UNIVERSIDADE ESTADUAL PAULISTA
"JULIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL

RESPOSTAS METABÓLICAS, MORFOLOGIA DO TECIDO MUSCULAR E EXPRESSÃO DOS GENES RELACIONADOS AO CRESCIMENTO E À ATROFIA MUSCULAR, DURANTE O JEJUM E REALIMENTAÇÃO EM JUVENIS DE TILÁPIA-DO-NILO

Caroline Nebo
Zootecnista

2015

UNIVERSIDADE ESTADUAL PAULISTA
"JULIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL

RESPOSTAS METABÓLICAS, MORFOLOGIA DO TECIDO MUSCULAR E EXPRESSÃO DOS GENES RELACIONADOS AO CRESCIMENTO E À ATROFIA MUSCULAR, DURANTE O JEJUM E REALIMENTAÇÃO EM JUVENIS DE TILÁPIA-DO-NILO

Caroline Nebo

Orientadora: Profa. Dra. Maria Célia Portella

Coorientadora: Profa. Dra. Maeli Dal-Pai

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Campus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Zootecnia.

2015

Nebo, Caroline

N361r Respostas metabólicas, morfologia do tecido muscular e expressão dos genes relacionados ao crescimento e à atrofia muscular, durante o jejum e realimentação em juvenis de tilápia-do-nilo. / Caroline Nebo. – – Jaboticabal, 2015

viii, 93 p.: il ; 28 cm

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2015

Orientador: Maria Célia Portella

Coorientador: Maeli Dal-Pai

Banca examinadora: Luis Roberto Furlan, Rodrigo Takata, Jaqueline Dalbelo Biller Takahashi, Fernanda Losi Alves de Almeida

Bibliografia

1. *Oreochromis niloticus*. 2. Jejum. 3. Crescimento Muscular. 4. Atrofia Muscular, 5. IGF-1. 6. Fatores Regulatórios Miogênicos. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 639.3.043

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação – Serviço Técnico de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.

unesp UNIVERSIDADE ESTADUAL PAULISTA

CAMPUS DE JABOTICABAL

FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS DE JABOTICABAL

CERTIFICADO DE APROVAÇÃO

TÍTULO: RESPOSTAS METABÓLICAS, MORFOLOGIA DO TECIDO MUSCULAR E EXPRESSÃO DOS GENES RELACIONADOS AO CRESCIMENTO E À ATROFIA MUSCULAR, DURANTE O JEJUM E REALIMENTAÇÃO EM JUVENIS DE TILÁPIA-DO-NILO

AUTORA: CAROLINE NEBO

ORIENTADORA: Profa. Dra. MARIA CÉLIA PORTELLA
CO-ORIENTADORA: Profa. Dra. MAELI DAL PAI

Aprovada como parte das exigências para obtenção do Título de DOUTOR EM ZOOTECNIA , pela Comissão Examinadora:

Profa. Dra. MARIA CÉLIA PORTELLA
Departamento de Biologia Aplicada À Agropecuária / Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal

Prof. Dr. LUIZ ROBERTO FURLAN
Centro de Aquicultura Da Unesp / Jaboticabal/SP

Prof. Dr. RODRIGO TAKATA
Fundação Instituto de Pesca do Estado do Rio de Janeiro / Cordeiro/RJ

Profa. Dra. JAQUELINE DALBELLO BILLER TAKAHASHI
Pós-doutoranda / Departamento de Biologia e Zootecnia / Faculdade de Engenharia de Ilha Solteira

Profa. Dra. FERNANDA LOSI ALVES DE ALMEIDA
Universidade Estadual de Maringá / Maringá/PR

Data da realização: 20 de fevereiro de 2015.

DADOS CURRICULARES DA AUTORA

CAROLINE NEBO – gêmea nascida em 31 de janeiro de 1983, na cidade de Bauru – São Paulo, filha de Dene Nebo e Olga Fumico Nebo, ingressou no curso de Zootecnia pela Universidade Estadual Paulista, Júlio de Mesquita Filho, Campus de Dracena, em agosto de 2004. Foi bolsista de Iniciação Científica pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Em fevereiro de 2009, ingressou no Curso de Pós-Graduação em Biologia Geral e Aplicada, em nível de Mestrado, no Instituto de Biociências de Botucatu, Universidade Estadual Paulista, Campus de Botucatu, sob a orientação da professora Dra. Maeli Dal Pai Silva, sendo bolsista do programa de Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), obtendo o título de mestre em fevereiro de 2011. Em março do mesmo ano, iniciou o curso de doutorado pela Pós-Graduação em Zootecnia, pela Universidade Estadual Paulista, Campus de Jaboticabal, sob a orientação da professora Dra. Maria Célia Portella, sendo bolsista da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Entre setembro de 2013 e maio de 2014 realizou o doutorado-sanduíche com bolsa de estágio no exterior da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), no centro experimental da Universidade de Idaho (Hagerman Fish Culture Experiment Station), em Hagerman, Idaho, Estados Unidos, sob a orientação do pesquisador Ken Overturf – USDA (United States Department of Agriculture), onde realizou estudos na área de biologia molecular em peixes.

Dedicatória

À minha mãe Olga pela paciência, amor e carinho.

Aos melhores irmãos que Deus pode me enviar: Meire Nebo Tokuhara (Nê), Edna Nebo Ikehara (Ed), Paulo Eduardo Nebo (Duda) e, em especial, Liliane Nebo (Lia), que é a minha cara metade e alma gêmea, que sempre compartilha meus momentos de alegria, tristeza, conquistas e decepções. Amo vocês!

Aos meus cunhados Paulo Toshio Tokuhara (Shio), Edson Yoshihiro Ikehara (Shiro) e Rosângela Nebo (Rô), que nunca mediram esforços para me ajudar. Muito obrigada!

Aos meus sobrinhos: Daniel Toshio Tokuhara (Danoninho), Heloise Heidi Ikehara (Poia), Douglas Kenji Tokuhara (Kbça), Edson Yoshihiro Ikehara Júnior (Juninho), Augusto Cesar Nebo (Chicão), Lilian Thiemi Tokuhara (Lilika Ripilika) e Beatriz Sayuri Nebo (Gordinha), que são meus amores, minha alegria, minha paixão.

Agradecimentos

À FAPESP pela concessão da bolsa de doutorado no Brasil e nos Estados Unidos (2011/08420-3 e 2013/13100-3) e pelo auxílio à pesquisa (2011/22326-0).

À Faculdade de Ciências Agrárias e Veterinárias – UNESP, Campus de Jaboticabal e ao Progrma de Pós-Graduação em Zootecnia.

Ao CAUNESP por oferecer a infraestrutura e apoio técnico para a realização do experimento.

À minha orientadora Profa. Dra. Maria Célia Portella pelo aprendizado e incentivo para o estágio de Doutorado Sanduíche.

À minha coorientadora Profa. Dra. Maeli Dal-Pai do Departamento de Morfologia do Instituto de Biociências da UNESP, Campus de Botucatu, pela ajuda e carinho.

Ao meu coorientador do estágio de Doutorado Sanduíche Prof. Dr. Ken Overturf da USDA – United States Department of Agriculture, por não medir esforços em me ajudar durante a minha estadia em seu laboratório e por me convidar para participar de datas comemorativas com sua família.

Ao Prof. Dr. Ron Hardy, diretor do centro de pesquisa (Hagerman Fish Culture Experiment Station), da University of Idaho – USA. Aos professores Dr. Shawn Narum (CRITFC – Columbia River Inter-Tribal Fish Commission) e Dr. Matt Powell (University of Idaho), muito obrigada a todos pelo convívio e por me receberem tão bem.

Ao Prof. Dr. Dalton José Carneiro do Laboratório de Nutrição do CAUNESP pela ajuda quando necessário.

Ao Prof. Dr. Alexandre Hilsdorf da Universidade de Mogi das Cruzes, por me ceder peixes para a realização do projeto piloto.

Aos meus velhos amigos da Pós-Graduação do Laboratório de Nutrição de Organismos Aquáticos: Juliana Tomomi Kojima (Rapô), Thiago Mendes de Freitas (Nogento), Natália de Jesus Leitão (Nati), Thyssia Bomfim Araújo da Silva Dairiki (Thissa), Raphael de Leão Serafini (Rapha), Tais Lopes, Olívia Menossi (Taxinha), Hellen Buzzolo (Hélen), Thiago Matias do Nascimento (Strumi) e Jesaias Ismael da Costa. Aos novos amigos do Laboratório: Amanda Elias Halum (Amandita), Ivã Guidini, Frederico Werneck Lima (Fred), Rudney Weiber Assis (Tortelete) e Juliano Coutinho (Juju). Obrigada a todos que contribuíram direta ou indiretamente com o trabalho e que me aturaram nos momentos de mau humor.

Ao meu amigo Rodrigo Yukihiro Gimbo, por me auxiliar durante a realização do experimento e nas análises fisiológicas. Muito obrigada!

Aos estagiários Andressa Innocente (Dêssa) e Thalys Vinicius da Cruz (Thalys José) do Laboratório de Nutrição.

À Fernanda Regina Carani pela ajuda nas coletas do experimento e no exame de qualificação do doutorado. Fer muito obrigada!

A minha amiga Raquel Tatiane Pereira (Marmotinha), que mesmo longe, sempre foi minha conselheira em todos os momentos de desespero. Muito obrigada!

Aos meus colegas de Jaboticabal que sempre me proporcionaram momentos de alegria e felicidade: Josimari Pasqualoto (Raxinha), Mariane Sespere (Mari), Gisele Cristina Fávero (Griselda), Mateus Medeiros (Paquito),

Gelcirene de Albuquerque (Xuxa), Talísia Martins (Talitinha) e Rafael Sabione (Rafito).

Aos meus colegas do Laboratório (Hagerman Fish Culture Experiment Station): Biswamitra Patro, Alejandro Vilajante e Ben Hecht pela convivência, caronas e ajuda. Ao meu amigo Andreas Brezas pelos ensinamentos, realização das análises molecures e pelo companheirismo nos vários finais de semana no laboratório.

Aos técnicos da USDA – United States Department of Agriculture, Karen Frank e Michael Bezerra pela ajuda nas análises em Hagerman.

À banca de qualificação e defesa do doutorado: Profa. Dra. Elisabeth Criscuolo Urbinati, Prof. Dr. Luis Roberto Furlan, Prof. Dr. Leonardo Sussumu Takahashi, Dra. Fernanda Regina Carani, Dr. Rodrigo Takata, Dra. Jaqueline Biller Takahashi e à Profa. Dra. Fernanda Losi Alves de Almeida.

Sumário

Página

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o Protocolo nº 009436/11 do trabalho de pesquisa intitulado **"Morfologia e expressão dos genes relacionados ao crescimento e à atrofia muscular, durante a restrição alimentar e realimentação em juvenis de tilápia-do-nilo, *Oerochromis niloticus*"**, sob a responsabilidade da Profª Drª Maria Célia Portella, está de acordo com os Princípios Éticos na Experimentação Animal, adotado pelo Colégio Brasileiro de Experimentação (COBEA) e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), em reunião ordinária de 12 de Maio de 2011.

Jaboticabal, 13 de Maio de 2011.

Prof. Dr. Jeffrey Frederico Lui
Presidente - CEUA

Med. Vet. Maria Alice de Campos
Secretária - CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n - CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209 2600 - fax 16 3202 4275 - www.fcav.unesp.br

RESPOSTAS METABÓLICAS, MORFOLOGIA DO TECIDO MUSCULAR E EXPRESSÃO DOS GENES RELACIONADOS AO CRESCIMENTO E À ATROFIA MUSCULAR, DURANTE O JEJUM E REALIMENTAÇÃO EM JUVENIS DE TILÁPIA-DO-NILO

RESUMO - O objetivo do trabalho foi investigar as características morfológicas e fisiológicas, e a expressão de genes envolvidos na atrofia muscular (MuRF-1 e atrogina-1) e genes que controlam o crescimento muscular (MyoD, Miogenina, IGF-1 e receptor de IGF-1) e a Miostatina, em juvenis de tilápia-do-nilo (*Oreochromis niloticus*), submetidos ao jejum, seguidos de realimentação. Foram utilizados juvenis de tilápia-do-nilo, com peso médio de 30.2 ± 0.9 g, e o experimento foi realizado em delineamento inteiramente casualizado (DIC), com quatro tratamentos e oito repetições: (AL) controle alimentado, alimentado até a saciedade aparente durante o período experimental; (J1) uma semana de jejum e 10 semanas de realimentação; (J2) duas semanas de jejum e 10 semanas de realimentação; (J3) três semanas de jejum e 10 semanas de realimentação. As biometrias foram realizadas após uma, duas e três semanas de jejum e após uma, duas, seis e 10 semanas de realimentação para cada tratamento. As coletas de amostras (n = 16/tratamento) foram realizadas nos finais dos períodos de jejum e após 10 semanas de realimentação. Foram observadas significativas reduções nos índices de desempenho nos peixes durante o jejum. Entretanto, após dez semanas de realimentação, os peixes que passaram por jejum apresentaram apenas crescimento compensatório parcial. O consumo alimentar e a taxa de crescimento específico aumentaram durante a realimentação, igualando-se ao consumo alimentar do tratamento AL, porém não foram suficientes para induzir o crescimento compensatório total. A taxa de sobrevivência não diferiu entre os tratamentos. Os níveis de triglicérides e o cortisol nos peixes do tratmento J1 foram significativamente menores em relação ao AL. Os índice hepatosomático (IHS), índices de gordura visceral (IGV), lipídio na carcaça e os níveis de glicogênio hepático reduziram nos peixes que passaram pelo jejum. Entretanto, após 10 semanas de realimentação, a quantidade de lipídios no IGV, na composição corporal e glicogênio hepático dos peixes dos tratamentos J1, J2 e J3 se igualaram ao AL. Na primeira semana de experimento, nos tratamentos J1 e AL, a análise morfológica das fibras musculares mostrou uma grande quantidade de fibras pequenas e com núcleo central e padrão de hiperplasia em mosaico. Entretanto, na análise morfométrica não foram identificadas diferenças nas distribuições das fibras musculares em classe de diâmetro, tanto nos peixes submetidos ao jejum como no controle. Após o período de realimentação por 10 semanas, houve um aumento no tamanho das fibras, indicando crescimento hipertrófico em todos os tratamentos. A expressão gênica do IGF-1 diminuiu nos tratamentos J1, J2 e J3 em relação ao AL, durante o jejum. Entretanto, as expressões gênica do MuRF1 e atrogina-1, durante o jejum, foram significativamente maiores em todos os peixes submetidos ao jejum que no AL. Após 10 semanas de realimentação, os níveis de mRNA do MuRF1, atrogina-1 e IGF-1 em todos os tratamentos não diferiram mais entre si. Diferentemente do IGF-1, a expressão gênica do receptor de IGF-1 durante o jejum foi significativamente maior nos tratamentos J1, J2 e J3 em relação ao tratamento AL. Os resultados mostram que diferentemente de outros protocolos, onde os peixes apresentaram crescimento compensatório total, as estratégias utilizada em juvenis de tilápia-do-nilo (1 a 3 semanas de jejum e 10 semanas de realimentação) foram capazes de causar somente crescimento compensatório

parcial nos peixes . Entretanto, o nosso trabalho indica que a estratégia utilizada não inibe o crescimento dos peixes durante a realimentação, mas ao mesmo tempo, os peixes que foram submetidos ao jejum, não foram capazes de atingir crescimento compensatório total ou sobrecompensação do peso como nos peixes do tratamento controle. O aumento da expressão dos genes relacionados a atrofia muscular (MuRF1 e atrogina-1) durante o jejum foi capaz de ativar a via da atrofia muscular, inibindo assim, a via de crescimento e desenvolvimento muscular dos peixes.

Palavras-chave: *Oreochromis niloticus*, jejum, crescimento muscular, atrofia muscular, IGF-1, Fatores Reguladores Miogênicos.

METABOLIC RESPONSES, MORPHOLOGY OF MUSCLE TISSUE AND EXPRESSION OF GENES RELATED TO GROWTH AND MUSCLE ATROPHY, DURING FASTING AND REFEEDING IN NILE TILAPIA JUVENILES

ABSTRACT – The aim of this study was to investigate the morphological and physiological characteristics and the gene expression involved in muscle atrophy (MuRF-1 and atrogin-1) and genes that control muscle growth (MyoD, Myogenin, IGF-1 and IGF-1 receptor) and the Myostatin, in Nile tilapia (*Oreochomis niloticus*) juveniles submitted to fasting, followed by refeeding. It was used Nile tilapia juveniles with average body mass 30.2 ± 0.9 g and the experiment was completely randomized with four treatments and eight repetitions: (FC) fed control, fish were fed continuously during the 13 weeks; (F1) one week of fasting and 10 weeks of refeeding; (F2) two weeks of fasting and 10 weeks of refeeding; (F3) three weeks of fasting and 10 weeks of refeeding. Biometric performance was made after one, two and three weeks of fasting and after one, two, six and 10 weeks of refeeding. Samples were collected (n = 16 fish/treatment) after the end of each period of fasting and after 10 weeks of refeeding. It was observed significant decrease of performance index of fish during fasting. However, after 10 weeks of refeeding, fish that were fasted showed only partial compensatory growth. The feed intake and specific growth rate increased during refeeding, equaling to the FC, but it was not enough to induce total growth compensation. The survival rate did not differ among all treatments. The triglycerides and cortisol levels in fish from F1 were significantly lower than FC. The hepatosomatic index (HSI), visceral fat index (VFI), carcass lipid and liver glycogen levels decreased in fish that was fasting. However, after 10 weeks of refeeding, the lipids amount on VFI, on body composition and liver glycogen in fish from F1, F2 and F3 treatments were similar to FC. At the first week of the experiment, in the F1 and FC treatments, the morphological muscle fibers analyses showed a high quantity of small muscle fibers with central nucleus, showing the mosaic hyperplasia pattern. However, the morphometric analyses did not indicate differences in muscle fibers diameters distributions, between fish from fasting and control. After refeeding for 10 weeks, an increasing in muscle fibers diameter was observed, indicating hypertrophic growth in all treatments. The IGF-1 gene expression decreased in the F1, F2 and F3 treatments compared to the FC during feed restriction. Nonetheless, MuRF1 and atrogin-1 genes expression during fasting were significantly higher in all fasted fish than FC. After 10 weeks of refeeding, the MuRF1 mRNA levels, atrogin-1 and IGF-1 in all treatments did not differ among them. Differently to the IGF-1, the IGF-1 receptor gene expression, during fasting, was significantly higher in F1, F2 and F3 in comparison to the FC treatments. These results showed that unlike other protocols that fish exhibit total compensatory growth, the feed strategies utilized in Nile tilapia juveniles (1-3 weeks of fasting and 10 weeks of refeeding) were able to cause only partial growth compensation. Therefore, our study indicates that the feed strategy utilized did not prevent fasted fish to grow during the refeeding, but at the same time, fasted fish were not able to achieve total or over compensation of the body mass as fed control treatment. The increase of expression of genes related to muscle atrophy (MuRF1 and atrogin-1) during fasting was able to activate the muscle atrophy pathway, inhibiting both fish muscle growth and development pathways.

