., Cervellati, C. 2004. Oxygen, reactive oxygen species and tissue damage. *Curr. Pharmaceutical Design* 10, 1611-1626.

Biewenga, G. P. H., Haenen, G. R. M. M., Bast, A. 1997. The pharmacology of the antioxidant lipoic acid. *Gen Pharmacol*. 29, 315–331

Blanchette, B., Feng X., Singh B. R., 2009. Marine glutathione S-transferase. *Mar. Biotech.* 9, 513-542.

Block, G., 1991. Vitamin C and cancer prevention: the epidemiologic evidence. *The American Journal of Clinical Nutrition* 53, 270-282.

Bustamante, J., Lodge, J. K., Marcocci, L., Tritschler H. J., Packer, L., Rihn, B. 1998. α-lipoic acid in liver metabolism and disease. *Free radical Biology and Medicine*24, 123-139.

Çakatai, U., Kayali R. 2005.Plasma protein oxidation in aging rats after alpha-lipoic acid administration. *Biogerontology* 6, 87-93.

Costa, L. F., Miranda-Filho, K. C., Severo, M.P.,Sampaio, L.A. 2008. Tolerance of juvenile pompano *Trachinotus marginatus* to acute ammonia and nitrite exposure at different salinity levels. *Aquaculture* 285, 270-272.

Cunha, V. L., Rodrigues, R. V., Okamoto, M. H., Sampaio, L.A. 2009. Consumo de oxigênio pós-pandrial de juvenis do pampo *Trachinotus marginatus*. *Ciência Rural* 39, 1257-1259.

Da Rocha, A. M., De Freitas D. P. S., Burns M., Vieira, J. P., de La Torre, F. R., Monserrat, J. M., 2009. Seasonal and organ variations in antioxidant capacity, detoxifying competence and oxidative damage in freshwater and estuarine fishes from Southern Brazil. *Comparative Biochemistry and Physiology Part C* 150, 512-520.

Diaz-Cruz, A., Serret, M., Ramirez, G., Ávila, E., Guinzberg, R., Pina E. 2003. Prophylactic action of lipoic acid on oxidative stress and growth performance in broilers at risk of developing ascites syndrome. *Avian Pathology*, 32(6) : 645-653.

Eaton, D.L & Brammler T.K.1999. Concise review of the glutathione S-transferase and their significance in toxicology. *Toxicological Sciences* 49, 156-164.

Ferreira, P. M. P., Militão, G. C. G., Freitas, R. M. 2009. Lipoic acid effects on lipid peroxidation level, superoxide dismutase activity and monoamines concentration in rat hippocampus. *Neurosci. Lett*. 464, 131-134.

Freitas, R. M. 2009. The evaluation of effects of lipoic acid on the lipid peroxidation, nitrite formation and antioxidant enzymes in the hippocampus of rats after pilocarpine-induced seizures. *Neurosci. Lett*. 455, 140-144.

Garling, D. L., Wilson, R. P. 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus.J. Nutr.* 106, 1368-1375.

Gupte, A. A., Bomhoff, G. L, Morris, J. K., Gorres, B. K., Geiger, P. C. 2009. Lipoic acid increase heat shock protein expression and inhibits stress kinase activation to improve insulin signaling in skeletal muscle from high-fat-fed rats. *J. Appl. Physiol.* 106, 1425-1434.

Ghibu, S., Richard, C., Vergely, C., Zeller, M., Cottin, Y., Rochette, L. 2009. Antioxidant properties of an endogenous thiol: Alpha-lipoic acid, useful in the prevention of cardiovascular diseases. *J. Cardiovasc. Pharmacol*, 54, 391-398.

Habig, W. H., Jakoby, W.B. 1981. Assays for differentiation of glutathione S-transferases. *Methods in Enzymology*77, 398-405.

Habig, W. H., Pabst, M. J., Jakoby, W. B. 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J*ournal of Biological Chemistry* 249, 7130-7139.

Hamano, Y. 2002. Influence of lipoic acid on lipid metabolism and b-adrenergic response to intravenous or oral administration of clenbuterol in broiler chickens. *Reprod. Nutr. Dev*., 42: 307–316.

Hill, A. S., Werner, J. A., Rogers, Q. R., O'Neil, S. L., Christopher, M. M. 2004. Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats. *Journal of Animal Physiology and Animal Nutrition* 88, 150-156.

Ibrahim, S. F., Osman, K., Das, S., Othman A. M., Majid, N. A., Rahman, M. P. A. 2008. A study of antioxidant effect of alfa lipoc acid on sperm quality. *Clinics* 64, 545-550.

Kagan, V. E., Shvedova A., Serbinova E., Khan, S., Swanson, C., Powell, R., Packer L., 1992. Dihydrilipoic acid: A universal antioxidant both in the membrane and in the aqueous phase. *Biochemical Pharmacology* 44, 1637-1649.

Kim, M. S., Park, J. Y., Namkoong, C., Jang, P. G. Ryu J. W, Song, H. S, Yun, J. Y., Namgoong, I. S., Ha, J., Park, I. S., Lee, I. K., Viollet, B., Youn, J. H., Lee, H. K, Lee,

K. U., 2004. Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nat. Med*. 10:727–733.

Lykkesfeldt & Svendsen 2007. Oxidants and antioxidant in diseases:oxidative stress in farm animals. *Veterinary Journal* 173, 502-511.

Linnane, A. W., Eastwood, H. 2006. Cellular redox regulation and prooxidant signaling systems: A new perspective on the free radical theory of aging. *Annals of the New York Academy of Sciences*1067, 47-55.

Merchie, G., Lavens, P., Storch, V., Übel, U., Nelis, H., Leenheer, A. De., Sorgeloos, P. 1996. Influence of dietary vitamin C dosage on turbot (*Scophthalmus maximus*) and European sea bass (*Dicentrarchus labrax*) nursery stages. *Comparative Biochemistry and Physiology* 114a, 123-133.

Moini, H., Packer, L., Saris, Nils- Erik L. 2002. Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid. *Toxicology and Applied Pharmacology* 182, 84-90.

Monserrat, J. M., Ventura, J. L., Ferreira, R., Acosta, D., Garcia, M. L., Ramos, P. B., Moraes, T. B., Dos-Santos, L. C., Amado, L. L. 2008. Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras paleatus* (Callychthyidae). *Comp. Biochem. Physiol*. C 148, 287-292.

Oakes, K. D., Van der Kraak, G. J. 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *AquaticToxicology*63, 447-463.

O'Donnell, E., Lynch M.A. 1998. Dietary antioxidant supplementation reverses aged-related neuronal changes. *Neurobiol Ageing* 19,416-467.

Okamoto, M. H., Tesser, M. B., Louzada, L. R., Santos, R. A., Sampaio, L. A., 2009. Benzocaína e eugenol como anestésicos para juvenis do pampo *Trachinotus marginatus*. *Ciência Rural* 39, 866-870.

Otto, D. M. E., Sen, C. K., Casley, W. L, Moon T. W. 1997. Regulation of 3,30,4,40-tetrachlorobiphenyl induced cytochrome P450 metabolism by thiols in tissues of rainbow trout. *Comp. Physiol. Biochem*. C 117, 299-309.

Packer, L., Witt, E. H., Tritschler H. J. 1995.Alpha-lipoic acid as a biological antioxidant. *Free Radical Biology and Medicine*19,227-250.

Park, K. H., Terjesen, B. F., Tesser, M. B., Portella, M. C., Dabrowski, K. 2006. α-Lipoic acid enrichment partially reverses tissue ascorbic acid depletion in pacu (*Piaractus mesopotamicus*) fed vitamin C-devoid diets. *Fish Physiol. Biochem*.32, 329-338.

Roberts, C. K., Sindhu, K. K. 2009. Oxidative stress and metabolic syndrome. *Life sciences* 84, 705-712.

Sampaio, L. A., Tesser, M. B., Burkert, D. 2003. Tolerância de juvenis do pampo *Trachinotus marginatus* (Teleostei, Carangidae) ao choque agudo de salinidade em laboratório. *Ciência Rural* 33,757-761.

Sheehan, D., Meade, G., Foley, V.M., Dowd, C.A. 2001. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of ancient enzyme superfamily. *Biochem. J.* 360,1-16.