Keywords: *Oreochromis niloticus*, fasting, muscle growth, muscle atrophy, IGF-1, Myogenic Regulatory Factor.

LISTA DE TABELAS

CAPÍTULO 2 - METABOLIC EFFECTS AND GROWTH DURING FASTING AND REFEEDING IN NILE TILAPIA (*Oreochromis niloticus*) JUVENILES

CAPÍTULO 3 - INCREASED EXPRESSION OF ATROGENES AND IGF-1 REDUCTION INDUCED BY FASTING IN NILE TILAPIA (*Oreochromis niloticus*) JUVENILES

LISTA DE FIGURAS

CAPÍTULO 2 - METABOLIC EFFECTS AND GROWTH DURING FASTING AND REFEEDING IN NILE TILAPIA (*Oreochromis niloticus*) JUVENILES

LISTA DE ABREVIAÇÕES

BM = Body mass

SGR = Specific growth ratio

WG = Weight gain

HSI = Hepatosomatic index

VFI = Visceral fat index

FC = Fed control

F1 = One weeks of fasting and 10 weeks of refeeding

F2 = Two weeks of fasting and 10 weeks of refeeding

F3 = Three weeks of fasting and 10 weeks of refeeding

1WF = One week of fasting

2WF = Two weeks of fasting

3WF = Three weeks of fasting

10WR = 10 weeks of refeeding

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. Introdução

1.1 Tilápia-do-nilo

Tilápia é o nome comum atribuído a três gêneros de peixes africanos da família dos Ciclídeos: *Oreochromis, Sarotherodon* e *Tilapia.* Dentre as mais de 70 espécies de tilápias, as mais importantes para a aquicultura pertencem ao gênero *Oreochromis*, incluindo a tilápia-do-nilo (*Oreochromis niloticus*), a tilápia moçambique (*Oreochromis mossambicus*), a tilápia azul (*Oreochromis aureus*) e a tilápia zanzibar (*Oreochromis uroleps hornorum*). Estas espécies são nativas da África, Israel e Jordânia e espalharam-se pelo mundo, sendo atualmente catalogados mais de 100 países produtores. A tilápia-do-nilo corresponde a cerca de 80% de toda a produção mundial da espécie, devido a sua adaptabilidade aos variados sistemas de produção e condições ambientais, facilidade de reprodução, alta prolificidade e rápido crescimento (Popma e Masser, 1999; Watanabe al., 2002).

No Brasil, a criação de tilápia teve como marco inicial a introdução de um plantel de *Tilapia rendalli*, na década de 50, seguida da linhagem de tilápia nilótica em 1972. Nas últimas décadas, a tilapicultura vem se intensificando de forma significativa, segundo o Ministério da Pesca e Aquicultura (MPA), sendo a espécie mais produzida no Brasil e a segunda espécie mais cultivada no mundo, perdendo somente para a carpa comum (*Cyprinus carpio*). A tilápia-do-nilo apresenta inúmeras qualidades zootécnicas, como rusticidade, crescimento rápido, adaptabilidade, baixa conversão alimentar e alto ganho em peso, além de apresentar carne branca, de excelente paladar e textura, com ausência de espinhos, facilitando assim a filetagem e sua industrialização (Zanoni et al., 2000; Boscolo et al., 2001).

A tilápia-do-nilo possui grande habilidade para filtrar as partículas do plâncton e, quando cultivada em viveiros de águas verdes, geralmente supera em crescimento e conversão alimentar as outras espécies. É uma espécie versátil para a piscicultura, pois se adapta tanto em cultivos extensivos, com baixa tecnologia, quanto em sistemas de criação em tanques-rede, com alta tecnologia de produção (Kubitza, 2000). Em 2011, o Brasil produziu cerca de

253,8 mil toneladas de tilápia, representando 46,6 % do total de pescado cultivado (Brasil, 2011).

Com o objetivo de intensificar a tilapicultura no mundo, várias linhagens de tilápia nilótica têm sido desenvolvidas através de melhoramento genético, dentre elas a tilápia tailandesa (*Oreochromis niloticus)* que foi desenvolvida no Japão e melhorada no Palácio Real de Chitrala na Tailândia. Esta linhagem foi introduzida no Brasil em 1996, a partir de juvenis doados pelo *Asian Institute of Technology* (AIT) (Zimmermann, 2000). O desempenho das linhagens melhoradas, como a tilápia tailandesa apresentou ótimos resultados como maior ganho empeso, sobrevivência e melhor conversão alimentar quando comparada com as demais linhagens existentes na época (Boscolo et al., 2001; Leonhardt et al., 2006).

1.2 Músculo Estriado Esquelético

O músculo estriado esquelético, nos peixes, pode representar cerca de 30-80% do peso corporal e essa massa muscular não representa somente um mecanismo específico de adaptação no meio aquático, mas também uma fonte proteica na alimentação humana (Weatherley e Gill, 1985).

Nos peixes, os tipos de fibras musculares estão distribuídos em regiões ou compartimentos distintos: superficial, intermediário e profundo. O compartimento vermelho ou superficial, localizado abaixo da pele, corresponde até 30% da musculatura total dos peixes e é responsável pelos movimentos lentos e de sustentação do corpo. As fibras musculares desse compartimento possuem muitas mitocôndrias, mioglobina e lipídios, apresentam metabolismo oxidativo e cadeia pesada de miosina (MHC) *slow*, contraindo as fibras lentamente, sendo numerosas na região do septo horizontal e aumentando em direção à nadadeira caudal (Close, 1972; Bahler et al., 1968; Fauconneau et al., 1995).

No compartimento profundo ou branco, concentra-se a maior quantidade da massa muscular, representando cerca de 60 a 70% do total. As fibras brancas possuem pouca mioglobina, mitocôndrias e lipídios. Possuem metabolismo glicolítico e cadeia pesada de miosina (MHC) *fast* para movimentos rápidos de locomoção, como na fuga de predadores e captura de alimentos (Johnston, 1999). As fibras do compartimento intermediário representam menos de 10% da massa corporal e estão localizadas entre o

compartimento superficial e o profundo. Possuem força de contração rápida e metabolismo glicolítico/oxidativo, podendo ser recrutadas para atividades de sustentação e movimentos rápidos (Driedzic e Hochchka, 1976; Johnston, 1999; Sanger e Stoiber, 2001).

Nos peixes, a formação e diferenciação muscular durante a embriogênese ocorrem em fases distintas. Na primeira fase ocorre a formação de fibras musculares do embrião, juntamente com a população de mioblastos indiferenciados, que serão a fonte para o crescimento muscular. Na segunda fase, observada em larvas com saco vitelínico, ocorre o recrutamento e proliferação de mioblastos presentes na superfície dorsal e ventral do miótomo. Esse processo é denominado de crescimento hipertrófico estratificado e é responsável pelo aumento dos compartimentos musculares em espessura; a terceira e última fase envolve o recrutamento e formação de novas fibras musculares entre as fibras já existentes, em posições dispersas no miótomo, dando assim uma aparência de mosaico à musculatura quanto ao tamanho das fibras musculares (Veggetti et al., 1990; Currie e Inghan, 2001; Johnston e Hall, 2004; Ochi e Westerfield, 2007; Steinbacher et al., 2007).

O crescimento pós-embrionário do músculo esquelético em peixes depende da proliferação e da diferenciação de uma população de mioblastos adultos ou células satélites, que permanecem quiescentes na membrana basal do músculo durante a miogênese. Essas possuem a capacidade de proliferar-se e diferenciar-se, servindo como fonte de novos mionúcleos que irão se unir às miofibras já existentes (Kadi, 2008 apud Stotzer, 2009). Esse processo, denominado hipertrofia, ocorre durante o crescimento pós-natal, aumentando assim o diâmetro e área das fibras. As células satélites podem, também, sofrer fusão para formar novos mionúcleos e nova fibra muscular, processo conhecido como hiperplasia (Veggeti et al., 1990; Alfei et al., 1994, Johnston, 1999; Rehfeldt et al., 2004).

Nas espécies de pequeno porte (poucos centímetros), o crescimento muscular pós-natal envolve principalmente a hipertrofia das fibras formadas durante as fases iniciais da embriogênese e o período de crescimento hiperplásico é mais curto. Nas espécies que atingem maior tamanho, novas fibras musculares são continuamente formadas em todas as fases do crescimento. Na maioria das espécies de peixes, o crescimento hipertrófico das fibras musculares ocorre em todos os estágios de crescimento, porém é

predominante durante as fases juvenil e adulta (Veggeti et al. 1993; Patruno et al., 1998). Por outro lado, a taxa de crescimento por hiperplasia diminui com o avançar da idade dos peixes e sua contribuição nunca excede 50% do crescimento muscular (Alfei et al., 1994; Mommsen, 2001).

1.3 Fatores que Regulam o Crescimento Muscular

1.3.1 Fatores Reguladores Miogênicos

O crescimento muscular ou a miogênese pós-natal envolve a proliferação e a diferenciação das células satélites. Esse processo é regulado por várias proteínas, como a Miostatina e os fatores transcricionais pertencentes à família MyoD, conhecidos como Fatores de Regulação Miogênica (MRFs). Destes fazem parte a MyoD, Myf5, Miogenina e MRF4. Os MRFs contêm um domínio conservado conhecido como "basic helix-loop-helix" (bHLH) que se liga às sequências de DNA conhecidas como Ebox (5´-CANNTG-3´) na região promotora de vários genes músculo específicos. A região helix-loop-helix dos MRFs forma um dímero com uma proteína E. A ligação MRF-Proteína (E) à sequência Ebox do DNA, ativa a transcrição de genes músculos específicos, levando a sua expressão. Esses fatores de transcrição desempenham um papel fundamental na regulação do desenvolvimento e crescimento do músculo esquelético (Rescan et al., 2001; Johansen e Overturf, 2005; Johnston et al., 2008; Campos et al., 2010).

A MyoD e o Myf5 são conhecidos como fatores primários, sendo expressos em mioblastos na fase de proliferação, que antecede a diferenciação, enquanto que a Miogenina e o MRF4 são expressos em células na fase de fusão e diferenciação (Rehfeldt et al., 2004; Johnston, 2006; Schierholt et al., 2008). A MyoD também regula positivamente a transcrição gênicada Miostatina durante a maturação do miócito (Bradley et al., 2008).

1.3.2 Miostatina

A Miostatina ou GDF-8 (Fator de Crescimento e Diferenciação-8), outro fator regulatório do crescimento, é um membro da superfamília TGF-β (Fator de Crescimento e Transformação beta) e controla a proliferação e a diferenciação das células satélites, atuando negativamente na regulação do crescimento muscular, atenuando tanto a hipertrofia como hiperplasia (McPherron e Lee,

1997). Nos peixes, a miostatina é expressa no tecido muscular, olhos, brânquias, ovários, testículo, intestino e cérebro, desempenhando um papel importante na manutenção da homeostase do crescimento, regulação da osmolaridade e na função dos tecidos reprodutivos (Maccatrozzo et al., 2001; Rios et al., 2002; Rodgers et al., 2001).

1.3.3 Fator de Crescimento Semelhante à Insulina 1 (IGF-1)

O crescimento nos peixes, como em outros vertebrados, é regulado em grande parte pelo eixo GH/IGF-1 (Fox et al., 2010). O GH secretado pela hipófise estimula o fígado a sintetizar e liberar fatores de crescimento, como o IGF-1 (Fator de Crescimento Semelhante à Insulina-1), que tem efeito direto sobre os tecidos somáticos, resultando em crescimento. O IGF-1 estimula a síntese de proteínas, sinalizando uma cascata anabólica para a hipertrofia muscular, através da ativação da via fostatidilinositol 3 quinase (PI3K)-Akt-mTOR. Quando ativada, essa cascata de sinalização exerce um papel determinante na regulação da síntese protéica e, consequentemente, na hipertrofia muscular.

A sinalização do IGF-1, é mediada primeiramente pelo receptor de IGF-1 (IGF-1R), que exibe estrutura homóloga ao receptor de insulina (InsR), envolvendo um mesmo gene ancestral (Fernandez et al., 1995). A presença dos receptores de IGF-1 em diversos tipos celulares, juntamente com a expressão do IGF-1, permite ao IGF-1 exercer ações autócrinas, parácrinas e endócrinas. A ligação do IGF-1 no seu receptor ativa a cascata de sinalização do PI3K-Akt-TOR e MAP quinase (*Mitogen Activated Kinase*), que são bem conservadas em peixes e mamíferos (Duan et al., 2010). Por outro lado, a inibição desta via aumenta a proteólise e a expressão dos atrogenes, genes responsáveis pelo catabolismo protéico e atrofia muscular (Sacheck, 2004; Glass, 2010; Souza, 2010). O IGF-1, além de estimular a síntese protéica, tem efeito marcante sobre crescimento do músculo esquelético, aumentando a proliferação de células satélites, a diferenciação dos mioblastos e a fusão dos miotubos (Otto e Patel, 2010).

1.4 Atrofia muscular

A atrofia muscular é caracterizada pela redução do volume muscular, devido ao aumento da degradação e diminuição da taxa de síntese proteica no músculo. Nos animais, em condições normais de alimentação, as proteínas estão em fluxo constante, sendo continuamente sintetizadas e degradadas em um processo conhecido como "*turnover*", e esta capacidade de regulação exerce um impacto significativo sobre o crescimento, perda de peso e conversão alimentar. A manutenção da massa muscular esquelética é realizada através do balanço entre a síntese proteica e quebra de proteínas musculares. Assim, a massa muscular é mantida pela ingestão de proteína contida na dieta (Rafaello et al., 2010).

A degradação proteica é altamente regulada e dependente da atividade de enzimas proteolíticas, que nos vertebrados, estão agrupados em 3 sistemas: Sistema das catepsinas (proteólise lisossomal), Sistema das calpaínas (proteólise dependente de cálcio), e Sistema da ubiquitina-proteossoma (proteólise dependente de ATP e ubiquitina). As possíveis contribuições para desses sistemas proteolíticos na atrofia muscular têm sido debatidas, com alta evidência de que a degradação proteica, responsável pela a atrofia muscular em mamíferos ocorre, predominantemente, pela via dependente de ATP, ou seja, a via da ubiquina-proteassoma (Seiliez et al., 2008).

Em períodos prolongados de jejum, a degradação proteica no músculo esquelético é maior que a síntese, resultando em alterações na morfologia e estrutura do tecido muscular. A atrofia muscular nos peixes está associada com o aumento da expressão de duas ubiquitinas ligases específicas do músculo: a atrogina-1 ou *Muscle Atrophy F-box* (MAFbx) e o *Muscle Ring Finger* (MuRF-1). A expressão dessas enzimas, denominadas E3, de ligação à ubiquitina, ocorre somente no músculo esquelético e sua quantidade aumenta em múltiplas situações de atrofia (Bodine et al., 2001; Nader, 2005).

A via da ubiquitina-proteossoma é ativada em resposta a estímulos provocados por diversos fatores como caquexia, inatividade, restrição alimentar, entre outros. Este sistema é caracterizado principalmente por realizar o processo de proteólise intracelular de modo altamente seletivo e com gasto de energia (Lecker et al., 2006). A maior parte dos estudos relacionados com a atrofia muscular em peixes foi realizada com espécies de água fria, como a truta-arco-íris (*Oncorhynchus mykiss*) e salmão do Atlântico (*Salmo salar*), e

estudos com espécies de peixes de águas quentes são escassos. Espécies de águas frias, como a truta-arco-íris e o salmão passam naturalmente por períodos de vários meses de jejum durante o ano, devido à diminuição da temperatura da água no inverno. Por esta razão, muitos estudos não evidenciam a perda significativa de peso corporal e alterações no padrão morfológico das fibras musculares durante o período de jejum.

1.5 Respostas Compensatórias em Peixes

Na natureza, períodos de jejum são comuns entre os peixes, especialmente quando há escassez de alimento durante algum período do ano. Entretanto, a capacidade de sobrevivência, durante esses períodos, é uma habilidade bem desenvolvida em muitas espécies (Dave et al.,1975).

O jejum prolongado pode levar à diminuição da síntese de proteínas e crescimento mais lento e o restabelecimento de condições favoráveis, após um período de restrição alimentar, promove um crescimento compensatório, *i.e*., uma fase de crescimento acelerado, onde as condições normais são restabelecidas, após uma fase de baixo crescimento. A taxa de crescimento compensatório varia com o estágio de desenvolvimento, o período de jejum e de realimentação e a espécie considerada. Após um período de jejum, a realimentação promove uma reversão nos processos de mobilização de reservas corporais para suprir as perdas ocorridas durante o catabolismo. Somente quando esta condição estiver satisfeita, o destino da dieta será o crescimento (Dobson e Holmes, 1984; Kim e Lovell, 1995; Montserrat et al., 2007; Hagen et al., 2009).

Há algumas hipóteses que tentam explicar a elevada taxa de crescimento que normalmente ocorre durante o ganho compensatório. Uma delas é que há um aumento de consumo de alimento (hiperfagia), promovendo maior taxa de crescimento nos animais que passaram por um período de jejum, em relação aos animais continuamente alimentados (Hayward et al., 2000). A hiperfagia pode ser observada nos primeiros dias de realimentação e é considerada, em muitas espécies de peixes, como o mecanismo responsável pelo crescimento compensatório (Jobling e Johansen, 1999; Gurney et al., 2003). Outra hipótese do crescimento compensatório é que a resposta

hormonal, durante e após o período de restrição alimentar, impulsiona o crescimento (Gaylord et al., 2001).

Comparado a sistemas convencionais de criação em pisciculturas, que adotam regime constante de alimentação, a incorporação de protocolos alimentares, que induz o crescimento compensatório, mostra uma promessa de reduzir a quantidade de alimento necessária para espécies de peixes cultivadas comercialmente. Apesar de diversas espécies animais apresentarem crescimento compensatório, os mecanismos que comandam as respostas compensatórias ainda são pouco conhecidos (Won and Borski, 2013).

1.6 Respostas metabólicas dos peixes

As respostas metabólicas, durante a restrição alimentar, variam significativamente entre os teleósteos (Sheridan e Mommsen, 1991) e alguns fatores como a idade, estações do ano, condições ambientais e experimentais e estado nutricional, também podem influenciar o metabolismo aumentando ou diminuindo o efeito do jejum no ajuste biológico dos animais (Bastrop et al., 1991). Muitos animais, que vivem em ambientes com flutuações de alimento, estão adaptados a fazer grandes refeições, quando encontram alimento disponível. Quando a falta de alimento é devido a mudanças de temperatura e disponibilidade de água, os animais podem entrar em dormência e, assim, os processos digestivos são reduzidos (Wang et al., 2006).

Durante a privação de alimento, os processos vitais essenciais são mantidos às custas das reservas energéticas endógenas, resultando em diminuição e desgaste dos tecidos corporais (Weatherley e Gill, 1987). A redução do gasto energético pode ocorrer via redução da temperatura corporal, com redução da taxa metabólica. Os animais também podem reduzir o gasto energético, com redução da atividade locomoção, bem como mudanças fisiológicas e comportamentais na reprodução (Wang et al., 2006).

Durante o período de jejum, muitas mudanças fisiológicas ocorrem nos peixes, fazendo com que os animais utilizem o depósito de energia para a manutenção do metabolismo (Navarro e Gutierrez, 1995). As respostas provocadas pela restrição alimentar podem variar entre as espécies, tanto no tipo de reserva utilizada quanto no tecido a partir do qual essas fontes são obtidas (Silva et al., 1997). Algumas espécies de peixes utilizam as proteínas

musculares como maior reserva energética, enquanto o estoque de glicogênio é mantido pela gliconeogênese; entretanto, outros conservam as proteínas musculares e consomem a gordura e os estoques de glicogênio (Stimpson, 1965). Em *sunshine bass* (*Morone chrysops* X *Morone saxatilis*) e no *catfish* (*Ictalurus punctatus*), foi demonstrado que o glicogênio hepático armazenado foi mobilizado em resposta à uma semana de privação alimentar (Gaylord e Gatlin, 2000; Davis e Gaylord, 2011). Independente da exigência de cada espécie, a mobilização das fontes energéticas ocorre da forma mais eficiente para cada espécie animal. De acordo com Rios et al. (2002), em teleósteos, a principal reserva energética são as estocadas no fígado na forma de gordura vísceral durante o período de abundância de alimento. Geralmente, o lipídeo hepático é a primeira fonte de energia utilizada, seguido pelo glicogênio do fígado e glicogênio do músculo branco (Black e Love, 1986). A dinâmica de utilização da energia endógena pode ser estimada monitorando-se os índices hepato-somático (IHS) e a gordura víscero-somática (IGVS), sendo que as alterações nesses índices refletem a utilização de lipídio, proteína e glicogênio (Collins e Anderson, 1995).

A manutenção dos níveis de glicose sanguínea durante o jejum está diretamente relacionada com a capacidade de mobilização de glicogênio hepático, pelo menos no estágio inicial da privação alimentar, dependendo posteriormente, da ativação da gliconeogênese (Higuera e Cardenas, 1985).

Respostas fisiológicas provocadas pelas práticas de manejo, presença de parasitas e a restrição alimentar, provocam uma série de alterações no metabolismo dos peixes. Além disso, a alta densidade nos tanques de criação, baixa qualidade da água, transporte e aclimatação, entre outros podem causar grande estresse nos peixes. A resposta ao estresse, em peixes, pode provocar a liberação de catecolaminas (norepinefrina e epinefrina) e glicocorticóides (cortisol), sendo o cortisol o mais frequentemente utilizado como indicador de estresse (Barton, 2002). Geralmente, a taxa de cortisol é relacionada com a inibição de vários constituintes do eixo IGF, diminuindo desta maneira o crescimento (Davis e Peterson, 2006). A elevação do cortisol no plasma é uma importante resposta primária ao estresse, enquanto a elevação dos níveis sanguíneos de açúcar e alterações nos níveis de glicogênio, lipídios e proteínas em alguns tecidos, são efeitos secundários característicos (Mommsen et al., 1999).

A primeira resposta ao estresse inclui um rápido aumento nos níveis plasmáticos de catecolaminas e cortisol, enquanto que as secundárias incluem mudanças metabólicas como aumento nos níveis de glicose e diminuição de glicogênio tecidual, mudanças hematológicas, distúrbio na osmorregulação dos peixes e mudanças nas funções do sistema imune (defesa inata e adquirida). A terceira resposta provoca mudanças de desempenho dos peixes (taxa de crescimento, resistência a doenças e capacidade natatória) e também, mudanças comportamentais na alimentação e agressão dos animais (Barton, 2002).

2. Justificativa

A compreensão da fisiologia e dos eventos celulares e moleculares que ocorrem no músculo esquelético da tilápia-do-nilo, durante o jejum e realimentação, pode embasar a proposição de estratégias alternativas de alimentação que beneficiem o crescimento muscular de espécies de peixes cultivadas comercialmente, ao mesmo tempo em que promovam redução do custo de produção.

3. Hipóteses

Juvenis de tilápia-do-nilo submetidos a períodos de jejum podem demonstrar crescimento compensatório total depois que as condições adequadas de alimentação forem reestabelecida.

O jejum pode inibir o desempenho e interferir na fisiologia, na morfologia das fibras musculares e na expressão de genes relacionados ao crescimento e atrofia muscular em juvenis de tilápia-do-nilo (*Oreochromis niloticus*).

4. Objetivos

4.1 Gerais

O objetivo desse trabalho foi investigar o desempenho, as características morfológicas, fisiológicas e a expressão dos genes envolvidos na atrofia muscular (MuRF-1 e MAFbx) e genes que controlam o desenvolvimento e crescimento muscular (MyoD, Miogenina, Miostatina e IGF-1) em juvenis de tilápia-do-nilo (*Oreochromis niloticus*), submetidos a períodos de restrição alimentar e realimentação.

4.2 Específicos

1 – Avaliar, em juvenis de tilápia-do-nilo, os índices de desempenho, durante os períodos de jejum e realimentação: taxa de crescimento específico, consumo alimentar, conversão alimentar aparente, ganho em peso e sobrevivência;

2 – Verificar as respostas fisiológicas e a mobilização de energia, através das análises sanguíneas, dos índices hepato-somáticos (IHS) e gordura víscero-somática (IGVS), bem como da composição corporal da tilápia-do-nilo, durante jejum e realimentação;

3 – Verificar a morfologia das fibras musculares e a expressão dos genes envolvidos na atrofia muscular MURF-1 e atrogina-1 e dos genes que controlam crescimento muscular: MyoD, Miogenina, Miostatina, IGF-1 e receptor de IGF-1;

Referências Bibliográficas

ALFEI, L., ONALI, A., SPANO, L., COLUMBARI, P.T., ALTAVISTA, P.L.; De VITA, R. PCNA/cyclin expression and BrdU uptake define proliferating myosatellite cells during hyperplastic muscle growth of fish (*Cyprinus carpio* L.). **European Journal of Histochemistry,** v. 38, p. 151-162, 1994.

BAHLER, A.S., FALES, J.T., ZIELER, K.L. The dynamic properties of mammalian skeletal muscle. **The Journal of General Physiology,** v. 51. p. 369-389, 1968.

BARTON, B.A. Stress in Fishes: A diversity of responses with particular reference to change in circulating corticosteroids. **Integrative and Comparative Biology**, v. 42, p. 517-525, 2002.

BASTROP, R., SPANGERBERG, R., JURSS, K.. Biochemical adaptation of juvenile carpa (*Cyprinus carpio L*) to food deprivation. **Comparative Biochemystri and Physiology**. v. 9, p.143-149, 1991.

BLACK, D., LOVE, M. The sequential mobilization and restoration of energy reserves in tissue of Atlantic cod during starvation and refeeding. **Journal of Comparative Physiology,** Part B, v. 156, p. 469-479, 1986.

BODINE, S.C., LATRES, E., BAUMHUETER, S., LAI, V.K.M., NUNEZ, L., CLARKE, B.A., POUEYMIROU, W.T., PANARO, F.J., NA, E., DHARMARAJAN, K., PAN, Z.Q., VELENZUELA, D.M., DeCHIARA, T.M., STITT, T.N., YANCOUPOULOS, G.D., GLASS, D.J. Identification of ubiquitin ligases required for skeletal muscle atrophy. **Science**, v. 294, (5547), p. 1704-1708, 2001.

BOSCOLO, W. R., HAYASHI, C., SOARES, C. M., FURUYA, W. M., MEURER, F. Desempenho e características de carcaça de machos revertidos de tilápias do Nilo (*Oreochromis niloticus*), linhagens tailandesa e comum, nas fases inicial e de crescimento. **Revista Brasileira Zootecnia,** v.30, p.1391-1396, 2001.

BRADLEY, L., YAWORSKY, P.J., WALSH, F.S. Myostatin as a therapeuctic target for musculoskeletal disease. Cell Mol. **Life Science,** v. 65, p.2119-2124, 2008.

BRASIL. Ministério da Pesca e Aquicultura. **Boletim Estatístico da Pesca e Aquicultura 2011**. Disponível online. Acesso em 07/04/2011. http://www.mpa.gov.br/#imprensa/2010/ AGOSTO/nt_AGO_19-08-. Producao-de-pescado-aumenta.

CAMPOS, C., VALENTE, L.M.P., BORGES, P., BIZUAYEHU, T., FERNANDES, J.M.O. Dietary lipid levels have a remarkable impact on the expression of growth-related genes in Senegalese sole (*Solea senegalensis Kaup*). **The Journal of Experimental Biology,** v. 213, p.200-209, 2010.

COLLINS, A.L., ANDERSON, T.A. The regulation of endogeneous energy stores during starvation and refeeding in the somatic tissues of the golden perch. **Journal of Fish Biology**, v. 47, p.1004-1015, 1995.

CLOSE, R.I. Dynamic properties of mammalian skeletal muscle. **Physiology Reviews,** v. 52, p.129-197, 1972.

CURRIE, P.D., INGHAN, P.W. **Induction and patterning of embryonic skeletal muscle cells in the zebrafish**. In: Johnston IA, Muscle Development and Growth, Academic Press, London, p.1-17, 2001.

DAVE, G., JOHANDDON-SJÖBECK, M.L, LARSSON, A., LEWANDER, K., LIDMAN, U. Metabolic and hematological effects of starvation in the European eel, Anguila L. I – Carbohydrate, lipid, protein and inorganic ion metabolism. **Comparative Biochemistry and Physiology,** v. 52, p.423-430, 1975.

DAVIS, K.B. PETERSON, B.C. The effect of temperature, stress, and cortisol on plasma IGF-1 and IGFBPs in sunshine bass. **General Comparative Endocrinology**, v.149, p.219-225, 2006.

DAVIS, K.B. GAYLORD, T.G. Effect of fasting on body composition and responses to stress in sunshine bass. **Comparative Biochemistry and Physiology**, Part A, v.158, p.30-36, 2011.

DOBSON, S.H., HOLMES, R.M. Compensatory growth in rainbow trout, Salmo gairdneri Richardson**. Journal of Fish Biology,** v.25, p.649-656, 1984.

DRIEDZIC, W.R., HOCHACHKA, P.W. Control of energy metabolism in fish white muscle. **American Journal of Physiology,** v.230, p.579-582, 1976.

DUAN, C., REN, H., GAO, S. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: Roles in skeletal muscle growth and differentiation. **General Comparative Endocrinology,** v.167, p.344-351, 2010.

FAUCONNEU, B., ALAMI-DURANTE, H., LAROCHE, M., MARCEL, J., VALLOT, D. Growth and meat quality relations in carp. **Aquaculture,** v.129, p. 265-297, 1995.

FERNANDEZ, R., TABARINI, D., AZPIAZU, N., FRASCH, M., AND SCHLESSINGER, J. The Drosophila insulin receptor homologue: A gene essential for embryonic development encodes two receptor isoforms with different signaling potential. **The EMBO Journal,** v.14, p.101–112, 1995.

FOX, B.K., BREVES, J.P., DAVIS, L.K., PIERCE, A.L., HIRANO, T., GRAU, E.G. Tissue-specific regulation of the growth hormone/insulin-like growth factor axis during fasting and re-feeding: Importance of muscle expression of IGF-I and IGF-II mRNA in the tilapia. **General Comparative Endocrinology,** v.166, p.573-580, 2010.

GAYLORD, T.G., GATLIN, D.M.III. Dietary protein and energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*). **Aquaculture,** v.194, p.337–348, 2001.

GLASS, D.J. Signaling pathways perturbing muscle mass**. Current Opinion in Clinical Nutrition & Metabolic Care,** v.13, p.225-229, 2010.

GURNEY, W.S.C., JONES, W., VEITCH, A.R., NISBET, R.M. Resource allocation, hyperphagia and compensatory growth in juveniles. **Ecology,** v.84, p.2777-2787, 2003.

HAGEN, O., FERNANDES, J.M.O., SOLBERG, C., JOHNSTON, I.A. Expression of growth-related genes in muscle during fasting and refeeding of juvenile Atlantic halibut, *Hippoglossus hippoglossus* L. **Comparative Biochemistry and Physiology,** v.152, p.47-53, 2009.

HAYWARD, R.S., WANG, N., NOLTIE, D.B. Group holding impedes compensatory growth of hybrid sunfish. **Aquacultur,** v.183, p.299-305, 2000.

HIGUERA, M., CARDENAS, P. Influence of dietary composition on gluconeogenesis from L-(U-C) Glutamate 14 in rainbow trout (Salmo gairdneri). **Comparative Biochemistry and Physiology,** v.81A, p.391– 395, 1985.

JOBLING, M., JOHANSEN, J.S. The lipostat, hyperphagia and catch-up growth. **Aquaculture Research,** v.30, p. 473-478, 1999.

JOHANSEN, K.A., OVERTURF, K. Quantitative expression analysis of genes affecting muscle growth during development of rainbow trout (*Oncorhynchus mykiss*). **Marine Biotechnology,** v.7, p.576-587, 2005.

JOHNSTON, I.A. Muscle development and growth: potential implication for flesh quality in fish. **Aquaculture,** v. 77, p.99-115, 1999.

JOHNSTON, I.A.; HALL, T.E. Mechanisms of muscle development and responses to temperature change in fish larvae. **American Fish Society Symposium,** v.40, p.85-116, 2004.

JOHNSTON, I.A. Environment and plasticity of myogenesis in teleost fish. **The Journal of Experimental Biology,** v.209, p.2249-2264, 2006.

JOHNSTON, I.A., MACQUEEN, D.J., WATABE, S. Molecular biotechnology of development and growth in fish muscle. **Fisheries for Global Welfare Environment, 5th World Fisheries Congress,** p.241-262, 2008.

KADI, F. Cellular and molecular mechanisms responsible for the action of testosterone on human skeletal muscle. A basis for illegal performance enhancement. **British Journal of Pharmacology,** v.154, p.522-528, 2008.

KIM, M.K, LOVELL, R.T. Effect of restricted feeding regimens on compensatory weigth gain and body tissue changes in channel catfish *Ictalurus punctatus* in ponds**. Aquaculture**, v.125, p.285-293, 1995.

KUBITZA, F. **Tilápia: tecnologia e planejamento na produção comercial**. Jundiaí, 285p., 2000.

LECKER, S.H., GOLDBERG, A.L., MITCH, W.E. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. **Jounal of the American Society of Nephrology,** v.17, n.7, 1807-1819, 2006.

LEONHARDT, J.H., FILHO, C.M., FROSSARD, H., MORENO, A.M. Características morfométricas, rendimento e composição do filé de tilápia-do-nilo, *Oreochromis niloticus*, da linhagem tailandesa, local e do cruzamento de ambas**. Seminario: Ciências Agrárias**, Londrina, 27, 125-132, 2006.

MACCATROZZO, L., BARGELLONI, L., RODAELLI, G., MASCARELLO, F., PATARNELO, T. Characterization of the Myostatin gene in the giltherdseabrean (*Sparus aurata*): sequence, genomic structure and expression pattern. **Marine Biotechnology,** v.3, p.224-230, 2001.

MCPHERRON, A. C., LAWLER, A.M., LEE, S. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. **Nature**, v.387, p.83-90, 1997.

MOMMSEN, T.P., VIJAYAN, M.M., MOON, T.W. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation**. Reviews in Fish Biology and Fisheries**, v.9, p.211-268, 1999.

MOMMSEN, T.P. Paradigms of growth in fish*.* **Comparative Biochemistry Physiology,** v.129, p.207-219, 2001.

MONTSERRAT, N., GABILLARD, J.C., CAPILLA, E., NAVARRO, M.I., GUTIÉRREZ, J. Role of insulin, insulin-like growth factors, and muscle regulatory factors in the compensatory growth of the trout (*Oncorhynchus mykiss*). **General Comparative Endocrinology,** v.150, p.462-472, 2007.

NADER, G.A. Molecular determinants of skeletal muscle mass: getting the "AKT" together. **The International Journal of Biochemistry & Cell Biology,** v.37 n.10, p.1985-1996, 2005.

NAVARRO, I., GUTIERREZ, J. **Fasting and starvation**. In: Biochemistry and molecular biology of fishes. Edited: P.W. Hochanchka and T.P. Mommsen Elsevier Science, Amsterdam. vol. 4, 393-434, 1995.

OCHI, H., WESTERFIELD, M. Signaling networks that regulate muscle development: Lessons from zebrafish. **Development Growth Different,** *v.*49, p.1-11, 2007.

OTTO, A., PATEL, K**.** Signalling and the control of skeletal muscle size. **Experimental Cell Research,** v.316, p.3059-3066, 2010.

PATRUNO, M., RADAELLI, G., MASCARELLO, F., CANDIA CARNEVALI, M.D.. Muscle growth in response to changing demands of functions in the teleost Sparus aurata (L.) during development from hatching to juvenile. **Anatomy Embryology**, v.198, p.487-504, 1998.

POPMA, T., MASSER, M. **Tilapia: life history and biology**. Local: SRAC – Southern Regional Aquaculture Center, p283, 1999.

RAFFAELLO, A., MILAN, G. MASIERO, E., CARNIO, S., LEE, D., LANFRANCHI, G., GOLDBERG, A.L., SANDRI, M. JunB transcription factor maintains skeletal muscle mass and promotes hypertrophy. **Journal of Cell Biology**, v.191, p.101-113, 2010.

REHFELDT, C., FIEDLER, I., STICKLAND, N.C. **Number and Size of muscle fibres in relation to meat production.** "In": PAS, M.F.W; EVERTS, M.E.; HAAGSMAN, H.P. Muscle development of livestock animals: physiology, genetics, and meat quality. Trowbridge: CAB Int. p1-62, 2004.

RESCAN, P.Y., JUTEL, I., RALLIERE, C. Two Myostatin genes are differentially expressed in myotomal muscles of the trout (*Oncorhynchus mykiss*). **Jounal of Experimental Biology,** v.204, p.3523-3529, 2001.

RIOS, R., CARNEIRO, I., ARCE, V.M., DEVESA, J.. Myostatin is an inhibitor of myogenic differentiation. **American Journal Physiology & Cell Physiology,** v.282, p.993-999, 2002.

RODGERS, B.D., WEBER, G.M., SULLIVAN, C.V., LEVINE, M.A. Isolation and characterization of Myostatin complementary deoxyribonucleic acid clones from two commercially important fish: *Oreochromis mossambicus* and *Morone chrysops*. **Endocrinoly,** v.142, p.1412-1418, 2001.