Shen, Q. W., Jones, C. S., Kalchayanand, N., Zhu, M. J., Du, M. 2005. Effect of dietary α-lipoic acid on growth, body composition, muscle pH, and AMP-actived protein kinase phosphorylation in mice. *Journal Animal Science*83, 2611-2617.

Strickland, J. D. H., Parsons, T. R. 1972. A practical handbook of seawater analysis.

Fishery Research Board of Canada. 2ª ed. Ottawa: Bulletin 167, 311p.

Terjesen, B. F., Park, K., Tesser, M. B., Portella, M. C., Zhang, Y., Dabrowski, K., 2004. Lipoic acid and ascorbic acid affect plasma free amino acids selectively in the teleost fish Pacu (*Piaractus mesopotamicus*). *Journal of Nutrition* 134, 2930-2934.

Tocher, D. R., Mourente, G., Van Der Eecken. A., Ovjemo, J. O., Diaz, E., Bell, J. G., Geurden, I., Lavens, P., Olsen, Y. 2002. Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (Scophthalmusmaximus L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). *Aquaculture Nutrition* 8, 195-207.

Trattner, S., Pickova, J., Park, K. H., Rinchard, J., Dabrowski, K. 2007. Effects of α-lipoic and ascorbic acid on the muscle and brain fatty acids and antioxidant profile of the South American pacu *Piaractus mesopotamicus*. *Aquaculture* 273, 158-164.

UNESCO, 1983. Chemical methods for use in marine environmental monitoring. Manual and Guides 12, Intergovernmental Oceanographic Commission. Paris, France.

Wollin, S. D., Wang, Y., Kubow, S., Jones P. J. H. 2004. Effects of a medium chain triglyceride oil mixture and α-lipoic acid diet on body composition, antioxidant status, and plasma lipid levels in the Golden Syrian hamster. *Journal of Nutritional Biochemistry* 15, 402-410.

Wang, Y., Xiaojie, L. Yuming, G., Lawrence, C., Xinfu, G. 2010. α-lipoic acid increase energy expenditure by enhancing adenosine monophosphate-actived protein kinase-peroxisome proliferators-actived receptor-γ coativator 1-α signaling in skeletal muscle of aged mice. *Metabolism clinical end experimental*59, 967-976.

Yasin, M., Asghar, A., Anjum, F. M., Butt, M. S., Khan, M. I., Arshad, M. S., Shahid, M., El-Ghorab, A. H., Shibamoto, T. 2012. Oxidative stability enhancement of broiler bird meats with α-lipoic acid α-tocopherol acetate supplemented feed. *Food Chemistry*, 131, 768-773.

Zar, J.H. 1984. Bioestatistical Analysis, 2nd. ed. Ed. Prentice Hall, New Jersey.

Zempleni, J., Trusty T. A., Mock D.M.1997. Lipoic acid reduces the activities of biotin-dependent carboxylases in rat liver. *J. Nutr.* 127, 1776-1781.

Zhang, W., Chen, Q., Mai, K., Xu, W., Wang, X. ,Liufu, Z., 2010.Effects of dietary α-lipoic acid on the growth and antioxidative responses of juvenile abalone *Haliotis discus hannai Ino. Aquaculture Research* 41,781-787.

3.Tempo de resposta do *status* antioxidante do peixe *Trachinotus marginatus* (Pisces, Carangidade) alimentado com dieta suplementada com α-ácido lipóico.

Time response of antioxidant status to diet supplementation with α-lipoic acid in *Trachinotus marginatus* (Pisces, Carangidae)

Kütter M.T[a*], Zamora-Sillero J[a], Tesser M.B[a,c],Monserrat J.M[a,b],

[a]Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande (FURG), Av. Itália km 8, Rio Grande, RS, Cx.P. 474, CEP 96.200-970, Brazil.

[b] Instituto de Ciências Biológicas (ICB), FURG, Brazil.

[c] Instituto de Oceanografia (IO), Laboratório de Nutrição de Organismos Aquáticos, FURG, Brazil.

Juan Zamora-Sillero- Phone: 55-5332368042; fax:55 5332368042; zamorasillero@gmail.com

Marcelo Borges Tesser- Phone: 55-5332368042; fax:55-5332368042; mbtesser@gmail.com

José Maria Monserrat- Phone:55-5332935196; fax: 55-5332336850;josemonserrat@gmail.com

Mateus Tavares Kütter*

***Corresponding author**: Phone: 55-5332368042; fax:55-5332368042

Email address: kutter.m.t@gmail.com

Artigo em preparação para Archives of Animal Nutrition

Resumo

O presente trabalho avaliou a resposta ao longo do tempo sobre a condição antioxidante do peixe *Trachinotus marginatus* alimentado com dieta suplementada com α-ácido lipóico (AL). Os peixes foram aliementados durante 30 dias com dieta sem adição de AL (controle) ou 524 mg AL/kg ração. O cérebro, fígado, brânquia e músculo foram coletados aos 10, 20 e 30 dias de alimentação. A atividade da glutationa-S-transferase (GST) no cérebro não apresentou diferenças significativas entre os tratamentos ($p>0,05$). No fígado o grupo AL registrou aumento da GST em comparação ao grupo controle aos 30 dias ($p<0,05$). A peroxidação lipídica reduziu significativamente no grupo AL e em relação ao grupo controle aos 30 dias no músculo ($p<0,05$). Os resultados obtidos sugerem que é necessário ao menos 30 dias de suplementação da dieta com AL para aumentar a capacidade antioxidante do peixe.

Summary

This study evaluated along the time the effect of antioxidant status in the fish *Trachinotus marginatus* fed with diet supplemented with α-lipoic acid (LA). The fish were fed for 30 days with diet without supplementation (control) or with 524 mg LA/kg dry food. The brain, liver, gill and muscle were sampled at 10, 20 and 30 days. The glutathione-S-transferase (GST) activity in brain showed no significant differences between groups along the time ($p>0.05$). In the liver, the LA group exhibited an increase in GST activity at day 30 in comparison to control groups ($p<0.05$). The lipid peroxidation in muscle exhibited a decrease in AL group at day 30 in comparison to control group ($p<0.05$). The results obtained suggest that are necessary almost 30 days to LA diet supplementation enhances antioxidant status of fish.

Keywords: fish, glutathione, lipid peroxidation, lipoate.

Introduction

Fish like all aerobic organisms are also susceptible to deleterious effects caused by accumulation of reactive oxygen species and have substantial internal defenses that are well described in the literature (Ross et al. 2001; Martínez-Álvarez et al. 2005). The reactive oxygen species are formed during aerobic metabolism by several biochemical processes, including oxidative phosphorylation. It has been suggested that the accumulation of these compounds participate broadly in the pathogenesis of a wide range of pathologies (Soffler 2007; Roberts & Sindhu 2009).

Cellular macromolecules as, proteins, DNA and lipids are targets to ROS damage. The fish tissues contain large quantities of polyunsaturated fatty acids (PUFAs) that are more susceptible to ROS oxidation (Bergamini et al. 2004). The peroxidation of unsaturated lipids in cell membranes produces unstable lipid hydroperoxides, that when decomposed present high reactivity, affecting the cell integrity.

The non-enzymatic pathways whereby the reactivity of ROS can be neutralized are represented mainly by diet antioxidants such as retinol, ascorbic acid and α-tocopherol, carotenoids and lipoic acid (LA). The LA and its reduced form dihydrolipoic acid (DHLA) are powerful antioxidants, scavenging hydroxyl radical, hipochlorous acid, peroxynitrite and singlet oxygen (Packer et al. 2001). Lipoic acid is readily absorbed from the diet and converted to DHLA in many tissues. The DHLA possess ability to regenerate endogenous antioxidants such as α-tocopherol, ascorbic acid and glutathione (Packer et al. 2001). Moreover, the relatively low molecular mass of LA makes it soluble in both water and fat, permitting an action across the membrane, strengthening the antioxidant network of cell (Navari-Izzo et al. 2002). Because of these

antioxidant attributes, this compound has been employed as a therapeutic for treatment of neurodegenerative disorders, and also liver and cardiovascular dysfunctions (Packer et al. 1997; Bustamante et al. 1998; Wollin & Jones 2003).

In fish different factors such as: diet, environmental conditions, age, xenobiotic compounds and parasites can influence antioxidant defense (Martínez-Álvarez et al. 2005). Furthermore, during aquaculture procedure fish is exposed to many of these factors making susceptible to reduce defense against ROS.

Recently studies in aquatic organisms have been carried out and demonstrated the improvement of antioxidant status by LA (Monserrat et al. 2008; Zhang et al. 2010; Amado et al. 2011; Kütter et al. 2012). Therefore, the present study analyzed the period necessary to dietary LA supplementation in fish to elict an antioxidant response.

2. Material and methods

2.1 Fish and experimental design

Juvenile Plata pompano (*Trachinotus marginatus*) (Cuvier, 1832) were caught at the Cassino Beach (32°12'S; 52°10'W, Rio Grande, RS, Southern Brazil)- Environment Ministery permission- SISBIO 269021. The fish were transferred to the Aquaculture Marine Station at the Universidade Federal do Rio Grande (FURG) and stored in a 310-L plastic tank containing 200 L of seawater (30 ‰, 18.8 ± 2.0 ºC) with continuous aeration and submersed heater thermostat. Fish were stock at density of 60 fish per tank. They were arranged as a static system with a daily water exchange of 90 %, before the first meal. Tanks were cleaned daily by siphoning the bottom to remove deposed organic matter. Fish were acclimated for ten days previous to experiment. During this

period, the fish were fed to apparent satiety, three times a day (08:00, 12:00 and 16:00 h) with experimental food (control). The photoperiod was adjusted to 14L:10D.

After acclimation a total of 90 juvenile *T. marginatus* fish (5.3 ± 2.0 g) were randomly distributed into two cylinder tanks (310 L) containing 200 L of seawater at a stock density of 45 fish per tank. The system was the same that described above. The water quality parameters were monitored daily. The temperature and dissolved oxygen was measured using multi-parameter electrode (550 A, YSI, Yellow Springs, OH, USA). The salinity was determined using refractometer (S/Mill-E, ATAGO, Tokyo, Japan).

2.2 Experimental diet

The experimental diet was the same utilized by Kütter et al (2012) that used purified ingredients and contain 47% of crude protein and 11% lipid. The diet was supplemented with 1,000 mg LA/kg of dry food whereas in the control diet LA was not included. After drying pellets they were crushed to a small diameter and stored at -20 °C until used. The final LA concentration of food was 524±38.2 mg LA/kg dry food.

2.3 Organs samples and homogenization

The brain, liver, gill and muscle were dissected at 10, 20 and 30 days. The fish were anesthetized following Okamoto et al (2009) and euthanized by cervical dislocation. Prior to sampling, the fish were fasted for 24h. During the dissection the organs (brain, liver and gill) were immediately stored in 3mL of physiological saline solution and maintained at 5 °C, with exception to muscle that was directed stored at -80 °C. The physiological saline solution for marine fish was prepared according to Forster & Hong (1958). After dissection, organs were dried quickly in toilet paper and stored at -80°C until biochemical analyses.

The organs were homogenized (1:5 w/v) in Tris–HCl (100 mM, pH 7.75) buffer with EDTA (2 mM) and Mg^{2+} (5 mM) (Amado et al. 2011). The homogenates were centrifuged at 10,000 × g for 20 min at 4 °C, and the supernatants were employed for all of the measurements described here. The total protein content was determined in triplicate using a commercial kit (Proteínas Totais®; Doles, Goiânia,GO, Brazil) based on the Biuret assay (550 nm), using a microplate reader (BioTek LX 800). The protein content was expressed as mg protein/g wet tissue.

2.4 Activity of glutathione-S-transferase (GST)

The activity of the enzyme glutathione-S-transferase (GST) was determined following the conjugation of 1 mM glutathione (GSH) and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), as described by Habig et al. (1974) and Habig & Jakoby (1981). The absorbance was measured at 340 nm using a spectrofluorometer with a microplate reader (Víctor 2, Perkin Elmer, MA, USA) at 25 °C. The GST activity was normalized based on the protein concentration in the supernatant, and is expressed in nanomols of conjugated GSH-CDNB/mg proteins/min.

2.5 Measurement of lipid peroxides concentration

The degree of lipid peroxidation was determined according to the method of Oakes & Van der Kraak (2003). The method includes the derivatization of a by-product of lipid peroxidation, malondialdehyde (MDA), with thiobarbituric acid, which is followed by determination of the fluorescence. The homogenized extract (15 µL) was added to a reaction mixture containing 150 µL of 20 % acetic acid, 150 µL of thiobarbituric acid (0.8%), 50 µL of Milli Q water and 20 µL of sodium dodecyl sulfate (SDS, 8.1 %). The samples were heated to 95 °C for 30 min, and after cooling for 10 min, 100 µL of Milli Q water and 500 µL of n-butanol were added. After centrifugation

(3,000 × g for 10 min at 15 °C), the organic phase (150 μL) was placed in a microplate reader and the fluorescence was registered after excitation at 520 nm and emission at 580 nm. The concentration of thiobarbituric acid-reactive substances (TBARS) (nmol/mg of wet tissue) was determined using tetramethoxypropane (TMP, Acros Organics) as a standard.

2.6 Data and statistical analyses

The data were expressed as the means ± standard error (SE). The differences between the treatments were evaluated using a factorial analysis of variances (factors: time and treatment) followed by the Newman-Keuls *post-hoc* test. The significance level adopted was 5% (α=0.05). The ANOVA assumptions (normality by Shapiro-Wilks and variance homogeneity by Levene) were previously validated (Zar, 1984).

Results

The GST activity in the brain exhibited no significant differences between groups along the time ($p>0.05$) (Fig.1a). In the liver, the LA group exhibited an increase in GST activity at day 30 in comparison to control groups at same time ($p<0.05$) (Fig.1b). In the gill, the group LA showed decrease in GST activity in comparison to control groups at day 10 ($p<0.05$), but this difference disappear at days 20 and 30. Furthermore, at days 20 and 30 the GST activitie of LA groups was significative higher than LA groups at 10 days (Fig.1c).

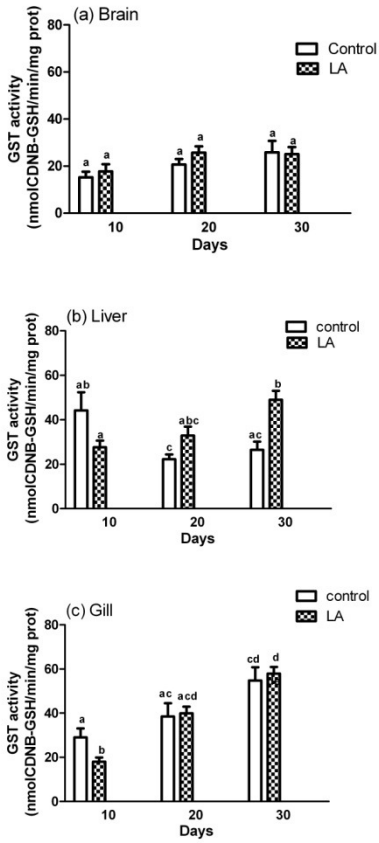

Figure 1. Activity of glutathione-S-transferase (GST) (nanomoles of conjugated GSH-CDNB mg of proteins^{-1} min^{-1}) in *Trachinotus marginatus*, fed with lipoic acid (LA) diet supplemented. **Control**: 0 mg/kg dry food; **LA**: 1000 mg/ kg dry food. The data are expressed as the mean ± the standard error (n=5-6). Similar letters indicate the absence of significant differences ($p>0.05$) after Newman-Keuls post-hoc test. Analysed organs: **(a)** brain, **(b)** liver, **(c)** gill.

The lipid peroxidation in brain exhibited no significant differences between groups ($p>0.05$) (Fig. 2a). In the liver, both treatments exhibited reduction in lipid peroxidation at day 20 in comparison to days 10 and 30. LA group also exhibited decrease of lipid peroxidation in comparison to control group at day 20 ($p<0.05$). The lipid peroxidation in liver exhibited significative reduction in LA group at 20 days in comparison to control at same period. Furthermore at this period lipid peroxidation was

smaller than at 10 and 30 days (Fig. 2b). In the gills, the lipid peroxidation did not differ between treatments in all period examined ($p>0.05$). The lipid peroxidation in gills exhibited higher values when compared to others tissues (Fig. 2c). The lipid peroxidation in muscle exhibited an increase in control group at day 30 in comparison to LA group at same period ($p<0.05$) (Fig.2d).