SACHECK, J.M., OHTSUKA, A., MCLARY, S.C., GOLDBERG, A.L. IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. **American Journal Physiology: Endocrinology and Metabolism,** v.287, p.591-601, 2004.

SANGER, A.M., STOIBER, W. **Muscle fiber diversity and plasticity**. *In:* Johnston, I.A. (Ed) Muscle development and growth. San Diego: Academic Press, p.187-250, 2001.

SCHIERHOLT, A.S., FONSECA, I., SILVA, P.V., PAIVA, S.R., CHAVES, L.C.S., LOPES, P.S., FARIA, D.A., GUIMARÃES, S.E.F. Análise filogenética do gene da miogenina. **Arquivo Brasileiro Medicina Veterinária e Zootecnia,** v.60, p.156-162, 2008.

SEILIEZ, I., PANSERAT, S., SKIBA-CASSY, S., FRICOT, A., VACHOT, C., KAUSHIK, S., TESSERAUD, S. Feeding status regulates the polyubiquitination step of the ubiquitin-proteasome-dependent proteolysis in rainbow trout (*Oncorhynchus mykiss*) muscle. **Journal of Nutrition,** v.138, p.487-491, 2008.

SHERIDAN, M.A., MOMMSEN, T.P. Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. **General Comparative Endocrinology,** v.81, p.473-483, 1991.

SILVA, S.D., Changes in the fatty acid profiles of hybrid red tilapia, *Oreochromis mossambicus* x *O.niloticus*, subjected to short-term starvation, and a comparison with changes in seawater raised fish. **Aquaculture,** Amsterdan, 153, 273-290, 1997.

SOUZA, E.O. **Efeito do treinamento concorrente na expressão gênica e protéica associada à hipertrofia muscular**. 2010. 88f. Dissertação (Mestrado em Educação Física). Escola de Educação Física e Esporte, Universidade de São Paulo, São Paulo, 2010.

STAINBACHER, P., HASHLETT, J.R., OBERMAYER, A., MARSCHALLINGER, J., BAUER, H.C., SANGER, A.M., STOIBER, W. MyoD and miogenina expression during myogenic phases in brown trout: a precocious onset of masaic hyperplasia is a prerequisite for fast somatic growth. **Development Dynamics,** v.236, p.1106-1114, 2007.

STIMPSON, J. Comparative aspects of the control of glycogen utilization in vertebrate liver. **Comparative Biochemistry and Physiology,** v.15, p.187-197, 1965.

STOTZER, U.S. **Efeitos do treinamento resistido associado com decanoato de nandrolona sobre a expressão gênica de moduladores de vias de hipertrofia e atrofia do músculo esquelético**. 2009. 63f. Dissertação (Mestrado em Ciências Fisiológicas). Centro de Ciências Biológicas e da Saúde, Universidade Federal de São Carlos, São Carlos, 2009.

VEGGETTI, A., MASCARELLO, F., SCAPOLO, P.A. Hiperplastic and hypertrophic growth of lateral muscle in *Dicentrarchus labrax*.. An ultrastructural and morphometric study. **Anatomy and Embriology,** v.182, p.1-10, 1990.

VEGGETTI, A., MASCARELLO, F., SCAPOLO, P.A., ROWLERSON, M.D., CARNEVALI, C.M.D. Muscle growth and myosin isoform transitions during development of a small teleost fish (*Poecilia reticulate)* (Atheriniformes, Poeciliidae): a histochemical, immunohistochemical, ultrastructural and morphometric study. **Anatatomy and Embryology,** v.187, p.353-361, 1993.

WANG, T., HUNG, C.C.Y., RANDALL, D.J. The comparative physiology of food deprivation: From feast to famine. **Annual Review of Physiology**, v.68, p.223-251, 2006.

WATANABE, W.O., LOSORDO, T.M., FITZSIMMONS, K., HANLEY, F. Tilapia production systems in the Americas: technological advances, trends, and challenges. **Review in Fisheries Science,** v.10, p.465-498, 2002.

WEATHERLEY, A.H., GILL, H.S. Dynamics of increase in muscle fibers in fishes in relation to size and growth. **Experientia**, v.41, p.353-354, 1985.

WEATHERLEY, A.H., GILL, H.S. **The biology of fish growth.** London: Academic Press, 1987.

WON, E.T., BORSKI, R.J. Endocrine regulation of compensatory growth in fish. **Frontier in Endocrinology,** v.4, p.1-13, 2013.

ZANONI, M.A., FILHO, M.C., LEONHARDT, J.H. Performance de crescimento de diferentes linhagens de tilápia-do-nilo, Oreochromis niloticus (Linnaeus, 1757), em gaiolas. **Acta Scientiarum**, Maringá, 22, 683-687, 2000.

CAPÍTULO 2 - METABOLIC EFFECTS AND GROWTH DURING FASTING AND REFEEDING IN NILE TILAPIA (*Oreochromis niloticus*) JUVENILES

Abstract

We evaluated growth performance and metabolic responses in Nile tilapia (*Oreochromis niloticus*) juveniles (initial weight 30.2 ± 0.9 g) submitted to one, two and three weeks of fasting (F1, F2 and F3, respectively) and subsequent refeeding for 10 weeks (10 WR). The survival rate did not differ ($P > 0.05$) among treatments, and the evaluation of body mass, weight gain and specific growth rate, as expected, were lower in all fasted fish compared to the fed control (FC). Hyperphagia was not observed in all fasted fish during the refeeding. However, the feed intake percentage in relation to fish body mass and the specific growth ratio increased in fish from F1, F2 and F3 during refeeding and induced to partial compensatory growth. The feed conversion ratio (FCR), at 10 WR, was significantly higher in F2 and F3 in comparison to fed control (FC). The hepatosomatic index (HSI), visceral fat index (VFI) and liver glycogen (LG) dropped in fasted fish in comparison to the FC ($P < 0.05$), showing a depletion of stored nutrients like fat and glycogen on liver. Nonetheless, at 10 WR, the variables of HSI, VFI and LG increased in all fasted fish and were similar in all treatments. The results on carcass composition showed an increased mobilization of lipid levels during fasting in comparison to the FC ($P < 0.001$). However, the carcass proximate lipid composition was recovered in the fasted fish after 10 WR, equaling to the FC. The blood levels of (cholesterol, glucose, total protein), muscle lipid and liver lipid were not affected by fasting and refeeding. Cortisol level was lower only in the F1 in comparison to the FC. Along with liver glycogen, the fat reserves were mobilized at 1 to 3 weeks of fasting to maintain basal metabolism and survival, but the lipids were restored to control levels after 10WR. These results showed that unlike other protocols with small tilapia juveniles, the feed strategies utilized in Nile tilapia juveniles (1-3 weeks of fasting and 10WR) were able to cause only partial compensatory growth. Therefore, our study indicates that the feed strategy utilized did not prevent fasted fish to grow during the refeeding, but fasted fish were not able to achieve total or over compensation of the body mass as fed control treatment.

Key-words: *Oreochromis niloticus*, fasting, metabolic energy, compensatory growth, hyperphagia.

1. Introduction

Fish are able to survive long periods of starvation, which is a common situation faced in the wild and also in farming. Wild fish may go through nutrient restriction due to limitation of available food, seasonal changes and during phases of the reproductive cycle (McCue, 2010). Furthermore, in intensively reared fish, fasting is a feeding strategy utilized to reduce water quality problems, decrease the effects of disease outbreaks, reduce production costs, and stimulate fish growth rates after refeeding (Cho and Heo, 2010; Fox et al., 2010; Davis and Gaylord, 2011).

Feed restoration to satiation after a period of depressed growth tends to reestablish the original fish growth trajectory, a mechanism known as compensatory growth or catch-up. Therefore, compensatory growth is a period of accelerated growth when favorable conditions are restored (Ali et al., 2003). However, the degree of growth compensation achieved depends on the fish species and the occurrence of hyperphagia, improving the feed conversion ratio (FCR) and increasing the specific growth rate (SGR). For this reason, there are numerous studies on compensatory growth, involving many farmed fish species. Compared to conventional methods of fish farming which deploy a constant feeding regime, the incorporation of rearing protocols inducing compensatory growth may be an alternative to reduce the amount of feed needed for growth in commercial farming (Won and Borski, 2013).

The metabolic rates of fishes may decrease during fasting and, in addition to fish; other ectotherms appear to experience similar metabolic responses. (Jobling, 1980; Wang et al., 2006; Drew et al., 2008). Fish metabolism is largely based on lipids and proteins, with lipid storage in the liver, viscera and muscle. However, the distribution between these body components varies among species (Love, 1970; Jobling and Johansen, 1999). During fasting, vital processes are assured by endogenous energy stored and it induces changes in the storage reserves, particularly the lipids (Navarro and Gutierrez, 1995; Ali et al., 2003; Nicieza and Alvarez, 2009). Gaylord and Gatlin (2000) found that channel catfish (*Ictalurus punctatus*) juveniles modulated visceral organ size and composition in response to feed deprivation and refeeding. Their energy source from the liver and visceral fat were mobilized to maintain basic metabolic functions, showing that in fasting, lipids break down occur earlier than other body components.

Tilapia farming has expanded across the world in response to the increasing demand for this fish as a substitute for all kinds of wild-caught fish and the global need to broaden the sources of animal-derived protein for human consumption (Fitzsimmons et al., 2011). Indeed, in 2012, farmed tilapia exceeds 3.2 million tons per annum, surpassing the salmon and catfish industries (FAO, 2012).

Efforts to improve tilapia meat quality and farming productivity have explored the fasting effects on compensatory growth as feed strategy and contributed to understand which energy source is primarily mobilized to maintain the vital processes and survival. Furthermore, fasting technique in fish is also used to improve the end product quality by reducing muscle lipid content (Grigorakis and Alexis, 2005; Zhang et al., 2008). Some studies have analyzed the fasting and refeeding effects on tilapia at different growth phases and found that compensatory growth varied from partial to total depending on the growth phase (Abdel-Hakim et al., 2009; Breves et al., 2014; Wang et al., 2000; Wang et al., 2009). A study with hybrid tilapia (4.3g) (*Oreochromis mossambicus* × *O. niloticus*) reared in seawater found total compensatory growth after one week of fasting and refeeding for four weeks (Wang et al. 2000). However, only partial compensatory growth was obtained when Nile tilapia (6.6g) (*Oreochromis niloticus*) was submitted to cyclical feed deprivation (1 to 4 days) and refeeding (2 to 8 days) (Wang et al., 2009). We conjectured whether a modified protocol could provide better compensatory growth results for farming of Nile tilapia (*Oreochromis niloticus*). Indeed, differences in compensatory growth may be related to different experimental protocols, environmental conditions or physiological conditions of fish.

Here we examined the effect of different periods of fasting and refeeding on the dynamics of growth depression and depletion of energy source in Nile tilapia (*Oreochromis niloticus*) juveniles due the commercially importance of the specie in Brazil and other countries.

2. Material and Methods

2.1 Experimental design

The experiment was conducted at the Laboratory of Nutrition of Aquatic Organisms of the Aquaculture Center, UNESP, SP, Brazil. Fish were obtained from a commercial farm and acclimated for 15 days to the experimental facilities. Nile tilapia (*Oreochromis niloticus*) juveniles, chitralada Thai strain (30.2 ± 0.9 g), were stored into 32 tanks with 150 liters of water and constant aeration. The experiment lasted 13 weeks, and a fixed period of 10 weeks of refeeding (10WR) was maintained for all treatments. Fish were randomly distributed into four experimental treatments and eight replicates: FC: fed control, fish were fed continuously during 13 weeks; F1: one week of fasting and 10WR, F2: two weeks of fasting and 10WR; F3: three weeks of fasting and 10WR. Fish were fed to apparent satiation with extruded commercial diet (crude protein (max) 320g/kg, moisture (min) 80g/kg, lipids (min) 65g/kg, ash (max) 100g/kg) three times per day (9 am, 2 pm, and 5 pm) at three different feeding schemes imposed for 13 weeks. Water variables were monitored weekly and maintained at: temperature 30.1 ± 0.32 °C, pH 7.89 ± 0.14 and dissolved oxygen 4.74 ± 0.86 mg/L.

2.2 Biometric measurements

Fish were anesthetized with benzocaine (0.1 g/L), individually weighted, measured and tissue samples (liver, muscle and visceral fat) were collected from euthanized fish at the beginning of the experiment and at one, two and three weeks of fasting (1WF, 2WF and 3WF) and after 10 weeks of refeeding (10WR). All biometric were evaluated and compared to the FC at the beginning of the experiment (0 day), at the end of each period of fasting: one (1WF), two (2WF) and three (3WF) weeks of fasting, and after one, two, six and ten weeks of refeeding (1WR, 2WR, 6WR and 10WR). The number of fish in all treatments was adjusted after collect of samples. The parameters evaluated were as follows:

Body mass (BM) (g) = body mass

Weight gain (WG) (g) = final BM – initial BM

Feed intake (FI) (g/fish) = total dry matter consumption / number of fish

Feed intake in relation to body mass (% BM) = [FI (g) / BM (g)] x 100

Feed conversion ratio (FCR) = dry matter consumption / WG

Specific growth rate (SGR) (% day^{-1}) = [(Ln final BM − Ln initial BM) x 100] / number of days between the biometric measurements

Hepatosomatic index (HSI, %) and Visceral fat index (VFI, %) = [tissue weight (g) / total BM (g)] x 100

To investigate the compensatory responses, we analyzed the feed intake during the first three days of refeeding and the survival rate was evaluated at the end of the experiment.

The study was approved by the Ethics Committee on Animal Use of the Faculty of Agricultural and Veterinarian Sciences of the São Paulo State University (CEUA/FCAV/UNESP, 009436/11).

2.3 Proximate carcass composition

Carcass samples were collected (n = 8 fish/treatment) and frozen at -20ºC. The proximate carcass composition was determined in dry matter. Crude protein (N x 6,25) content was measured using Dumas method by (LECO FP-528) nitrogen analyzer, lipid by ether extraction using Soxhlet, ash by combustion in muffle at 600°C) and moisture (dried to a constant weight 105°C) on fish carcass were analyzed following the Association of Analytical Chemists procedures (AOAC, 2000).

2.4 Blood collection and tissue analysis

The blood samples (n = 8/ treatment) were taken from the caudal vessel of each fish, after one (1WF), two (2WF) and three (3WF) weeks of fasting and after ten weeks of refeeding (10WR). Blood was dispensed in microtubes, containing anticoagulant (Glistab – Labtest, São Paulo, Brazil, code 29) and microtubes without anticoagulant. Blood samples with anticoagulant were centrifuged (10 min at 3.000 xg) for plasma separation and, subsequently, were used to determine the glucose and triglycerides levels (Labtest, São Paulo, Brazil, codes 84 and 87, respectively). The blood without anticoagulant was allowed to clot at room temperature for 3 hours, and then centrifuged. The acquired serum was stored at –20º C for further determination of cholesterol, total protein levels (Labtest, São Paulo, Brazil, codes 76 and 99) and cortisol (DRG® Cortisol ELISA – EIA-1887). All assays were performed according to the manufacturer's instructions.

Liver and dorsal muscle were collected and stored at –20 ºC for further determination of lipid and glycogen levels. Liver glycogen levels were measured by lysis in amyloglycosidase after extraction with perchloric acid (7%) (Moon et al., 1989). Liver and muscle lipids and muscle protein levels were determined after extraction in chloroform and methanol solution (2:1), following Bligh and Dyer (1959) and Bradford methods (Bradford, 1976), respectively.

3. Statistical analysis

The results obtained for each parameter were expressed as mean ± SEM per each experimental treatment. All data were tested for normality and homogeneity of variances by Cramér-von Mises test and Brown-Forsythe's test and then submitted to a one-way ANOVA. When these tests showed significance ($P < 0.05$), means were compared using Tukey's test. Logarithmic transformations (natural logarithm) were used in all growth performance analysis (body mass, weight gain, specific growth rate, feed intake, and feed conversion ratio), carcass composition (lipid and crude protein), biochemical parameters (cholesterol, total protein and cortisol), hepatosomatic index and visceral fat index as relevant correction in original data.

4. Results

4.1 Performance drop after fasting and recovery after refeeding of Nile tilapia

Survival rate of Nile tilapia juveniles was higher than 84% in the FC, F1, and F2 treatments whereas in the F3, it was approximately 79%. However, these differences were not significant.

As expected, no BM increase was observed during the first until the third week of fasting; in fact, fish did not lose but also did not gain weight during fasting (Fig.1 A). The BM and WG increased during refeeding periods (1WR, 2WR, 6WR and 10WR), but it were lower in all fasted fish than FC treatment. At 1WR, the BM and WG in fish from F1 and F2 were significantly higher compared to the F3 treatment. At 2WR, the BM was still higher in fish from F1 and F2 treatments and the WG did not differ in all fasted fish. However, at 6WR, the BM and WG increased in fish from F3 compared to F1 and F2 treatments. After 10WR, the BM and WG in fish from F1 were significantly higher compared to the F2 and F3 treatments (Fig. 1 A, B).

The SGR was lower in all fasted fish compared to the FC treatment and it was different among the FC groups. At 1WR, the SGR in fish from the F1 and F2 was higher than FC treatments. At 2WR, the SGR were similar in fish from F1 and F3 and in the F2 it was lower compared to FC treatment. However, at 6WR, the SGR was higher in all fasted fish than FC treatment and at 10 WR the SGR was lower in the F2 and F3 compared to FC treatment (Fig. 1 C).

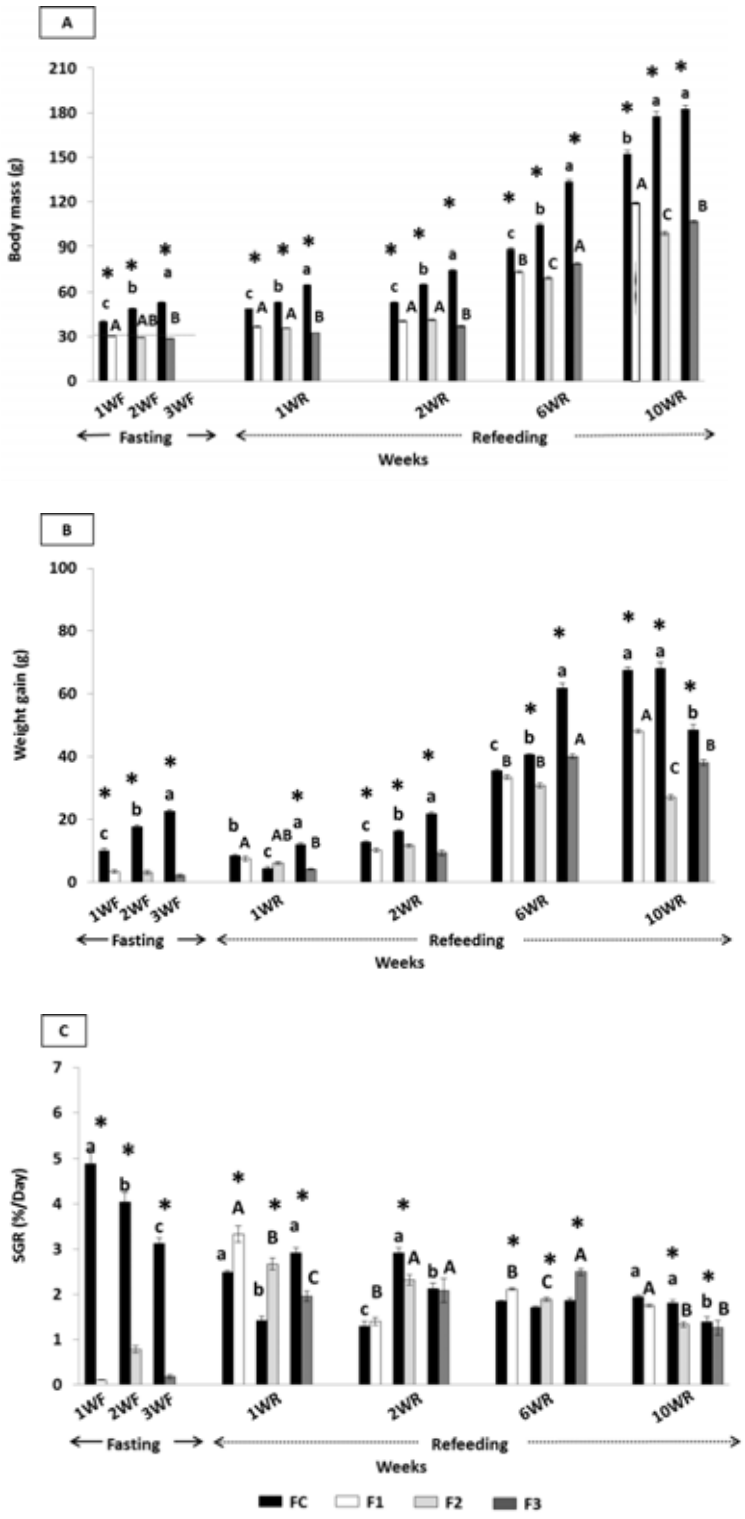

Figure 1 - Body mass, (g, BM), weight gain (g, WG) and specific growth ratio (SGR, % day-1) of Nile tilapia (*Oreochromis niloticus*) juveniles during fasting and refeeding periods. FC: control, continuously fed during 13 weeks of treatment; F1: one week of fasting and 10WR; F2: two weeks of fasting and 10WR; F3: three weeks of fasting and 10WR. Uppercase letters compare between fasting treatments after fasting and refeeding. Lowercase letters compare FC treatment after fasting and refeeding. (*) denotes significant difference (P<0.05) between FC and fasting treatments. Data are means ± SEM (n=8).

After the refeeding, fasting fish did not exhibit hyperphagia, and feed intake at the 1st, 2nd and 3rd day of refeeding was lower in the F1, F2 and F3 than in the FC treatments. However, feed intake percentage respective to fish body mass was significantly different among the treatments. At the 1st refeeding day, the feed intake percentage respective to on body mass was significantly lower in F1 and F3 treatments, but in F2 did not differ from FC. However, at the 2nd and 3rd days of refeeding, the F1 showed similar percentage of feed intake respective to body mass in relation to FC.

The feed conversion ratio (FCR) was significantly higher in F2 and F3 in comparison to the FC, but the F1 was similar to FC, after 10 WR (Table 1).

Table 1 – Feed performance recovery during refeeding of Nile tilapia (*Oreochromis niloticus*) juveniles.

Treatments	1st day	2nd day	3rd day	10 WR	FCR
			Feed Intake (g/fish)		
FC	1.24 ± 0.14 Ba	1.76 ± 0.13 Aba	1.51 ± 0.18 Ba	139.94 ± 2.62	1.43 ± 0.05
F1	0.63 ± 0.10 Bb	1.16 ± 0.40 Ab	1.12 ± 0.05 Ab	134.12 ± 1.22	1.51 ± 0.05
FC	1.28 ± 0.03Ba	2.32 ± 0.08 Aa	1.87 ± 0.23 Aa	157.52 ± 1.51	1.30 ± 0.07 b
F2	0.89 ± 0.19 Bb	0.95 ± 0.14 Ab	0.77 ± 0.12 ABb	145.11 ± 1.50	2.04 ± 0.07 a
FC	2.45 ± 0.16 Ba	4.00 ± 0.21 Aa	2.11 ± 0.17 Ba	153.53 ± 1.65	1.30 ± 0.11 b
F3	0.42 ± 0.07 Bb	0.68 ± 0.09 Ab	0.68 ± 0.13 Ab	127.98 ± 1.57	1.52 ± 0.09 a

			Feed Intake/body mass (%, BM)	
Treatments	1st day	2nd day	3rd day	10 WR
FC	3.16 ± 0.23 Ba	4.34 ± 0.23 Aa	4.06 ± 0.29 Aa	12.65 ± 0.40
F1	2.30 ± 0.17 Bb	3.86 ± 0.21 Aa	3.86 ± 0.23 Aa	12.50 ± 0.33
FC	2.65 ± 0.14 Ca	4.69 ± 0.25 Aa	3.65 ± 0.20 Ba	12.78 ± 0.34 b
F2	2.12 ± 0.24 Ba	3.32 ± 0.27 Ab	2.72 ± 0.20 Abb	14.85 ± 0.26 a
FC	4.54 ± 0.25 Ba	7.41 ± 0.32 Aa	3.95 ± 0.25 Ba	10.69 ± 0.39 b
F3	1.47 ± 0.15 Bb	2.40 ± 0.19 Ab	2.50 ± 0.23 Ab	21.63 ± 0.49 a

Feed intake (g/fish) and Feed Intake/body mass (%, BM) was measured after the 1st, 2nd and 3rd day of refeeding of Nile tilapia submitted to 1, 2 and 3 weeks of fasting and 10 weeks of refeeding (10WR). Feed conversion ratio (FCR) was measured after 10WR. BM = body mass; FC: control, continuously fed during the 13 weeks; F1: one week of fasting and 10WR; F2: two weeks of fasting and 10WR; F3: three weeks of fasting and 10WR. Uppercase letters compare treatments during the refeeding (in rows) and lowercase letters compare between FC and fasting treatments (F1, F2 and F3) during the 3-days refeeding (in columns). The same letter in column or in line shows absence of significant difference ($P > 0.05$). Data are means ± SEM (n=8).

4.2 Lipid as the primary energy source during fasting

Proximate composition analyses showed significant carcass lipid reduction ($P< 0.001$) in F1, F2 and F3 treatments during the fasting periods. However, the body lipid percentage was higher only in F3 in comparison to FC treatment after 10WR (Fig. 2 A). There was no difference in the percentage of carcass crude protein throughout the experiment among all treatments (Fig. 2 B). Carcass ash percentage was higher ($P < 0.001$) in F2 and F3 treatments compared to the FC ($P < 0.05$). Nonetheless, after 10WR, no differences were observed among the treatments (Fig. 2 C). Moisture percentage was higher in F3 than FC after fasting ($P < 0.001$), and lower after 10WR (Fig. 2 D).

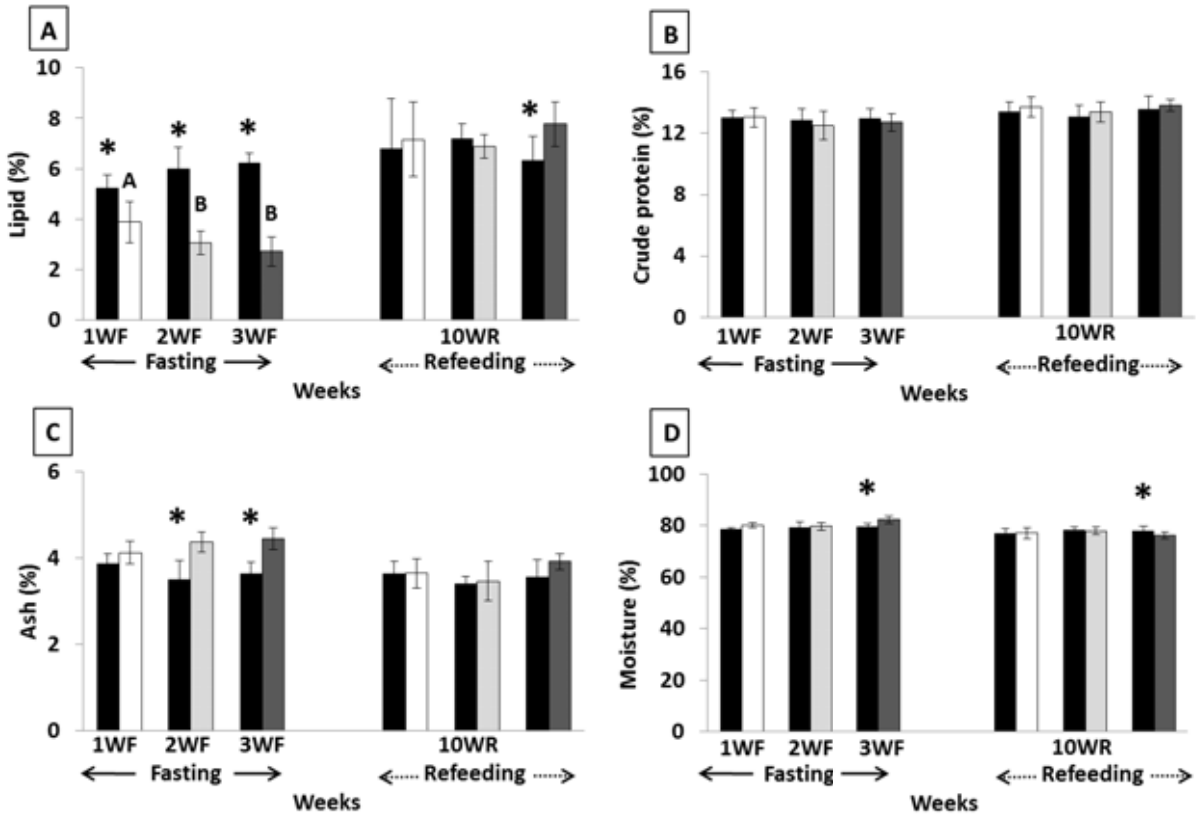

Figure 2 - Lipid as the primary energy source during fasting of Nile tilapia (*Oreochromis niloticus*). Proximate composition (lipid, crude protein, ash and moisture %) of carcass of Nile tilapia (*Oreochromis niloticus*) juveniles at the beginning of the experiment (0 day), after 1, 2 and 3 weeks of fasting and after 10WR; FC: control, continuously fed during the 13 weeks; F1: one week of fasting and 10WR; F2 two weeks of fasting and 10WR; F3: three weeks of fasting and 10WR.Lowercase letters compare the FC treatment during fasting and refeeding periods and uppercase letters compare between fasted fish after fasting and refeeding. (*) denotes significant difference ($P < 0.05$) between FC and fasting treatments. Data are means ± SEM (n=8).

4.3 Blood biochemical parameters

Plasma glucose levels in all fasted fish were similar to FC at the end of each fasting and refeeding periods. However, it was significantly higher in fish from the F3 compared to the F1 and F2 treatments after fasting (Fig. 3 A).

Plasma triglycerides were significantly lower only in the F1 compared to the FC treatment after fasting. Among the fasting treatments, the triglycerides levels were higher in fish from the F3 than the F1 treatments. At the end of each refeeding period, no differences were found between FC and fasted fish, but the comparison between the fasted fish showed higher levels of plasma triglycerides in F1 and F3 ($P < 0.05$) (Fig. 3 B).

Serum cholesterol levels did not differ between fasted and FC fish at the end of each fasting and refeeding periods. Nonetheless, it was significantly higher in fish from F3 in comparison to the F1 and F2 treatments. Cholesterol levels increased in the FC with the highest value in the F3 compared to F1 and F2 treatments (Fig. 3 C).

Serum total protein did not differ between fasted and FC fish at the end of each fasting and refeeding periods. However, among the fasting treatments, the total protein level in the F2 was higher than in the F1 and F3 treatments. Moreover, after refeeding, the total protein level was higher in the F1 and F2 than in the F3 treatments (Fig. 3D).

During the fasting phase, cortisol levels were higher only in the FC than in the F1 treatments (Fig. 3 E).

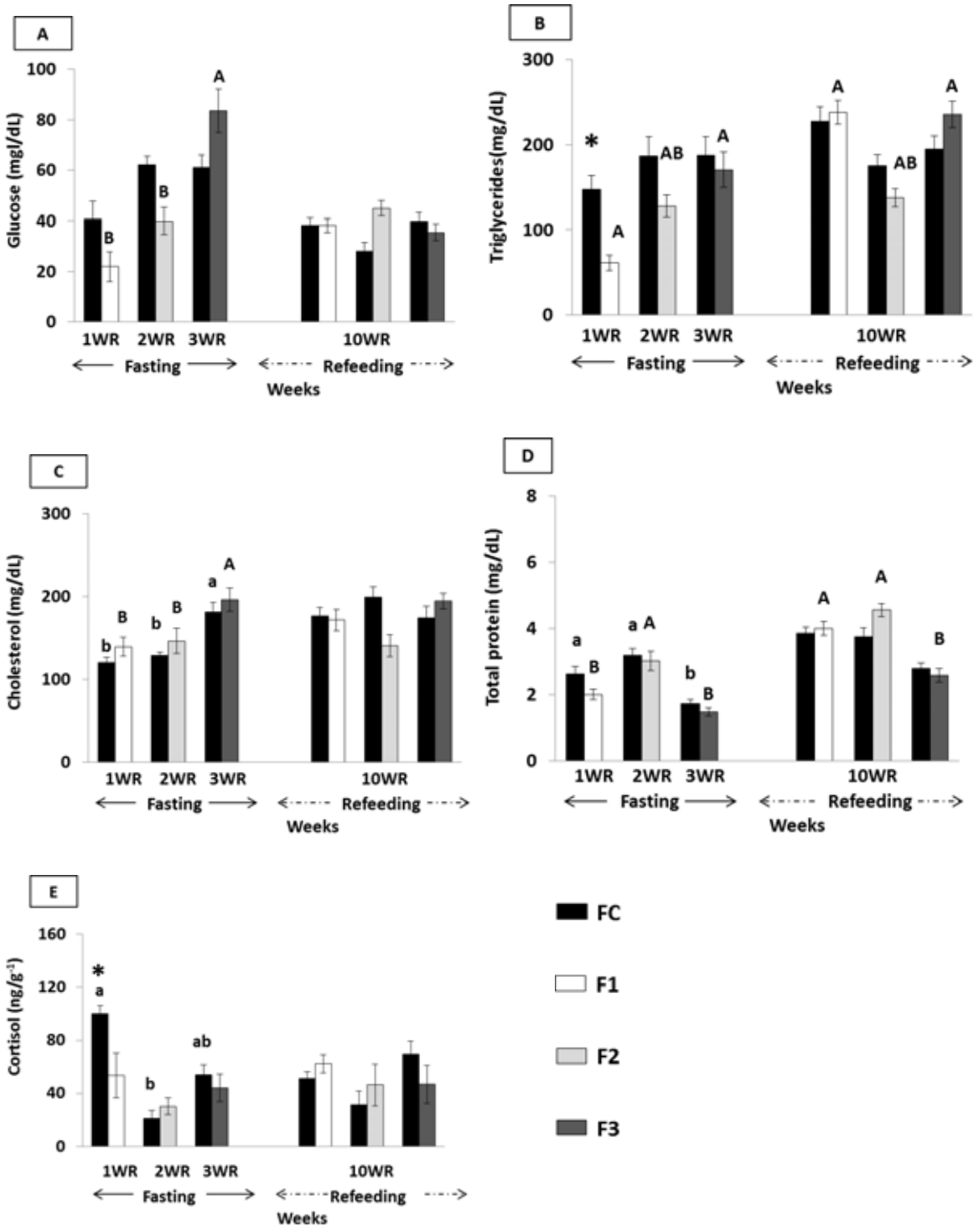

Figure 3 – Changes in plasma glucose, triglycerides, serum cholesterol, total protein and cortisol levels of Nile tilapia (*Oreochromis niloticus*) juveniles after 1, 2 and 3 weeks of fasting and after 10WR. FC: control, continuously fed during the 13 weeks; F1: one week of fasting and 10WR; F2: two weeks of fasting and 10WR; F3: three weeks of fasting and 10WR. Lowercase letters compare the FC treatment during fasting and refeeding periods and uppercase letters compare between fasted fish after fasting and refeeding. (*) denotes significant difference ($P < 0.05$) between FC and fasting treatments. Data are means ± SEM (n=8).

4.4 Hepatosomatic index (HIS) and Visceral fat index (VFI) drop after fasting and recovery after refeeding

The HSI decreased significantly in fish submitted to fasting, with the highest value found in the FC followed by F3, F2, and F1 treatments in this order. However, after 10WR, the HSI increased in the F1, F2 and F3 treatments, with no significant differences among them, to become higher than in the FC ($P < 0.05$) (Fig 4 A). The VFI was significantly lower in the F2 and F3 treatments compared to the FC after fasting ($P < 0.001$) (Fig. 4 B).

4.5 Liver glycogen mobilization during fasting and reestablishment after refeeding

Liver glycogen levels dropped significantly in all fasted fish. However, after 10WR, no differences were found between the FC and fasted fish ($P > 0.05$). Indeed, after refeeding, the highest liver glycogen level was observed in the F3 treatment (Fig. 4 C). Liver lipid levels did not differ between the FC and fasted fish throughout the experiment ($P > 0.05$) (Fig. 4 D).

Muscle lipid also did not differ from fasting and FC fish during fasting and refeeding. However, after the end of refeeding, muscle lipid levels were higher in the F1 than in the F3 treatments (Fig. 4 E).

Muscle protein level was significantly higher in the fish of the F2 treatment during the fasting period, and in the fish of the F1 treatment after refeeding, in comparison to the levels presented by the FC fish of the same conditions. Muscle protein varied among fasted fish during fasting and it was higher in the F3 than in the F1 during refeeding (Fig. 4 F).