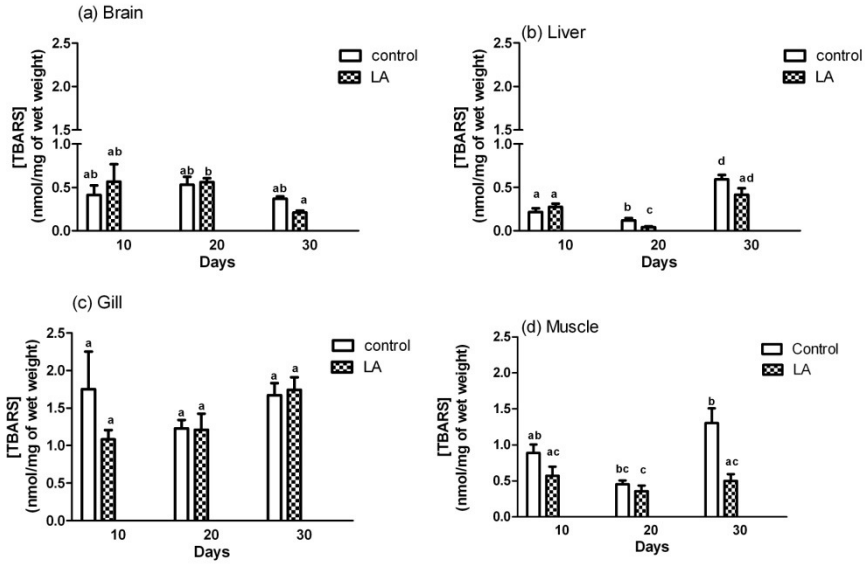

Figure 2. Lipid peroxidation (TBARS) in *Trachinotus marginatus*, fed with lipoic acid (LA) diet supplemented. **Control**: 0 mg/kg dry food; **LA**: 1000 mg/ kg dry food. The data are expressed as the mean ± the standard error (n=4-6) to brain, liver and gill, (n=7-12) muscle. Similar letters indicate the absence of significant differences ($p>0.05$) after Newman-Keuls post-hoc test. Analysed organs: **(a)** brain, **(b)** liver, **(c)** gill, **(d)** muscle.

Discussion

The glutatione-S-transferases (GSTs) are a multi-gene family of enzymes involved in the detoxification, and activation of a wide variety of chemicals (Eaton & Bammler 1999). In aquatic organisms, GSTs are know to play a main role in metabolism of xenobiotics in phase II detoxification, protecting cells against chemically induced toxicity (Blanchette et al. 2007). The GSTs also play an important role in detoxification of endogenous products of lipid peroxidation such as 4-hydroxy-2-nonenal (Eaton & Bammler 1999).

The results obtained of GST activity in brain exhibited no differences between treatments. The increase in GST activity in the brain of fish fed diet supplemented with LA had been reported by Monserrat et al (2008) and Kütter et al (2012). Moreover, previously study has reported that for this species a concentration of 890 mg LA/kg dry food is necessary to increase GST activity in brain (Kütter et al. 2012). In present study the employed concentration LA was 524 mg LA/ kg of dry food. This concentration was chosen because in this species is the best concentration to be employed in diet supplementation to enhance antioxidant status of some organs without reducing growth performance (Kütter et al. 2012).

The present study showed difference in organ response to LA diet supplementation. According to Bustamante et al (1998), when LA is offered via diet the absorption occur rapidly in the gut, taken up into various organs were metabolically altered and then excreted. The biotransformation of AL occurs mainly via β-oxidation of the carboxylic acid side chain and S-methylation of the dithiole moiety (Teichert et al. 2003). Furthermore, different animal metabolized LA in metabolic with distinct proprieties. The study of metabolic pathway of LA after oral administration, showed

that rat and mice forming predominatly derivatives of bisnorlipoic, whereas dogs the metabolic predominant are related with tetranorlipoic acid (Schupke et al. 2001). The liver is one of the main organs were LA accumulated after oral dose (Hill et al. 2004). Also, it is possible that LA in diet can present interference from others composts that could reduce its bioavailability (Shay et al. 2009). These statements could explain the effect on GST activity in liver occurred only after 30 days of LA diet supplementation.

The reduction in lipid peroxidation in the muscle of fish fed diet supplemented with LA had been reported by Kütter et al (2012). Similar with the response of liver the reduction in lipid peroxidation in muscle occurred only at 30 days. This reduction could be related with the interference of LA in body fatty acid compositions that increase the percentage of saturated fat acids that are less susceptible to oxidation (Çelik & Özkaya 2002; Özkan et al. 2005; Trattner et al. 2007).

The lipid peroxidation in gill exhibited higher values when compared to others tissues. Similar result has been previously described for trout *Oncorhynchus mykiss* (Trenzado et al 2006). According to the cited author the gill present less enzymatic antioxidant activity than liver and muscle turning this tissue more susceptible to oxidation.

Considering the explanation above we could suggest that are necessary almost 30 days to LA diet supplementation to enhance the antioxidant status of Plata pompano. Furthermore, the finding of reduction in lipid peroxidation in muscle can improve meat stability. The use of LA diet supplemented to increase meat quality had been reported for steer (Rentfrow et al 2004) and broiler (Arshad et al 2011; Yasin et al 2012).

Acknowlegments

J.M. Monserrat receives a productivity research fellowship from the Brazilian agency CNPq. Part of this study was supported by a grant from the Brazilian Agency FAPERGS (PROCOREDES VI Program, process number 0905160), which was given to J.M. Monserrat. A grant from the Brazilian Agency CNPQ (UNIVERSAL, process number 473680/2010) given to M.B. Tesser.

References

Amado LL, Garcia ML, Pereira TCB, Yunes JS, Bogo MR, Monserrat JM. 2011. Chemoprotection of lipoic acid against microcystin-induced toxicosis in common carp (*Cyprinus carpio*, Cyprinidae). *Comp. Biochem. Physiol.* 154: 146-153.

Arshad MS, Anjum FM, Asghar A, Khan MI, Yasin M, Shahid M, El-Ghorab AH. 2011. Lipid stability and antioxidant profile of microsomal fraction of broiler meat enriched with α-lipoic acid and α-tocopherol acetate. *J. Agric. Food Chem.* 59: 7346-7352.

Bergamini, CM, Gambetti S, Dondi A, Cervellati C. 2004. Oxygen, reactive oxygen species and tissue damage. *Curr. Pharmaceutical Design* 10:1611-1626.

Blanchette, B., Feng, X., Singh, B.R. 2007. Marine glutathione S-transferase. *Mar. Biotechnol.* 9: 513-542.

Bustamante J, Lodge JK, Marcocci L, Tritschler HJ, Packer L, Rihn B. 1998. α-lipoic acid in liver metabolism and disease. *Free Rad. Biol. Med.* 24: 123-139.

Çelik S, Özkaya A. 2002. Effects of intraperitoneally administratered lipoic acid, vitamin E, and linalool on the level of total lipid and fatty acids in guinea pig brain with oxidative stress induced by H_2O_2. *J. Biochem. Mol. Biol.* 6: 547-552.

Eaton DL, Bammler TK. 1999. Concise Review of the glutathione S-transferase and their significance to toxicology. *Toxicol. Sci*. 49: 156-164.

Foster RP, Hong SK. 1958. In vitro transport of dyes by isolated renal tubes of the flounder as disclosed by direct visualization: intracellular accumulation and trancellular movement. *Cell.Comp. Physiol.* 51: 259-272.

Habig WH, Jakoby WB. 1981. Assays for differentiation of glutathione S-transferases. *Methods in Enzymology* 77: 398-405.

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J. *Biol. Chem.* 249: 7130-7139.

Hill AS, Werner JA, Rogers QR, O'Neil SL, Christopher MM. 2004. Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats. *J. Animal Physiol. Animal Nutri.* 88: 150-156.

Kütter MT, Monserrat JM, Primel EG, Caldas SS, Tesser MB. 2012. Effects of dietary α-lipoic acid on growth, body composition and antioxidant status in the Plata pompano *Trachinotus marginatus* (Pisces, Carangidae). *Aquaculture* 368-369: 29-35.