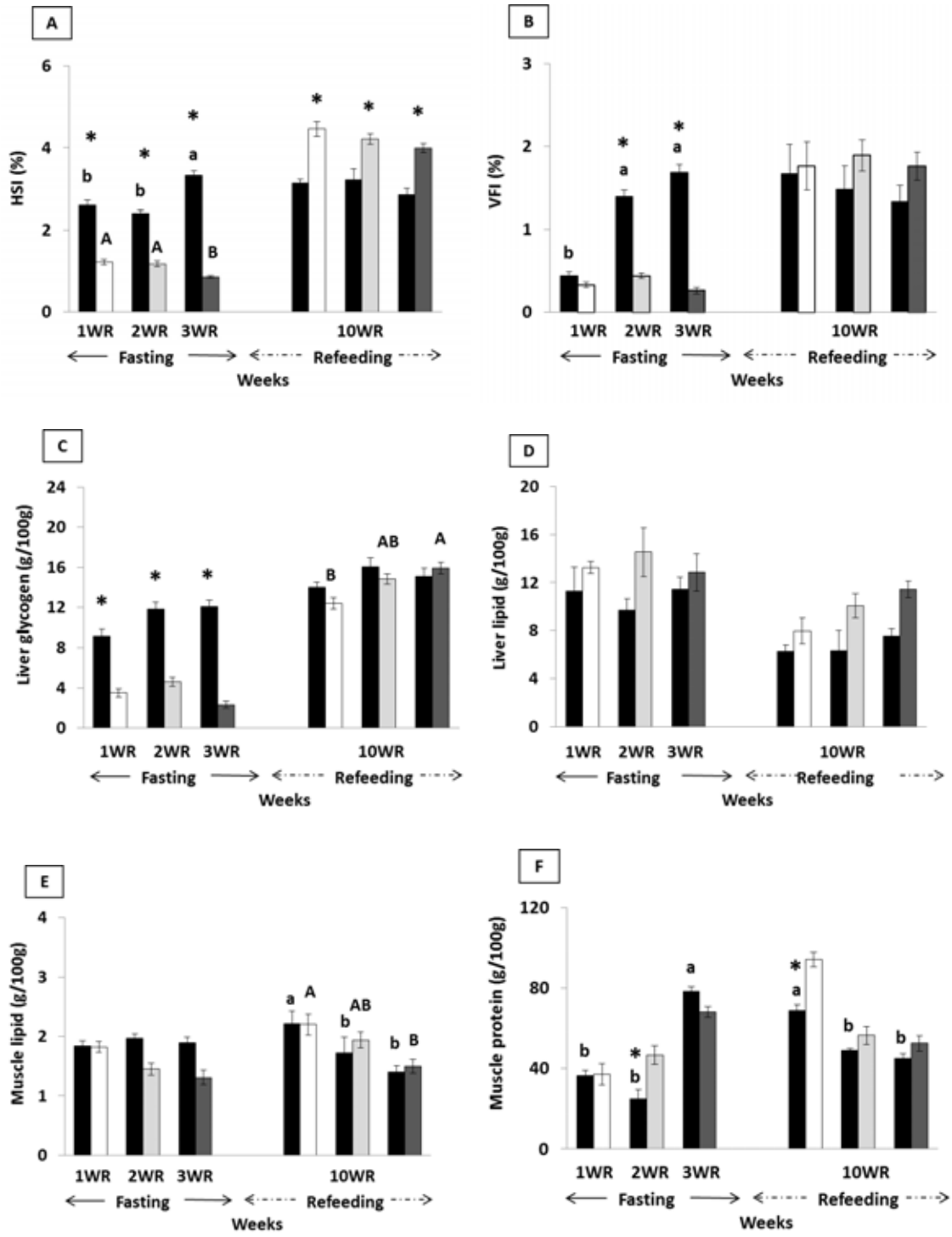

Figure 4 - Changes in hepatosomatic index (HSI, liver weight/body mass x 100 to achieve the percentage %), visceral fat index (VFI, weight/body mass x 100 to achieve the percentage %), liver glycogen, liver lipid, muscle lipid and muscle protein of Nile tilapia (*Oreochromis niloticus*) juveniles after 1WF, 2WF and 3WF and after 10WR. FC: control, continuously fed during the 13 weeks; F1: one week of fasting and 10WR; F2: two weeks of fasting and 10WR; F3: three weeks of fasting and 10WR. Lowercase letters compare the FC treatment during fasting and refeeding periods and uppercase letters compare between fasted fish after fasting and refeeding. (*) denotes significant difference ($P < 0.05$) between FC and fasting treatments. Data are means ± SEM (n=8).

5. Discussion

The purpose of use the fasting technique, that can manipulate the muscle proximate composition and lipid content, is also an important strategy to improve fish meat quality and farming productivity (Gricorakis and Alexis, 2005; Zhang et al., 2008). In this study, we analyzed the dynamics of growth and mobilization of energy source in Nile tilapia (*Oreochromis niloticus*) juveniles during different periods of fasting and after refeeding.

Fasted tilapia lost weight for one to three weeks and it was observed significantly reduction of lipid and increase in moisture of carcass. In *Dentex dentex*, the weight loss after fasting is result of energy mobilization from different body compartments to maintain important processes of life (Pérez-Jiménez et al., 2012). In our study, fasting for one to three weeks was sufficient to reduce SGR and induce the mobilization of energy sources to maintain metabolic process in Nile tilapia (*O. niloticus*) juveniles. The same results of decreasing of SGR was observed with hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus*) juvenile (14.5 g), reared in freshwater and fasting cycles for three days per week for four months (Abdel-Hakim et al., 2009).

The lower body mass founded in all fasted fish indicated that the fasting periods affected fish growth and it was also reported in Nile tilapia juveniles (6.6g) when a cyclical feed deprivation strategy, with three different protocols of fasting and refeeding during 12 weeks (Wang et al., 2009). Other protocols with fasting showed decrease of body mass in Mozambique tilapia (*O. mossambicus*) juveniles submitted to one to four weeks of food restriction (Fox et al., 2010), and with juveniles (40-60g) during 7 to 28 days of fasting (Breves et al., 2014). These decreases of body mass suggest that tissue degradation was higher than synthesis.

During refeeding, in our experiment, the SGR and BM in all fasted fish increased, but the BM was lower compared to FC treatment, suggesting growth recovered capacity and partial compensatory growth. Similarly to our results, in other fasting and refeeding protocols with Mozambique tilapia (*O. mossambicus*) (50 g) only partial compensatory growth was observed after food reestablishment. These fasted juveniles exhibited higher SGR until six weeks of refeeding, but at the eight weeks of refeeding, the SGR did not differ to the control, showing that the compensatory growth levels are dependent of the SGR at the first weeks of refeeding (Fox et al., 2010).

In our study we evaluated food consumption in the first 3 days of refeeding and did not observe hyperphagia in refed fish compared to control. However, the percentage of feed intake in relation to fish body mass increased in fish from F1, F2 and F3 treatments during refeeding being similar to FC. Even compensatory growth been typically characterized by hyperphagia, improved feed conversion ratio and increased specific growth rate (Ali et al., 2003; Picha et al., 2006; Won and Borski, 2013), studies reveal that hyperphagia produced a weak growth compensation (Gurney et al., 2003; Urbinati et al., 2014). Our results are in agreement with a study with hybrid tilapia (*O. niloticus x O. aureus*) juvenile (14.5 g), starved for 4 days (Abdel-Hakim et al., 2009). In this study, no increase in feed intake and specific growth ratio was observed in comparison to fed control treatment, after 4 months of refeeding. In our experiment, however, feed intake after 10 weeks of refeeding in F1, F2 and F3 were 4.16%, 7.87% and 16.64%, respectively, lower than in FC treatment, exhibiting also lower body mass, in F1 (12,03%), F2 (19,06%) and F3 (36,6%) in relation to FC.

Survival rates of Nile tilapia juveniles (30.2 ± 0.9 g) were not affected by fasting up to three weeks, and were high at the end of the experiment (> 84% in FC, F1 and F2 treatments and approximately 79% in F3), showing the rusticity of the species. However, in smaller Nile tilapia (6.6 g) submitted to 4 weeks of fasting with subsequently refeeding for 8 weeks, Wang et al. (2009) reported lower survival rate (60%). In territorial species, as Nile tilapia, dominant animals typically suppress the growth of subordinates. In such stressing situation, the subordinate Nile tilapia showed higher consumption of carbohydrate reserves (Fernandes and Volpato, 1993), increase the opercular movement that require higher energy expenditure, and in prolonged hypoxia subordinate fish die before the dominants (Volpato et al., 1989).

Liver glycogen and lipid storages in the carcass appear to be the main source of energy mobilized by tilapia during fasting to maintain the vital processes, as carcass lipid composition showed significant reduction in fasted fish during food restriction periods. In agreement to our results, the body lipid levels were significant lower in juvenile hybrid tilapia (*O. niloticus* x *O. aureus*) deprived of feed for 2 and 3 days per week during 4 months in comparison to control fed fish (Abdel-Hakim et al., 2009). In contrast, in Nile tilapia juveniles subjected to different cycles of fasting and refeeding, no differences in carcass lipid composition was noticed (Wang et al., 2009). In other hand, we also observed that carcass lipid increased in all fasted fish

at the end of the experiment, and in fish from F3 treatment it was higher compared to FC treatment. The quick restoration of liver glycogen and body lipid reserves in refed fish are indicative of the importance of both reserves in tilapia.

Two to three weeks of fasting also decreased fat on viscera, but did not affect fish muscle protein. Visceral fat index (VFI) at 2 and 3 weeks of fasting in fasted fish was lower than FC. Moreover, the hepatosomatic index (HSI) decreased significantly in all fasted fish in comparison to FC, showing that HSI decreased due to depletion of stored nutrients like fat and glycogen on liver. The reduction of liver weight is immediately affected by fasting and this effect has been also observed in other species (Francesco et al., 2004; Peterson and Small, 2004; Guroy et al., 2010; Han et al., 2010). Sturgeon (*Huso huso*) is able to conserve carcass protein better than lipid in different schemes of fasting and refeeding (Falahatkar, 2012). In the present study, carcass protein percentage and muscle protein in fish from all fasted treatments did not decrease along the experiment. In previous study, carcass protein reduced in Nile tilapia after 4 weeks fasting (Wang et al., 2009). Thus, in our study, tilapia preferred to save muscle protein and carcass protein using mainly lipids from peritoneal cavity.

During fasting periods, the glucose levels in fasted tilapia did not differ in relation to control fish. This finding may be related to the liver glycogen mobilization, being a way to maintain the blood glucose levels. Likewise, in other fish species traíra *(Hoplias malabaricus,* Rios et al., 2006), cod (*Gadus morhua,* Black and Love, 1986), channel catfish (*Ictalurus punctatus,* Gaylord and Gatlin III, 2000) and in sunshine bass (*Morone chrysops x Morone saxatilis,* Davis and Gaylord, 2010) the liver glycogen also decreased during fasting. Nonetheless, after food reestablishment the glycogen stores were completely recovered in fasted tilapia.

Cortisol is the main corticosteroid in teleost fish and rise during stress situation (Barton, 2002). Nile tilapia fasted for 1 to 3 weeks showed different physiological responses among the treatments. Cortisol levels, at the first week of fasting (1WF), were lower in F1 compared to FC. At the second week of fasting (2WF), it was higher in fish from F2 treatment than in FC, showing an increasing in cortisol levels as the time of starvation increased. Likewise, in larvae of Mozambique tilapia fasted for 3 to 6 days a rise in cortisol levels was observed with increasing of fasting, suggesting that the corticotrophin response to fasting is proportional to the degree of nutritional stress (Rodgers et a., 2003). Peterson and Small (2004) reported in channel catfish increased plasma cortisol level at 30 days of fasting, and

this increasing was related to the boost in glucose levels, possibly a result of cortisol induced gluconeogenesis. At the third week of fasting in the present experiment, no differences were found between F3 and FC treatment, similarly to the observed in sunshine bass, in which cortisol concentration was unaffected by 4 weeks of fasting (Davis and Gaylord, 2011). Possibly the higher glucose levels in fasted fish were maintained by cortisol-induced gluconeogenesis.

In summary, fasting for 1 to 3 weeks with subsequently refeeding for 10 weeks induced partial compensatory growth in Nile tilapia juveniles. Along with liver glycogen, the fat reserves were mobilized at 1 to 3 weeks of fasting to maintain basal metabolism and survival, but the lipids were restored to control levels after 10 weeks of refeeding. These results showed that unlike other protocols with other species that exhibit total compensatory growth, the feed strategies utilized in Nile tilapia juveniles (1-3 weeks of fasting and 10 weeks of refeeding) were able to cause only partial growth compensation. Therefore, our study indicates that the feed strategy utilized did not prevent fasted fish to grow during the refeeding phase. But at the same time, fasted fish were not able to achieve total or over compensation of the body mass as fed control treatment.

Acknowledgment

The authors are grateful to the Aquaculture Center of the São Paulo State University by facilities and technical assistant support. We also want to thank the staff of the laboratory of the Department of Morphology and Physiology (FCAV/Unesp) laboratory support. This work was funded by the São Paulo Research Foundation for student doctoral scholarships (2011/08426-3 and 2013/13100-3) and research grant (2011/22326-0).

References

A.O.A.C. **Official Methods of Analysis**. Association of Official Analytical Chemistry. EUA, 2000.

ABDEL-HAKIM., N.F., ABO-STATE., H.A., AL-AZAB., A.A., EL-KHOLY, KH.F. Effect of feeding regimes on growth performance of juvenile hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus)*. **World Journal of Agricultural Science**. v.5, n.1, p.49-54, 2009.

ALI M, NICIEZA A, WOOTON, R.J,,Compensatory growth in fishes: a response to growth depression. **Fish and Fisheries,** v.4, p.147-190, 2003.

BARTON, B.A. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. **Integrative and Comparative Biology,** v.42, p.517-525, 2002.

BLACK, D., LOVE., R.M. The sequential mobilisation and restoration of energy reserves in tissues of Atlantic cod during starvation and refeeding. Journal **Comparative Physiology B,** v.156, p.469-479, 1986.

BLIGH, E.G., DYER, W.J. A rapid method for total lipd extration and purification. **Canadian Journal of Biochemistry and Physiology**. v.37, p.911-917, 1959.

BRADFORD, M. A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye-binding. **Analytical Biochemistry**. v.72, p.248-254,1976.

BREVES, J.P., TIPSMARK, C.K., STOUGH, B.A., SEALE, A.P., FLACK, B.R., MOORMAN, B.P., LERNER, D.R., GORDON GRAU, E. Nutritional status and growth hormone regulate insulin-like growth factor binding protein (igfbp) transcripts in Mozambique tilapia. **General Comparative and Endocrinology,** v.207, p.66-73, 2014.

CHO, S.H., HEO, T.Y. Effect of dietary nutrient composition on compensatory growth of juvenile olive flounder *Paralichthys olivaceus* using different feeding regimes. **Aquaculture Nutrition,** p.1-8, 2010.

DAVIS, K.B., GAYLORD, G.T. Fasting on body composition and responses to stress in sunshine bass. **Comparative Biochemistry and Physiology, Part A,** v.158, p.30-36, 2011.

DREW., R.E., RODNICK, K.J., SETTLES, M., WACYK, J., CHURCHILL, E., POWEL, M.S., HARDY, R.W., MURDOCH, G.K., HILL, R.A., ROBISON, B.D. Effect of starvation on transcriptomes of brain and liver in adult female zebrafish (*Danio rerio*). **Physiology Genomics,** v.35, p.283-295, 2008.

FALAHATKAR, B. The metabolic effects of feeding and fasting in beluga *Huso huso.* **Marine Environmental Research,** v.82, p.69-75, 2012.

FERNANDES, M.O., VOLPATO, G.L. Heterogeneous growth in the Nile tilapia: Social Stress and Carbohydrate Metabolism. **Physiology & Behavior, v.**54, p.319-323, 1993.

FOOD AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, 2012. Global aquaculture production: 1950-2012. Available ftp://ftp.fao.org/FI/STAT/summary/a-6.pdf

FOX., B.K., BREVES, J.P., DAVIS, L.K., PIERCE., A.L., 2010. Tissue-specific regulation of the growth hormone/insulin-like growth factor axis during fasting and re-feeding: Importance of muscle expression of IGF-I AND IGF-II mRNA in the tilapia. **General Comparative Endocrinology,** v.166, p.573-580, 2010.

FRANCESCO, M., PARISI, G., MÉDALE, F., LUPI, P., KAUSHIK, S.J., POLI, B.M. Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*). **Aquaculture,** v.236, p.413-429, 2004.

GAYLORD, T.G., GATLIN III, D.M. Dietary protein and energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*). **Aquaculture,** v.194, p.337-348, 2001.

GAYLORD, T.G., GATLIN III, D.M. Assessment of compensatory growth in channel catfish *Ictalurus punctatus* R. and Associated changes in body condition indices. **Journal World Aquaculture Society,** v.31, n.3, p.326-336, 2000.

GURNEY, W.S.C., JONES, W., VEITCH, A.R., NISBET, R.M. Resource allocation, hyperphagia, and compensatory growth in juveniles. **Ecology,** v.34, n.10, p.2777–2787, 2003.

GUROY, D., GUROY, B., MERRIFIELD, D.L., ERGUN, S., TEKINAY, A.A., YIGIT, M. Effect of dietary Ulva and Spirulina on weight loss and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum) during a starvation period. **Journal of Animal Physiology and Animal Nutrition,** v.95, p.320-327, 2011.

HAN, C., WEN, X., ZHENG, Q., LI, H. Effect of starvation on activities and mRNA expression of lipoprotein lipase and hormone-sensitive lipase in tilapia (*Oreochromis niloticus x O. areus*). **Fish Physiol. Biochem**. 37, 113-122, 2011

JOBLING, M. Effects of starvation on proximate chemical composition and energy utilization of Plaice, *Pleuronectes platessa* L. **Journal of Fish Biology,** v.17, p.325-334, 1980.

JOBLING, M., JOHANSEN, J.S. The lipostat, hyperphagia and catch-up growth. **Aquaculture Research,** v.30, p.473-478, 1999.

LOVE, R.M. **The chemical biology of fishes**. Academic Press, London. v.1, 547p, 1970.

MONTSERRAT, N., GABILLARD, J.C., CAPILLA, E.; NAVARRO, M.I.; GUTIÉRREZ, J. Role of insulin, insulin-like growth factors, and muscle regulatory factors in the compensatory growth of the trout (*Oncorhynchus mykiss*). **General Comparative Endocrinology,** v.150, p.462-472, 2007.

MOON, T.W., FOSTER, G.D., PLISETSKAYA, E.M. Changes in peptide hormone and liver enzymes in the rainbow trout deprived of food 6 weeks. **Canadian Journal of Zoology**. V. 67, n. 9, p. 2198-2193, 1989.

NAVARRO, I., GUTIERREZ, J. **Fasting and starvation**. In: Biochemistry and molecular biology of fishes. Edited: P.W. Hochanchka and T.P. Mommsen Elsevier Science, Amsterdam. v.4, p.393-434, 1995.

NICIEZA, A.G., ALVAREZ, D.,2009. Statistical analysis of structural compensatory growth: how can we reduce the rate of false detection? **Ecology,** v.159, p.27-39, 2009.