Martínez-Álvarez RM, Morales AM, Sanz A. 2005. Antioxidant defenses in fish: biotic and abiotic factors. *Rev. Fish Biol. Fisheries* 15: 75-88.

Monserrat JM, Ventura JL, Ferreira R, Acosta D, Garcia ML, Ramos PB, Moraes TB, Dos-Santos LC, Amado LL. 2008. Modulation of antioxidant and detoxification

responses mediated by lipoic acid in the fish *Corydoras paleatus* (Callychthyidae). *Comp. Biochem. Physiol*. 148: 287-292.

Navari-Izzo F, Quartacci M, Sgherri C. 2002. Lipoic acid: a unique antioxidant in the detoxification of activated oxygen species. *Plant Physiol. Biochem.* 40: 463-470.

Oakes KD, Van der Kraak GJ. 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquat.Toxicol.* 63: 447-463.

Okamoto MH, Tesser MB, Louzada LR, Santos RA, Sampaio LA. 2009. Benzocaína e eugenol como anestésicos para juvenis do pampo *Trachinotus marginatus*. *Ciência Rural.* 39, 866-870.

Özkan Y, Yilmaz Ö, Öztürk AI, Erşan Y. 2005. Effects of triple antioxidant combination (vitamin E, vitamin C and α-lipoic acid) with insulin on lipid and cholesterol levels and fatty acid composition of brain tissue in experimental diabetic and non-diabetic rat. *Cell Biol. Int*. 29: 754-760.

Packer L, Tritschler HJ, Wessel K. 1997. Neuroprotection by the metabolic antioxidant α-lipoic acid. *Free Radic. Biol. Med.* 22: 359-378.

Packer L, Kraemer K; Rimbach G. 2001. Molecular aspects of lipoic acid in the prevention of diabetes complications. *Nutrit*. 17: 888-895.

Rentfrow G, Linville ML, Stahl CA, Olson KC, Berg EP. 2004. The effects of the antioxidant lipoic acid on beef longissimus bloom time. *J. Anim. Sci.* 83: 3034-3037.

Roberts CK, Sindhu KK. 2009. Oxidative stress and metabolic syndrome. *Life Sci.* 84, 705-712.

Ross SW, Dalton DA, Kramer S, Christensen BL. 2001. Physiological (antioxidant) responses of estuarine fishes to variability in dissolved oxygen. *Comp. Biochem. Physiol.* 130: 289-303.

Schupke , Hempel R, Peter G, Hermann R, Wessel K, Engel J, Kronbach T. 2001. New metabolic pathways of α-lipoic acid. *Drug Metabol. Dispos*. 29:855-862.

Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM. 2009. Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. *Biochem. Biophys Acta.* 1790: 1149-1160.

Soffler C.2007. Oxidative stress. *Vet. Clin. Equine*. 23: 135-157.

Teichert J, Hermann R, Ruus P, Preiss R. 2003. Plasma kinetics, metabolism, and urinary excretion of alpha-lipoic acid following oral administration in healthy volunteers. *J. Clin.Pharmacol*.43:1257-1267.

Trenzado C, Hidalgo MC, García-Gallego M, Morales AE, Furné M, Domezain J, Sanz A. 2006. Antioxidant enzymes and lipid peroxidation in sturgeon Acipenser naccarii and trout Oncorhynchus mykiss. A comparative study. *Aquaculture* 254: 758-767.

Trattner S, Pickova J, Park KH, Rinchard J, Dabrowski K. 2007. Effects of α-lipoic and ascorbic acid on the muscle and brain fatty acids and antioxidant profile of the South American pacu *Piaractus mesopotamicus*. *Aquaculture* 273: 158-164.

Wollin SD, Jones PJH. 2003. α-lipoic acid and cardiovascular disease.J. Nutr. 133, 3327-3330.

Yasin M, Asghar A, Anjum FM, Butt MS, Khan MI, Arshad MS, Shaid M, El-Ghorad AH, Shibamoto T. 2012. Oxidative stability enhancement of broiler bird meats with α-lipoic acid and α-tocopherol acetate supplemented feed. *Food Chem.* 131: 768-773.

Zar JH. 1984. Bioestatistical Analysis, 2nd. ed. Ed. Prentice Hall, New Jersey.

Zhang W, Chen Q, Mai K, Xu W, Wang X, Liufu Z. 2010. Effects of dietary α-lipoic acid on the growth and antioxidative responses of juvenile abalone *Haliotis discus hannai* Ino. *Aquacult. Res.*41: 781-787.

4. Alpha-Ácido lipóico: antioxidante com potencial uso na aquicultura

Alpha-lipoic acid: antioxidant with potential uses in aquaculture

Kütter M.T[a], Tesser M.B[a,c], Monserrat J.M[a,b*]

[a]Programa de Pós-Graduação em Aquicultura, Universidade Federal do Rio Grande (FURG), Av. Itália km 8, Rio Grande, RS, C.P. 474, CEP 96.200-970, Brazil.

[b] Instituto de Ciências Biológicas (ICB), FURG, Brazil.

[c] Instituto de Oceanografia (IO), Laboratório de Nutrição de Organismos Aquáticos, FURG, Brazil.

***Corresponding author**: Phone: 55-5332368042; fax:55-5332368042

Email address: josemonserrat@gmail.com

Artigo em preparação para Journal Aquatic Living Resources

Resumo

O ácido lipóico (AL) é um dissulfito derivado do ácido octanóico que é sintetizado na mitocôndria, atuando como cofator das enzimas mitocondriais que catalizam a dexcarboxilação oxidativa no ciclo de Krebs. As propriedades antioxidantes do AL fazem com este composto seja conhecido como "antioxidante ideal". Diversos benefícios terapêuticos associados a sua ação antioxidante têm sido encontrados em uma variedade de patologias. Estudos recentes demonstraram que este composto pode ser empregado na aquicultura para aumentar a capacidade antioxidante dos organismos. Desta forma o presente manuscrito apresenta os potenciais usos do AL na aquicultura.

Abstract

Lipoic acid (LA) is a disulphite derived from octanoic acid that is synthesized in mitochondria. LA acts as cofactor of mitochondrial enzymes that catalyses oxidative decarboxylation in the Krebs cycle. The antioxidants properties of LA make this compound be consider an "ideal antioxidant". Several therapeutic benefits associated with its antioxidant action have been investigated in wide range of pathologies. Recently researches have demonstrated that this compound can be also employed for enhance antioxidant status in aquatic organisms. The present manuscript relates the potential uses of LA in aquaculture.

Key-words: antioxidant status, aquaculture, aquatic organism, lipoate.

Introduction

The α-lipoic acid or 1,2-dithiolane-3-pentanoic acid, is a disulphate derived from octanoic acid synthesized in the mitochondria, found in microorganisms, plants and animals (Reed 1974; Navari-Izzo et al. 2002). The lipoic acid (LA) presents two enantiomers: R that occur naturally and S that is synthetically synthesized. This molecule contains two sulfur atoms (at C6 and C8) connected by dissulfite bond. (Fig.1a). Intracellular the thiol group of lipoic acid can be reduced in dihydrolipoic acid (DHLA)(Fig.1b).

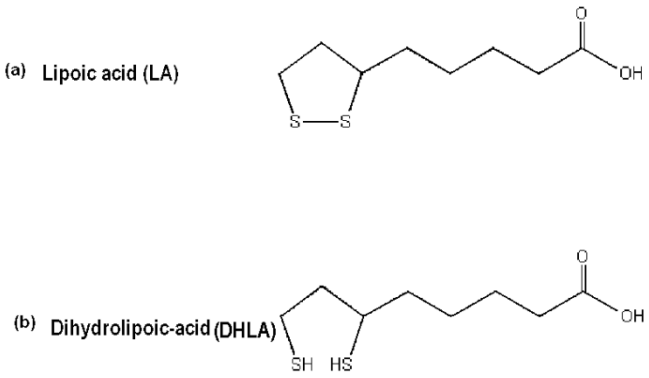

Figure 1- The structure of α- lipoic acid- oxized form (a); dihydrolipoic acid- reduced form.

The LA acts as cofactor of mitochondrial enzymes that catalyses oxidative decarboxylation in the Krebs cycle (Reed 1974; Christensen, 1983). Furthermore LA and DHLA have antioxidant proprieties including free radical quenching, metal chelation and interaction with other antioxidants. Together with characteristics cited above the amphiphilic character and bioavailability and safety properties of LA, make this compound an "ideal antioxidant" (Packer et al. 2001). The phisiologycal pathway of LA are multifaceted including the induction of cellular signaling pathways of glicogenesis and lipid metabolism, a vasorelaxant/anti-hypertensive compound, and an adjuvant for neuro-cognitive function (Shay et al 2009). Considering all these characteristics, the use of LA to improve the antioxidant status of cells, allows important applications for disease prevention in aquaculture organism.

In contrast to other antioxidants, like ascorbic acid, α-tocopherol, the use of LA in pets and farm animals is restricted to experimental studies (Diaz-Cruz et al. 2003; Wollin et at. 2004; Hill et al. 2004; Hamano 2002, Hamano 2007, Ibrahim et al. 2008).

The first studies investigating the effects of LA in aquatic organism started in 1960's. In these studies were investigated the effects of LA on animalization in sea urchin (Runnstrom 1957), in the regenerative capacity of hydra (Ham and Eakin 1958) and planaria (Henderson and Eakin 1959), on morphogenetic of frog embryo and acetabularia (Brachet 1961; Brachet 1962). Although, after this period a lack of publications focusing on LA effects in aquatic organisms occurred in subsequent decades. Only a few decades lather, information regarding the effects of LA on organisms utilized in aquaculture was published. Although, to our knowledge, these last published information is limited to only a few species: raibow trout *Onchorhynchus mykiss* (Otto et al. 1997), Pacu *Piaractus mesopotamicus* (Terjesen et al. 2004; Park et al. 2006; Trattner et al. 2007), corydora *Corydora paleatus* (Monserrat et al. 2008), habalone *Haliotis discus hannai* (Zhang et al. 2010), Carp *Cyprinus carpio* (Amado et al. 2011, Longaray et al 2013), zebrafish *Danio rerio* (Francis et al. 2012) and Plata pompano *Trachinotus marginatus* (Kütter et al. 2012; Kütter et al. 2013,).

Oxidative stress in aquatic organism

Like all aerobic organisms, cultured aquatic organisms are also susceptible to damage effects caused by oxidative stress and have substantial internal defenses that are well described in the literature (Ross et al 2001; Lushchak & Bagnyukova 2006). In homeostasis the rates of reduction and oxidation are equal. The oxidative stress occurs when the concentration of reactive oxygen species (ROS) become greater than the concentration of antioxidants (Sies 1985). For other point of view, oxidative stress can be consider a perturbation in signaling and redox control of cell (Jones 2006). This condition has potential deleterious effects if important biomolecules are oxidized (Sies 1991). The ROS are formed during aerobic metabolism by several biochemical

processes, including oxidative phosphorylation; ROS are also involved in the regulation of cellular signaling and gene expression and in the antimicrobial defense elicited by the immune system (Butler et al. 2009; Cui et al. 2009). However, it has been suggested that high ROS concentration are behing the etiology of several pathologies (Soffler 2007; Roberts & Sindhu 2009; Lu et al. 2006).

In farm animals, oxidative stress may be involved in several pathological circumstances including conditions that are relevant for animal production and the general welfare of the individuals (Lykkesfeldt & Svendsen 2006). During the rearing process temporal and spatial alterations in water quality make the organism cultured susceptible to oxidative stress. Variations in salinity, oxygen and temperature can alter the ability to detoxify ROS (Da Rocha et al. 2009). Furthermore, parasite infestation and toxins produced by cyanobacteria, such as microcystin, can also affect the antioxidant capacity of fish (Secombes & Chappell 1996; Belló et al 200; Amado et al. 2011; Garcia et al. 2011).

Considering that during the intensification of farm animal disease that normally have low incidence in wild populations can occur in large number of individuals in confined environment, any changes in antioxidant response of fish will be favorable to disease infestation. To prevent pro-oxidant condition the utilization of exogenous dietary antioxidants had been employed (Linnane & Eastwood 2006). The exogenous non-enzymatic antioxidants include ascorbic acid, α-tocopherol, carotenoids and α-lipoic acid (LA). Dietary supplementation with exogenous non-enzimatic antioxidants has been extensively used for humans, farm animals and pets (Block 1991; Merchie et al. 1996; Moini et al. 2002; Tocher et al. 2002).

Uptake and toxicity

The LA is considered a safety dietary supplement with non-observed adverse effect in human (Cremer et al. 2006). It has been large utilized due to its antioxidant and anti-hyperglicaemic proprieties in humans. Meanwhile, some authors have reported adverse effects depending of dose for others species. The rat is more tolerant to LA followed by dog, mice and cat that are very sensitive to LA (Hill et al. 2004). In broilers no limit concentration has been described. However, a dose of 150 mg/ kg exhibited growth reduction (Yasin et al. 2012).

In aquatic organism, studies investigating dose response have been reported for habalone and Plata pompano (Zhang et al. 2010; Kütter et al. 2012; Kütter et al. 2013). In the habalone was reported that high LA doses in diet, (between 1,600 and 3,200 mg LA Kg^{-1} dry food- nominal concentration), decreased antioxidant capacity and growth. In Plata pompano a single dose injected intraperitonelly, 40 or 60 mg LA kg^{-1} body weight, showed a pro-oxidant effect in liver with increase in lipid peroxidation and free iron concentrations (Kütter et al. 2013), whereas in diet doses of 890 and 1,367 mg kg^{-1} dry food cause growth delay and decreased food intake (Kütter et al. 2012).

The antioxidant/pro-oxidant properties of LA, in different circumstances, are dependent on its concentration (Çakatay & Kayali 2005; Atukeren et al. 2010; Valdecantos et al. 2010). The pro-oxidant effect of LA is considered to be related to its ability to reduce Fe^{3+} to Fe^{2+}, resulting in the generation of a hydroxyl radical via Fenton reactions that may propagate chain reactions including lipid peroxidation (Goralska et al. 2003).

Furthermore, the decrease caused by diet LA is related to reduction in the activity of the hypothalamic AMP-activated protein kinase that is responsible for

signaling the cell to consume energy (Kim et al. 2004). In addition, some reports indicate that dietary LA enhances energy expenditure (Kim et al. 2004; Wang et al. 2010).

Considering that aquaculture employ a large number of species it is important to determinate the appropriated dose to different species. The studies cited above indicated that both fish and habalone are tolerant to diets supplemented with doses of 3,000 and 3,200 mg kg^{-1} (nominal dose) respectively. However, these doses althought did not induced mortality, decrease growth and antioxidant status. In both cases, the optimal concentration was considered to be between 500 to 1,000 mg LA kg^{-1} dry food (nominal concentration) for Plata pompano and 709 mg LA kg^{-1} dry food for habalone (Zhang et al. 2010; Kütter et al. 2012). Furthermore, acute LA dose in Plata pompano exhibited optimal response with a single intraperitoneal injection of 20 mg kg^{-1} body weight (Kütter et al 2013).

Possible benefits of lipoic acid in fish farming

Researches with other antioxidants in aquatic organisms have demonstrated that these compounds increasing growth, survival, alter behavior and immunology, and also increase nutritional value of fish (Trenzado et al 2009, Rostamzad et al. 2010; Celada et al. 2013).

The reduced form of LA, dihydrolipoic acid (DHLA), can act in the regeneration of other antioxidants, such as α-tocopherol, ascorbate, glutathione and ubiquinol (Packer et al. 2001). Studies with LA in Pacu *Piaractus mesopotamicus* suggest that dietary LA suppress symptoms of ascorbic acid deficiency (Park et al 2006). Furthermore, other studies in aquatic organisms have been carried out and demonstrated

the improvement of antioxidant status by LA (Monserrat et al. 2008; Zhang et al. 2010; Amado et al. 2011; Kütter et al. 2012; Kütter et al. 2013, Longaray et al. 2013).

In corydoras *Corydoras paleatus* was reported that fish fed with dietary LA had antioxidant status of brain improved (Monserrat et al. 2008). Furthermore, in abalone, *Haliotis discus hannai* the supplement diet with LA present a positive correlation between the LA concentration and antioxidant capacity and growth in doses ranging from 200 to 800 mg kg^{-1}(Zhang et al. 2010). Whereas in Plata pompano, LA diet supplementation triggered reduction in lipid peroxidation in muscle and enhance antioxidant status of brain (Kütter et al. 2012). In other work was reported that treatment with LA could reduce the toxic effects of cyanotoxins in carp *Cyprinus carpio* (Amado et al. 2011). The application of LA in antioxidant-enriched media during embryonic incubation was tested in zebrafish, *Danio rerio* and showed enhancement of cell proliferation and growth (Francis et al. 2012).