PÉREZ-JIMÉNEZ, A., CARDENETE, G., HIDALGO, M.C., GARCÍA-ALCÁZAR, A., ABELLÁN, E., MORALES, A.E. Metabolic adjustments of Dentex dentex to prolonged starvation and refeeding. **Fish Physiology and Biochemistry,** v.38, n.4, p.1145-1157, 2012.

PETERSON, B.C., SMALL, B.C., WALDBIESER, G.C., BOSWORTH, B.G. Endocrine responses of Fast- and Slow-Growing Families of Channel Catfish. **North American Journal of Aquaculture,** v.70, p.240-250, 2008.

RIOS, F.S.A., MORAES, G., OBA, E.T., FERNANDES, M.N., DONATTI, L., KALININ., A.L., RANTIN, F.T. Mobilization and recovery of energy in traíra, *Hoplias malabaricus* Block (Teleostei, Erythrinidae) during long-term starvation and after re-feeding. **Journal of Comparative Physiology, Part B,** v.176, p.721-728, 2006.

TEROVA, G., RIMOLDI, S., LARGHI, S., BERNARDINI, G., GORNATI, R., SAROGLIA, M. Regulation of progastricsin mRNA levels in sea bass (*Dicentrarchus labrax*) in response to fluctuations in food availability. **Biochemical and Biophysical Research Communications,** v.363, p.591-596, 2007.

URBINATI, E.C., SARMIENTE, S.J., TAKAHASHI, L.S. Short-term cycles of feed deprivation and refeeding promote full compensatory growth in the Amazon fish matrinxã (*Brycon amazonicus*). **Aquaculture,** v.433, p.430-433, 2014.

VOLPATO, G.L.; FRIOLI, P.M.A.; CARRIERI, M.P. Heterogeneous growth in fishes; some new data in the Nile tilapia (*Oreochromis niloticus*) and a general view about the causal mechanisms. **Bol. Fisiol. Anim**. 13, 7-22, 1976.

WANG, T., HUNG, C.C.Y., RANDAL, D.J., 2006. The comparative physiology of food deprivation: from feast to famine. **Annual Review of Physiology**, v.68, p.223-251, 2006.

WANG, Y., QIN, J.G., HAN, H. Cyclical feed deprivation and refeeding fails to enhance compensatory growth in Nile tilapia, *Oreochromis niloticus* L. **Aquaculture,** v.40, p.204-201, 2009.

WON, E.T., BORSKI, R.J., Endocrine regulation of compensatory growth in fish. **Frontiers Endocrinology,** v.4. p.1-13, 2013.

CAPÍTULO 3 - INCREASED EXPRESSION OF ATROGENES AND IGF-1 REDUCTION INDUCED BY FASTING IN NILE TILAPIA (*Oreochromis niloticus*) JUVENILES

Abstract

The growth rate of farmed fish is one of the most important factors for aquaculture success. The understanding of the cellular events that occur in skeletal muscle when fish undergo periods of fasting and refeeding can provide information to develop alternative feeding strategies to improve muscle growth of commercially cultivated species. To evaluate the effect caused by one to three weeks of fasting and 10 weeks of refeeding in Nile tilapia juveniles, we analyzed the growth performance and changes in muscle cellularity and the expression of growth-related genes (MyoD, myogenin, IGF-1 and IGF-1 receptor), atrogenes (MuRF-1 and atrogin-1/MAFbx) and the myostatin. Lower body mass and specific growth rate (SGR) was observed in all fasted fish during the fasting periods, and 10 weeks of refeeding resulted in partial compensatory growth. No differences in white muscle fiber frequencies in diameter classes were observed between fasted and fed control fish treatments. However, changes in gene expression induced by fasting and refeeding were found. IGF-1 receptor and ubiquitin ligases MuRF1 and atrogin-1 expression increased during 1-3 weeks of fasting, while IGF-1 levels dropped significantly ($P < 0.001$) in all fasted fish compared to the control treatment. However, the expression of the MRFs MyoD did not differ throughout the experiment, while myogenin mRNA level in fish submitted to three weeks of fasting was higher in comparison to the control treatment at the end of fasting ($P < 0.05$). Overall, our results showed that one to three weeks of fasting can induce muscle atrophy activation in Nile tilapia juveniles, and 10 weeks of refeeding it is enough to induce only partial compensatory growth.

Key-words: Muscle atrophy, Fasting, Muscle Regulatory Factors, IGF-1, MuRF-1, atrogin-1.

Introduction

Fasting is a phenomenon that can occur naturally in the environment due to low food supply, during fish migration, winter or reproductive, but fish have the ability to survive without food during lengthy periods of time (Love, 1970; Bower et al. 2009). After a period of growth depression and when favorable conditions are restored, there occurs a phase of accelerated growth, called compensatory growth (Ali et al., 2003). In teleosts, the maintenance of skeletal muscle development and growth is a complex process, and may be influenced by environmental factors and nutritional and physiological state of the animal (Johnston 2008; Johnston et al. 2011). A significant amount of research effort has been expended in understanding the regulatory factors surrounding muscle accretion, specifically evaluation of the genes involved. Myogenic regulatory factors (MRFs) are a family of four transcription factor (MyoD, myogenin, myf5 and MRF4/myf6) that are highly conserved in vertebrates and are responsible for the control of myogenesis and muscle growth. Each gene has evolved to regulate a specific area of myogenesis. For instance, MyoD and myf5 are essential regulators of muscle cell proliferation determination, whereas Myogenin and myf4 acts as regulators of muscle cell differentiation (Rudnicki et al., 1993; Steinbacher et al., 2007; Johnston, 2008).

Muscle growth is also controlled by the regulator myostatin. Myostatin is a member of the transforming growth factor-β (TGF- β) superfamily that functions as a negative regulator of skeletal muscle development and growth in mammalian; however, in fish, myostatin is also present in other tissues and in other forms (Maccatrozzo et al., 2001; Rios et al., 2002; Acosta et al., 2005; De Santis et al., 2012). Another regulator of muscle growth in vertebrates is the insulin-like growth factor-1 (IGF-1) that represents the main autocrine and paracrine regulator mechanisms of skeletal muscle by activation of the PI3k/Akt pathway which consequently increases protein synthesis, associated with muscle hypertrophy (Glass, 2003; Sacheck et al., 2004; Kandarian and Jackman, 2006).

Muscle protein degradation is regulated by several processes, one of which is the ubiquitin-proteasome pathway that includes two muscle-specific ubiquitin ligases E3, such as muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx/atrogin-1). Thus, the increase of MuRF1 and atrogin-1 expression causes fiber reduction due to catabolic conditions (Glass, 2005).

The Nile tilapia (*Oreochromis niloticus*) is one of the most important fish species that has been found to effectively adapt to a wide range of rearing conditions (Abdel-

Hakim et al., 2009; Fox et al., 2009). However, tilapia culture is affected by water shortage, low food supply in floating cages, prolonged winter, fish reproductive period and other conditions. Understanding the molecular mechanisms that control muscle growth in tilapia would be beneficial for improving production under different conditions as fasting.

Based on this context, we tested the hypothesis that the ubiquitin-proteasome pathway is activated by the increase of ubiquitin ligases (MuRF1 and atrogin-1) expression in tilapia juveniles during periods of fasting, and IGF-1 is overexpressed during the refeeding to induce compensatory growth.

2 - Material and Methods

2.1 Experimental design

The experiment was conducted at the Laboratory of Nutrition of Aquatic Organisms of the Aquaculture Center, UNESP, SP, Brazil. Fish was obtained from a commercial farm and acclimated (15 days) to the experimental conditions. Nile tilapia (*Oreochromis niloticus*) juveniles, chitralada Thai strain (initial weight 30.2 ± 0.9 g) were stored (32 fish/tank) into 32 tanks with 150 liters of water and constant aeration, and randomly distributed into four experimental treatments and eight replicates: (FC) control, fish were fed continuously during the 13 weeks; (F1) one week of fasting and 10 weeks of refeeding, (F2) two weeks of fasting and 10 weeks of refeeding; (F3) three weeks of fasting and 10 weeks of refeeding. The experiment lasted 13 weeks, and a fixed period of 10 weeks of refeeding was maintained for all fasting treatments (Figure 1). Fish were fed to apparent satiation with an extruded commercial diet (32% crude protein) three times per day (9 am, 2 pm, and 5 pm). White muscle samples were collected to analyze morphology, morphometry and the expression of growth related genes and muscle atrophy, at the end of each period of fasting (1WF, 2WF and 3WF) and after 10WR. Water variables were monitored weekly and maintained at: temperature 30.1 ± 0.32 °C, pH 7.89 ± 0.14 and dissolved oxygen 4.74 ± 0.86 mg/L. Fish were euthanized (0.1 g/L of benzocaine) and individually weighed and measured. The body mass (g), weight gain (g) and the specific growth ratio (SGR) [(Ln final body mass – Ln initial body mass) x 100/ experimental period in days] were evaluated at the beginning of the experiment (0 day) and after each period of fasting: one (1WF), two (2WF) and three (3WF) weeks, and after 10 weeks of refeeding (10WR). The study was approved by the Ethics

Committee on Animal Use (CEUA) of the Faculty of Agricultural and Veterinarian Sciences of the São Paulo State University (CEUA/FCAV/UNESP, 009436/11).

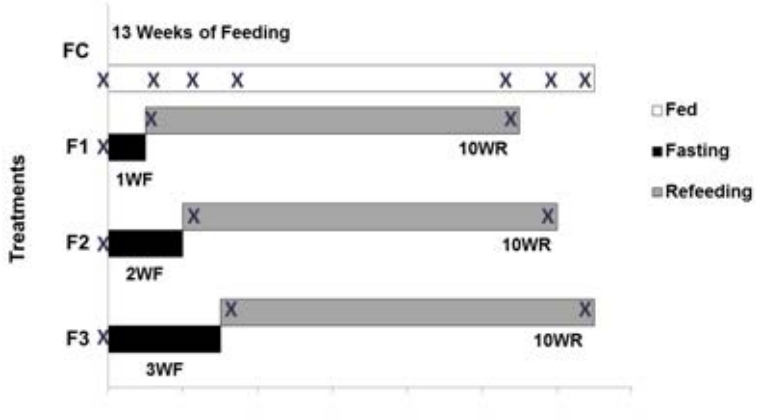

Figure 1 - Experimental design: (FC) fed control; fish were fed continuously during 13 weeks; (F1) one week of fasting and 10 weeks of refeeding; (F2) two weeks of fasting and 10 weeks of refeeding; (F3) three weeks of fasting and 10 weeks of refeeding. (x) indicates each sampling period.

2.2 Morphology and Morphometry of muscle fibers

White muscle samples were collected from the dorsal region, fixed in Karnovsky solution (8% paraformaldehyde and 2.5% glutaraldehyde in PBS – phosphate buffer saline) and embedded in Historesin® (Leica, Germany). Histological transverse sections (4 µm) were obtained and stained with hematoxilyn-phloxine B to analyze muscle fiber diameter. To estimate the degree of muscle hypertrophy, hyperplasia or atrophy the diameter of 700 white muscle fibers from each animal (n=8 per treatment) were measured into three to four fields. The fibers were distributed into classes according to their diameter (d, µm): class 10 = $d < 10$, class 30 = $10 \leq d < 30$, class 50 = $30 \leq d < 50$ and class 60 = $d \geq 50$, and white muscle fibers frequency in different diameter classes was evaluated. Images were captured with a Zeiss Axioplan 2 microscope and analyzed in Zeiss Axiovision 4.7 software (Carl Zeiss Microscope, LLC, USA).

2.3 Quantitative gene expression

White muscle samples were collected from the dorsal region of fish during the experiment and frozen in liquid nitrogen. Total RNA was extracted using TRIzol® Reagent according to the manufacturer's protocol (Life Technologies, Carlsbad, CA, USA). Extracted RNA integrity was confirmed by electrophoresis on a 1% agarose

gel stained with GelRed (Biotium, Hayward, CA, USA) and visualized under ultraviolet light. The amount of RNA extracted was determined using a NanoVue™ Plus Spectrophotometer (GE Healthcare, Piscataway, NJ, USA). RNA purity was ensured by obtaining a 260/280 nm OD ratio ≥ 1.8. Total RNA was solubilized in RNase-free water, incubated in Dnase I (Life Technologies, Carlsbad, CA, USA) to remove any DNA present in the samples. Total RNA (2µg) was reverse transcribed using the High Capacity cDNA archive kit with RNase inhibitor (Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. At the end of the reaction, the products were stored at -20 °C.

Gene expression analysis was evaluated by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). For each gene, specific primers were tested at different concentration levels to determine optimal amplification. Samples of cDNA were amplified with specific primers for *MyoD, myogenin, myostatin* (Nebo et al., 2013), IGF-1, IGF-1 receptor, and ubiquitin genes designed from cDNA nucleotide sequences, available in GenBank (http://www.ncbi.nlm.nih.gov/pubmed/nucleotide). The gene expression analyses were performed in ABI 7500 Fast Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). The reactions were run in duplicate and using Fast SYBR Green Master Mix (Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. Target gene expression was normalized to ubiquitin gene expression and melting dissociation curves were performed to confirm that only a single product was amplified. Control reactions lacking cDNA template were evaluated to check for reagent contamination.

For the analysis of MuRF1, atrogin-1 and beta-actin genes expression primers and probes were utilized. Reactions were run with the real-time qPCR ABI Prism 7900HT Sequence Detection System (Life Technologies, Carlsbad, CA, USA) and Verso 1-Step RT-qPCR ROX Mix (Thermo Fisher Scientific, Waltham, MA, USA). The reactions were run in duplicate with kit Taqman Assay (Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. Samples were normalized to beta-actin gene expression. Relative gene expression was calculated using the ($2^{-\Delta\Delta CT}$) method (Livak and Schimittgen, 2001). For IGF-1, IGF-1 receptor, beta-actin, MuRF1, and atrogin-1 genes primers and probes were designed using PrimerQuest (Integrated DNA Technologies, Coralville, IA) (Table 1).

Table 1 - Oligonucleotide primers and probes used for RT-qPCR amplification.

Primer-Probe Sequence (5'- 3')	GenBank Accession No
Atrogin-1	
Forward: GTGGGCTCGTTCAGGACAT	XM005450592.1
Reverse: CGGTGTACCCAGATGTTGATGTTC	
Probe: CAGCACAGACTTGCCC	
B-actin	
Forward: GCCCCACGCCATCCT	XM003443127.2
Reverse: GAGTAGCCACGCTCTGTCA	
Probe: CTGGCCGTGACCTCAC	
MuRF1	
Forward: AGGGTGAACTGAGTAATGCTATCGA	XM005456465
Reverse: GCAGGCATCTTCCATCTG	
Probe: CAGGCGACCATTGCTG	
IGF1	
Forward: CAAGAGCACCCAAGGTTAGTAG	EU272149.1
Reverse: CAGGTGAAGGTCTCTTCTTGTT	
IGF1 receptor	
Forward: GTGGTCAAAGACGAACCAGA	KC506777.1
Reverse: CTCATGACGGAGGCTTCATT	
Myogenin	
Forward: GCAGCCACACTGAGGGAGAA	*Nebo et al., 2013*
Reverse: AAGCATCGAAGGCCTCGTT	
Myostatin	
Forward: TGTGGACTTCGAGGACTTTGG	*Nebo et al., 2013*
Reverse: TGGCCTTGTAGCGTTTTGGT	
MyoD	
Forward: TCAGACAACCAGAAGAGGAAGCT	*Nebo et al., 2013*
Reverse: CCGTTTGGAGTCTCGGAGAA	
Ubiquitin	
Forward: CCTGCGGCTGATTTTGGT	XM005463259
Reverse: CAGAGTCGTTGGGAGAGAAGA	

3. Statistical Analysis

Gene expression data were submitted to analysis of variance (one-way ANOVA), and differences between means were compared by Tukey's test (P <0.05), using the Statistical Analysis System SAS v.9 software (SAS Institute Inc., Cary, NC, USA). All data were tested for homogeneity of variances by Brown-Forsythe's test (P<0.05) and normal distribution using Cramer-von Mises test (P <0.05). Logarithmic transformations (natural logarithm) were used, in analysis of body mass, SGR, IGF-1, IGF-1 receptor, myogenin, atrogin-1 and MuRF1, as relevant correction in original data. Morphometric data were analyzed by non-parametric Kruskal-Wallis ANOVA followed by Bonferroni multiple comparison test.

Results

4.1 Body mass response to fasting and refeeding

Survival rate of Nile tilapia juveniles was higher than 84% in the FC, F1, and F2 treatments whereas in the F3, it was approximately 79%. However, these differences were not significant.

As expected, no BM increase was observed during the first until the third week of fasting; in fact, fish did not lose but also did not gain weight during fasting (Fig. 2 A). The BM and WG increased during refeeding periods (1WR, 2WR, 6WR and 10WR), but it were lower in all fasted fish than FC treatment. At 1WR, the BM and WG in fish from F1 and F2 were significantly higher compared to the F3 treatment. At 2WR, the BM was still higher in fish from F1 and F2 treatments and the WG did not differ in all fasted fish. However, at 6WR, the BM and WG increased in fish from F3 compared to F1 and F2 treatments. After 10WR, the BM and WG in fish from F1 were significantly higher compared to the F2 and F3 treatments (Fig. 2 A, B).

The SGR was lower in all fasted fish compared to the FC treatment and it was different among the FC groups. At 1WR, the SGR in fish from the F1 and F2 was higher than FC treatments. At 2WR, the SGR were similar in fish from F1 and F3 and in the F2 it was lower compared to FC treatment. However, at 6WR, the SGR was higher in all fasted fish than FC treatment and at 10 WR the SGR was lower in the F2 and F3 compared to FC treatment (Fig. 2 C).