Recently studies demonstrated that LA applied intraperitoneal also can enhance antioxidant status in brain of Plata pompano *Trachinotus marginatus* (Kütter et al. 2013). Other study conduced by Longaray et al. (2013) reported that carp injected intraperitoneally with LA or LA nanocapsuled exhibited diference response at different organs. It was showed that at short time the liver was the principal organ to provide antioxidant responses, whereas at long time, the brain and muscle are more responsible.

Conclusions and future directions

The ROS damage can trigger apoptosis and cell dysfunction, making oxidative stress a primary factor in the pathogenesis or a secondary contributor mechanism in disease etiology. The utilization of compounds that can enhance antioxidant defense should provide benefits against a wide range of pathologies. Furthermore, the present data demonstrated that LA could be employed at different stages of development in fish (Francis et al. 2012, Kütter et al. 2012). The LA can be applicated using different route of administration: intraperitoneally, diet supplemented and water solution. These characteristic permit the application at different circumstances, short time or long time response, and at different stages of aquaculture practices.

The beneficial proprieties of LA reported in current researches with aquatic organisms, turn this compound suitable to be used in aquaculture. Like others antioxidants LA can be applicated to improve growth, survival, immunology, and also increase nutritional value of fish. However, it is necessary determinate the appropriated dose to different species an even organ. Furthermore, economically profitable of LA employ in aquaculture need more research. Regardless of actual high price of this compound it would be an alternative to improve health in fish farm.

References

Amado L.L, Garcia M.L, Pereira T.C.B, Yunes J.S, Bogo M.R, Monserrat J.M, 2011, Chemoprotection of lipoic acid against microcystin-induced toxicosis in common carp (*Cyprinuscarpio*, Cyprinidae). Comp. Biochem. Physiol. 154,146-153.

Atukeren P., Aydin S., Uslu E., Gumustas M.K, Çakatay U., 2010, Redox homeostasis of albumin in relation to alpha-lipoic acid and dihydrolipic acid. Oxid. Med. Cell. Long. 3, 206-213.

Belló A.R.R, Fortes E, Belló-Klein A., Belló A.A, Llesuy S.F, Robaldo R. B, Bianchini A, 2000, Lipid peroxidation induced by Clinostomum detruncatum in muscle of the freshwater fish Rhamdia quelen. Dis. Aqus. Org. 42, 233-236.

Brachet J,1961, Morphogenic effects of lipoic acid on amphibian embryos.Nature 189,156-157.

Brachet J, 1962, Effects of β-mercaptoethanol and lipoic acid on morphogenesis.Nature193, 87-88.

Block G, 1991, Vitamin C and cancer prevention: the epidemiologic evidence. Am. J. Clin. Nutri. 53, 270-282.

Butler J.A, Hagen T.M, Moreau R, 2009, Lipoic acid improves hypertriglyceridemia by stimulating tryacilglycerol clearance and downregulating liver triacylglycerol secretion. Arch. Biochem. Bioph. 485, 63-71.

Çakatai U, Kayali R, 2005, Plasma protein oxidation in aging rats after alpha-lipoic acid administration. *Biogerontology* 6, 87-93.

Celada J.D, Fuertes J.B, Carral J.M, Sáez-Royuela M, González Á, González-Rodríguez Á, 2013, Effects of vitamin C in practical diets on survival and growth of juvenile crayfish (*Pacifastacus leniusculus* Dana, Astacidae) from the onset of exogenous feeding. Aquacult. Nutr. 19, 110-116.

Christensen K, 1983, Vitamins, in: *Dynamic biochemistry of animal production*, Riis P.M. (Ed.), Elsevier Science, Amsterdam, pp. 215–272.

Cremer, D.R, Rabeler R, Roberts A, Lynch B, 2006, Long-term safety of α-lipoic acid (ALA) consumption: A 2-year study. Regulatory Toxicol. Pharmacol. 46, 193-201

Cui J, Guowei L, Yang R, Shi Y, 2009, Lipoic acid attenuates high fat diet-induced chronic oxidative stress and immunosuppression in mice jejunum: A microarray analysis. Cell. Immunol. 260, 44-50.

Da Rocha A.M, De Freitas D.P.S, Burns M, Vieira, J.P, de La Torre F.R, Monserrat J.M, 2009, Seasonal and organ variations in antioxidant capacity, detoxifying competence and oxidative damage in freshwater and estuarine fishes from Southern Brazil. *Comp. Biochem. Physiol.*150*,* 512-520.

Diaz-Cruz A, Serret M, Ramirez G, Ávila E, Guinzberg R, Pina E, 2003, Prophylactic action of lipoic acid on oxidative stress and growth performance in broilers at risk of developing ascites syndrome. *Avian Pathol.* 32, 645-653.

Francis S, Delgoda R, Young R, 2012, Effects of embryonic exposure to α-lipoic acid or ascorbic acid on hatching rate and development of zebrafish (*Danio rerio*). Aquacult. Res. 43, 777-788.

Garcia L.O, Becker A.G, Bertuzzi T, Cunha M.A, Kochhann D, Finamor I.A, Riffel A.P.K, Llesuy S, Pavanato M.A, Baldisserotto B, 2011, Oxidative stress parameters in

silver catfish (*Rhamdia quelen*) juveniles infected with *Ichthyophthirius multifiliis* and maintained at different levels of water pH. Vet. Parasitol. 178, 15-21.

Goralska M, Dackor R, Holley B, McGahan M.C, 2003, Alpha lipoic acid changes iron uptake and storage in lens epithelial cells. Experiment. Eye Res. 76, 241-248.

Ham R.G, Eakin R.E, 1958, Loss of regenerative capacity in hydra treated with lipoic acid. J. Exp. Zool.139, 55-68.

Hamano Y, 2002, Influence of lipoic acid on lipid metabolism and b-adrenergic response to intravenous or oral administration of clenbuterol in broiler chickens. *Reprod. Nutr. Dev*. 42, 307–316.

Hamano Y, 2007, Continuous infusion of lipoic acid rapidly reduces plasma β-hydroxybutyrate with elevation of non-esterified fatty acids in broiler chickens. British J. Nutr. 97, 495-501.

Henderson R.F, Eakin R.E, 1959, Alteration of regeneration in planaria treated with lipoic acid. J. Exp. Zool. 141, 175-190.

Hill A.S, Werner J.A, Rogers Q.R, O'Neil S.L, Christopher M.M, 2004, Lipoic acid is 10 times more toxic in cats than reported in humans, dogs or rats. J. Animal Physiol. Animal Nutr. 88, 150-156.

Ibrahim S.F, Osman K, Das S, Othman A.M, Majid N.A, Rahman M.P.A, 2008, A study of antioxidant effect of alfa lipoc acid on sperm quality. Clinics 64, 545-550.

Jones D.P, 2006, Redefining oxidative stress. Antiox Redox Signal 8, 1865-1879.

Kim M.S, Park J.Y, Namkoong C, Jang P.G, Ryu J.W, Song H.S, Yun J.Y, Namgoong I.S, Ha J, Park I.S, Lee I.K, Viollet B, Youn JH., Lee HK, Lee KU, 2004, Anti-obesity

effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nat. Med*. 10,727–733.

Kütter M.T, Monserrat J.M, Primel E.G, Caldas S.S, Tesser M.B, 2012, Effects of dietary α-lipoic acid on growth, body composition and antioxidant status in the Plata pompano *Trachinotus marginatus* (Pisces, Carangidae). Aquaculture 368-369: 29-35.

Kütter M.T, Monserrat J.M, Santos R.A, Tesser M.B, 2013, Dose-response effects of the antioxidant α-lipoic acid in the liver and brain of pompano *Trachinotus marginatus* (Pisces, Carangidae). J. Appl. Ichthyol. DOI:10.1111/jai.12137

Linnane A.W, Eastwood H, 2006, Cellular redox regulation and prooxidant signaling systems: A new perspective on the free radical theory of aging. Ann. New York Acad. Sci.1067, 47-55.

Longaray-Garcia M, Flores J.A, Külkamp-Gerreiro I.C, Guterres S.S, Pereira T.C.B, Bogo M.R, Monserrat J.M, 2013, Modulation of antioxidant and detoxifying capacity in fish Cyprinus carpio (Cyprinidae) after treatment with nanocapsules containing lipoic acid. Comp. Biochem. Physiol. DOI: 10.1016/j.cbpa.2013.02.004.

Lu C-Y, Lee C-F, Ma Y-S, Liu C-Y, Wei C-Y,Chen Y-C, Wu S-B, Wei Y-H, 2006, Oxidative stress and mitochondrial diseas. In:Keshav Singh (Ed), Oxidative stress disease and cancer, Imperial College Press, pp.649-672.

Lushchak V.I, Bagnyukova T.V, 2006, Effects of different environmental oxygen levels on free radical process in fish. Comp. Biochem. Physiol.B 144 , 283-289.

Lykkesfeldt J, Svendsen O, 2007, Oxidants and antioxidant in diseases: oxidative stress in farm animals. Vet. J. 173, 502-511.

Merchie G, Lavens P, Storch V, Übel U, Nelis H, Leenheer A.De, Sorgeloos P, 1996, Influence of dietary vitamin C dosage on turbot (*Scophthalmus maximus*) and European sea bass (*Dicentrarchus labrax*) nursery stages. Comp. Biochem. Physiol. A 114, 123-133.

Moini H, Packer L, Saris Nils- Erik L, 2002, Antioxidant and prooxidant activities of α-lipoic acid and dihydrolipoic acid. Toxicol. Appl. Pharmacol. 182, 84-90.

Monserrat J.M, Ventura J.L, Ferreira R, Acosta D, Garcia M.L, Ramos P.B, Moraes T.B, Dos-Santos L.C, Amado L.L, 2008, Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras paleatus* (Callychthyidae). Comp. Biochem. Physiol. C 148, 287-292.

Navari-Izzo F, Quartacci M, Sgherri C, 2002, Lipoic acid: a unique antioxidant in the detoxification of activated oxygen species. Plant Physiol. Biochem. 40, 463-470.

Otto D.M.E, Sen C.K, Casley W.L, Moon T.W, 1997, Regulation of 3,30,4,40-tetrachlorobiphenyl induced cytochrome P450 metabolism by thiols in tissues of rainbow trout. Comp. Physiol. Biochem. C 117, 299-309.

Packer L, Witt E.H, Tritschler H.J, 1995, Alpha-lipoic acid as a biological antioxidant. Free Rad. Biol. Med.19,227-250.

Packer L, Kraemer K, Rimbach G, 2001, Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutr. 17, 888-895.

Park K.H, Terjesen B.F, Tesser M.B, Portella M.C, Dabrowski K, 2006, α-Lipoic acid enrichment partially reverses tissue ascorbic acid depletion in pacu (*Piaractus mesopotamicus*) fed vitamin C-devoid diets. Fish Physiol. Biochem.32, 329-338.

Reed L.J, Florkin M, Stotz E.H, 1974, Multienzyme complexes. Acc. Chem. Res. 7, 40-46.

Roberts C.K, Sindhu K.K, 2009, Oxidative stress and metabolic syndrome. *Life Sci.* 84, 705-712.

Roztamzad H, Shabanpour B, Kashaninejad M, Shabani A, 2010, Inhibitory impact of natural antioxidant (ascorbic acid and citric acid) and vaccum packaging on lipid oxidation in frozen Persian sturgeon fillets, Iranian J. Fisheries Sci. 9, 279-292.

Ross S.W, Dalton D.A, Kramer S, Christensen B.L, 2001, Physiological (antioxidant) responses of estuarine fishes to variability to dissolved oxygen. Comp. Biochem. Physiol. C 130, 289-303.

Runnstrom J., 1957, The animalizing effect of α-lipoic acid on sea urchin eggs. Exp. Cell. Res. 11, 660-664.

Secombes C.J, Chappell L.H, 1996, Fish immune responses to experimental and natural infection with helminth parasites. Ann. Review Fish Dis. 6, 167-177.

Shay K.P, Moreau R.F, Smith E.J, Smith A.R, Hagen T.M, 2009, Alpha-lipoic acid as dietary supplement: molecular mechanism and therapeutical potencial. Biochimica Biophys. Acta. 1790, 1149-1160.

Sies H, 1985, Oxidative stress: introductory remarks. In *Oxidative Stress*, ed. SIES, H., Academic Press, London, pp. 1-8.

Sies H, 1991, Oxidative stress: oxidants and antioxidants. Ed. Sies, H., Academic Press, London, 650pp.

Soffler C, 2007, Oxidative stress. Vet. Clin. Equine. 23, 135-157.

Terjesen B.F, Park K, Tesser M.B, Portella M.C, Zhang Y, Dabrowski K, 2004, Lipoic acid and ascorbic acid affect plasma free amino acids selectively in the teleost fish Pacu (*Piaractus mesopotamicus*). J. Nutr. 134, 2930-2934.

Tocher D.R, Mourente G, Van Der Eecken A, Ovjemo J.O, Diaz E, Bell J.G, Geurden I, Lavens P, Olsen Y, 2002, Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (Scophthalmusmaximus L.),halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). Aquacult. Nutr. 8, 195-207.

Trattner S, Pickova J, Park K.H, Rinchard J, Dabrowski K, 2007, Effects of α-lipoic and ascorbic acid on the muscle and brain fatty acids and antioxidant profile of the South American pacu *Piaractus mesopotamicus*. Aquaculture 273, 158-164.

Trenzado C.E, Morales A.E, Palma J.M, de la Higuera M, 2009, Blood antioxidant defenses and hematological adjustments in crowded/uncrowded rainbow trout (Oncorhynchus mykiss) fed on diets with different levels of antioxidant vitamins and HUFA. Comp. Biochem. Physiol. C 149, 440-447.

Valdecantos M.P, Perez-Matute P, Quintero P, Martinez J.A, 2010, Vitamin C, resveratrol and lipoic acid actions on isolated rat liver mitochondria: all antioxidants but different. Redox Report 15, 207-216.

Wang Y, Xiaojie L, Yuming G, Lawrence C, Xinfu G, 2010, α-lipoic acid increase energy expenditure by enhancing adenosine monophosphate-actived protein kinase-peroxisome proliferators-actived receptor-γ coativator 1-α signaling in skeletal muscle of aged mice. Metab. Clin. Exp. 59, 967-976.

Wollin S.D, Wang Y, Kubow S, Jones P.J.H, 2004, Effects of a medium chain triglyceride oil mixture and α-lipoic acid diet on body composition, antioxidant status,

and plasma lipid levels in the Golden Syrian hamster. J. Nutritional. Biochem. 15, 402-410.

Yasin M, Asghar A, Anjum F.M, Butt M.S, Khan M.I, Arshad M.S, Shahid M, El-Ghorab A.H, Shibamoto T, 2012, Oxidative stability enhancement of broiler bird meats with α-lipoic acid α-tocopherol acetate supplemented feed. Food Chem. 131, 768-773.

Zhang W, Chen Q, Mai K, Xu W, Wang X, Liufu Z, 2010, Effects of dietary α-lipoic acid on the growth and antioxidative responses of juvenile abalone *Haliotis discus hannai* Ino. Aquacult. Res.41: 781-787.

Considerações finais

Os resultados encontrados no presente estudo demonstraram que é necessária a determinação da concentração de α-ácido lipóico para que este apresente efeito benéfico ao organismo. Sendo assim, podemos concluir que a utilização de α-ácido lipóico no peixe *Trachinotus marginatus* melhora a capacidade antioxidante dos animais quando utilizado de forma aguda ou crônica. Além disso, a utilização de α-ácido lipóico na ração apresenta aumento da capacidade antioxidante após 30 dias de tratamento.

As doses de AL recomendadas para juvenis de *T. marginatus* são de 20 mg/kg em uma aplicação i.p enquanto que a inclusão de AL na dieta deve ser entre 316 e 524 mg AL/ kg de ração. O α-ácido lipóico pode ser utilizado na aquicultura, pois apresenta potenciais benefios relacionados à sua capacidade antioxidante que pode prevenir diversas patologias.