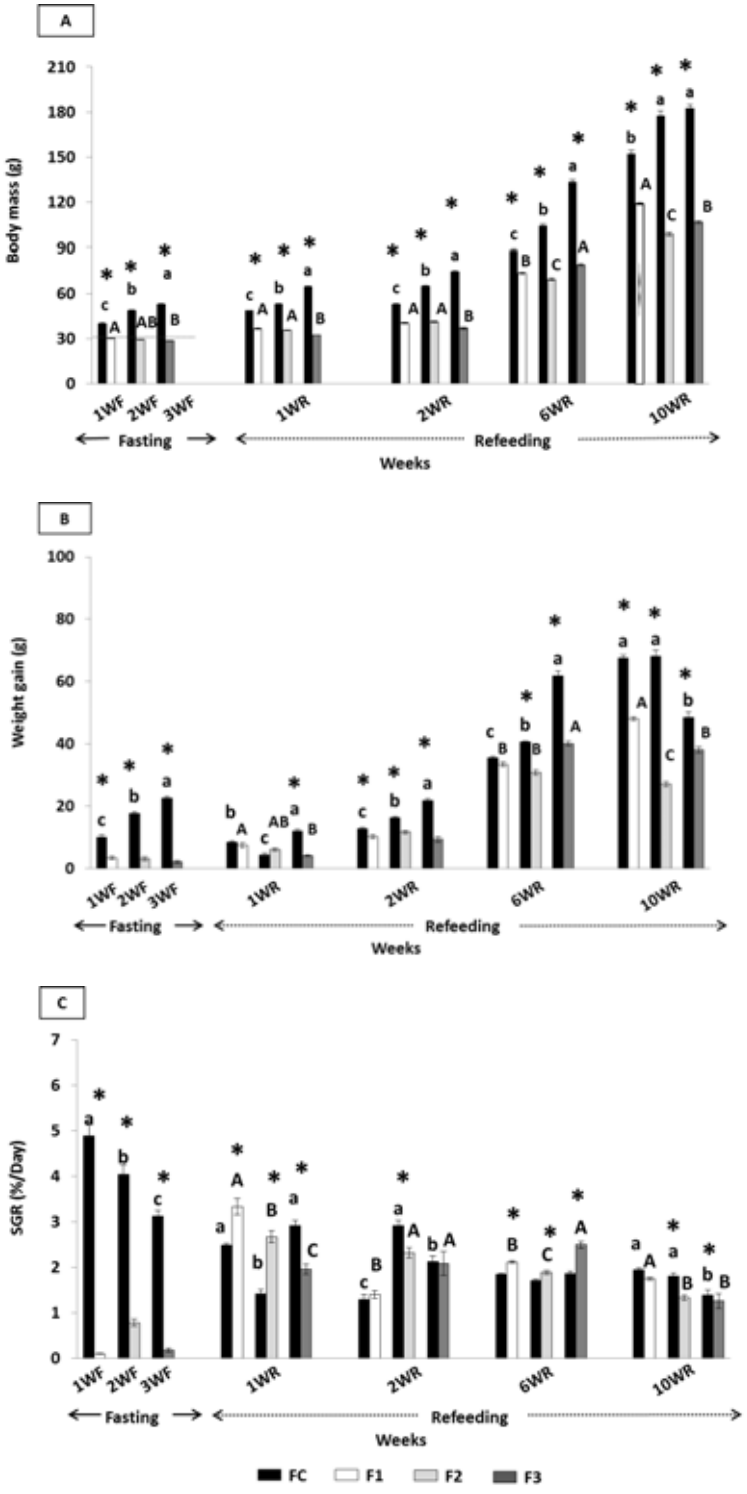

Figure 2 - Body mass (g, BM), weight gain (g, WG) and specific growth ratio (SGR, % day^{-1}) of Nile tilapia (*Oreochromis niloticus*) juveniles during fasting and refeeding periods. FC: control, continuously fed during the 13 weeks of treatment; F1: one week of fasting and 10WR; F2: two weeks of fasting and 10WR; F3: three weeks of fasting and 10WR. Uppercase letters compare between fasting treatments after fasting and refeeding. Lowercase letters compare FC treatment after fasting and refeeding. (*) denotes significant difference (P < 0.05) between FC and fasting treatments. Data are means ± SEM (n=8).

4.2 Morphological and morphometric analyses

Muscle morphology revealed at 1WF in the F1 and in the FC treatments a high quantity of muscle fibers with central nucleus, exhibiting a pattern of mosaic hyperplasia (Fig. 3 A, B). Nonetheless, the morphology of muscle fibers at 2WF or 3WF in fish from F2, F3 and in their fed control (FC) treatment was different than it was observed one week before, showing increased number of mature muscle fibers with multinucleated cells with peripheral nuclei (Fig. 3 C, D, E, F). After 10WR, in fish from FC and in the fasting treatments (F1, F2 and F3), muscle fiber exhibited a typical mosaic pattern with polygonal shaped fibers, small fibers adjacent to big fibers (Fig. 4 G, H, I, J, K, L).

After 1WF, 2WF and 3WF, morphometric analyses showed a higher quantity (> 95%) of smaller muscle fibers in classes 10 and 30, and a lower quantity (< 5%) in classes 50 and 60 (Fig. 5 A, B, C). The frequency of muscle fibers in diameter classes did not differ between fasted fish and respective FC ($P > 0.05$). However, after 10WR the distribution of white muscle fibers frequency in diameter classes changed in fish from F1, F2 and F3 treatments, showing higher numbers of big fibers in classes 50 and 60 compared to classes 10 and 30. Similar to the fasting period, no differences were found among FC and the fasted fish, after 10WR (Fig. 5 D, E, F).

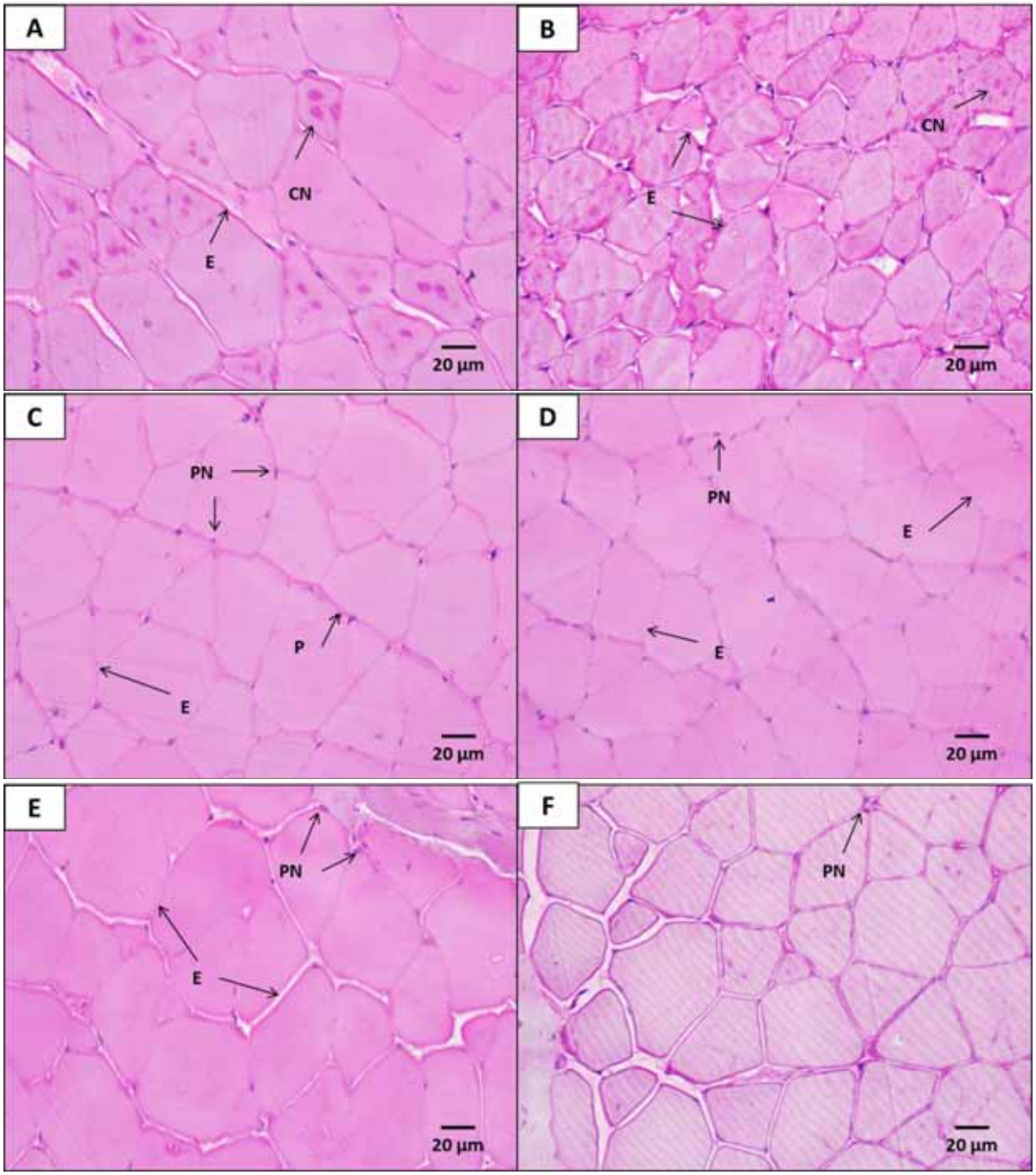

Figure 3 - Transverse section of white muscle fiber of Nile tilapia (*Oreochromis niloticus)* juveniles submitted to fasting and refeeding: (FC) control, feeding continuously during the 13 weeks; (F1) one week of fasting and 10WR, (F2) two weeks of fasting and 10WR; (F3) three weeks of fasting and 10WR. Small fibers around big fibers, forming a growth mosaic pattern. Central nucleus (CN), peripheral nucleus (PN), perimysium (P) and endomysium (E). (A, B) FC and F1 at 1WF; (C, D) FC and F2 at 2WF; (E, F) FC and F3 at 3WF. Hematoxylin-floxin stain. Scale bars: 20 µm, 40 X.

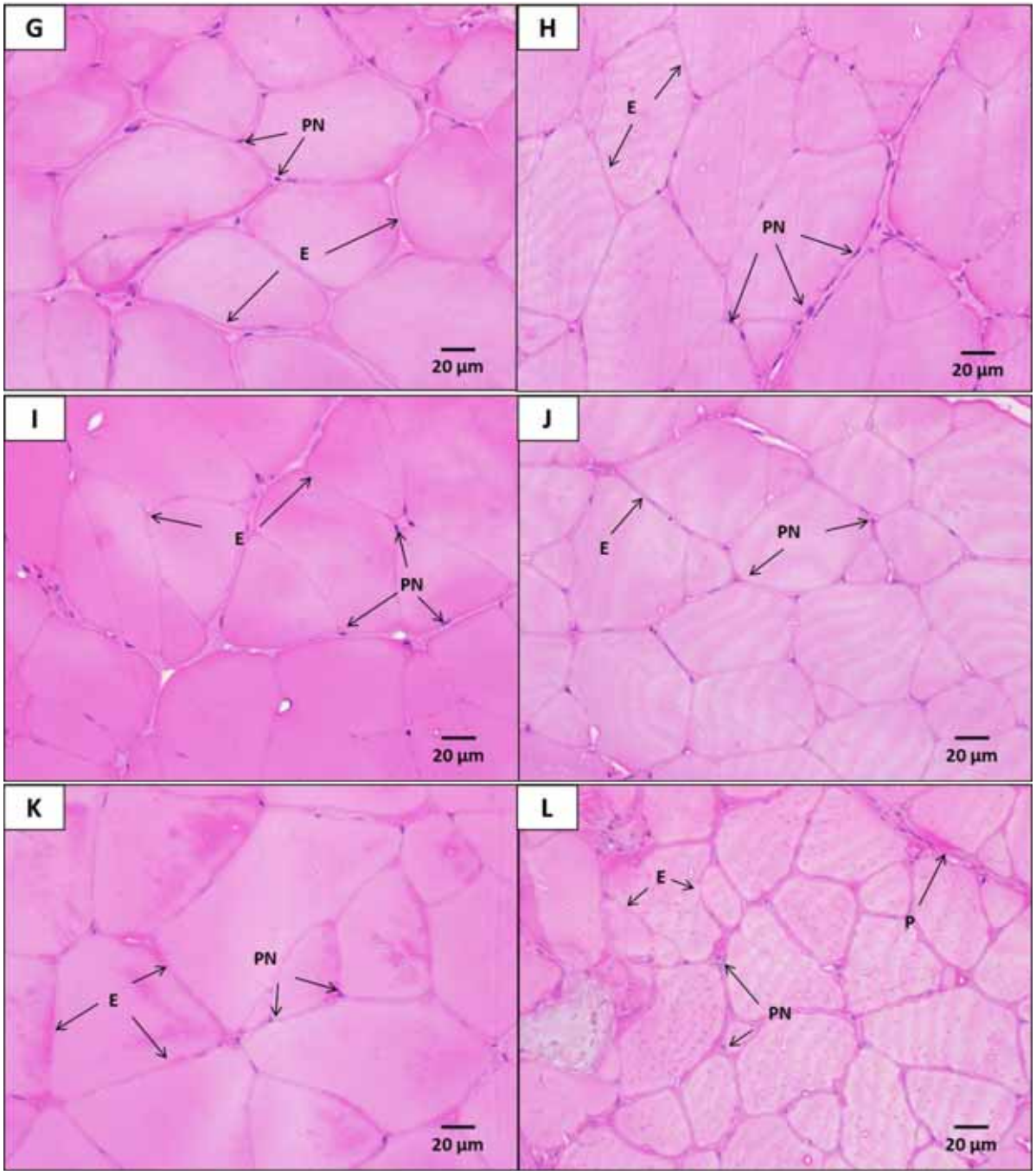

Figure 4 - Transverse section of white muscle fiber of Nile tilapia (*Oreochromis niloticus)* juveniles submitted to fasting and refeeding*:* (FC) control, feeding continuously during the 13 weeks; (F1) one week of fasting and 10WR; (F2) two weeks of fasting and 10WR; (F3) three weeks of fasting and 10WR. *S*mall fibers around big fibers, creating a growth mosaic pattern. Peripheral nucleus (PN), endomysium (E), and perimysium (P). (G, H) FC and F1 at 10WR; (I, J) FC and F2 at 10WR; (K, L) FC and F3 10WR. Hematoxylin-floxin stain. Hematoxylin-floxin stain. Scale bars: 20 µm, 40X.

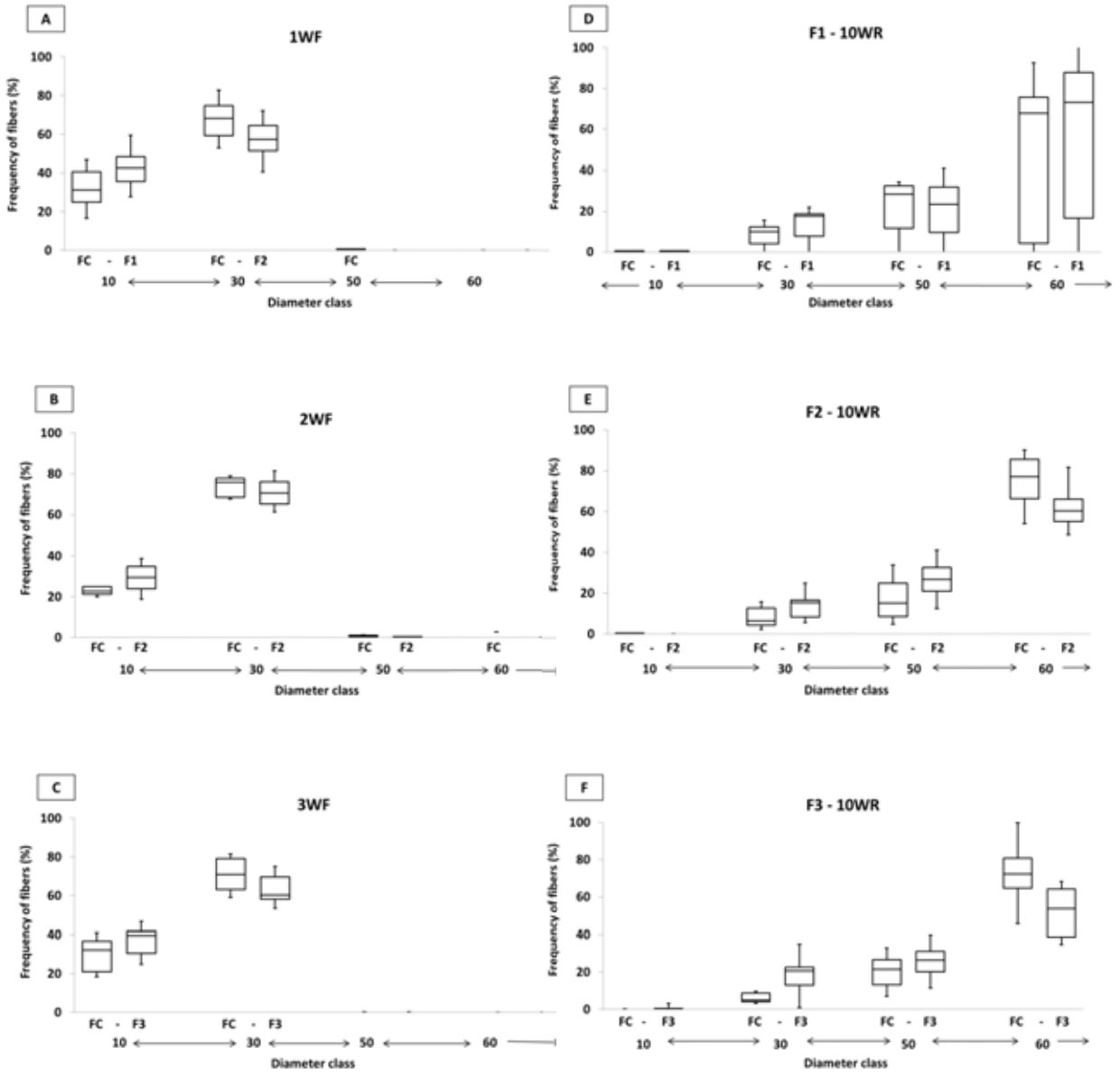

Figure 5 - Frequency of white muscle fibers in diameter classes of Nile tilapia (*Oreochromis niloticus*) juveniles submitted to fasting and refeeding: (FC) control, feeding continuously during the 13 weeks; (F1) one week of fasting and 10 weeks of refeeding; (F2) two weeks of fasting and 10 weeks of refeeding; (F3) three weeks of fasting and 10 weeks of refeeding. Muscle diameters are grouped into four diameter classes (d, μm): class 10 = d < 10, class 30 = 10 ≤ d < 30, class 50 = 30 ≤ d < 50 and class 60 = d ≥ 50. (A) FC and F1 at 1WF; (B) FC and F2 at 2WF; (C) FC and F3 at 3WF; (D) FC and F1 at 10WR; (E) FC and F2 at 10WR; (F) FC and F3 at 10WR. Data are presented with median values (minimum, 1st quartile, median, 3rd quartile and maximum).

4.3 Increased expression of atrogenes and IGF-1 reduction during fasting

The IGF-1 gene expression, at 1WF, 2WF and 3WF, was downregulated in all fasted fish, exhibiting significant differences ($P < 0.05$) in comparison to the FC; and after 10WR, the IGF-1 levels increased in all fasted fish, being similar to the IGF-1 mRNA level in the FC, showing a substantial response to refeeding. Furthermore, at 10WR, the F1 treatment was now found to be express IGF-1 significantly higher than F2 (Fig. 6 A). Conversely, the IGF-1 receptor was upregulated in fish from F1, F2 and F3 treatments during fasting periods, but after 10WR, only the expression of IGF-1 receptor in the F3 was significantly reduced ($P < 0.05$) in comparison to the FC treatment (Fig. 6 B).

MyoD gene expression was found to not differ among all treatments both in the fasting and refeeding (Fig. 6 C). Myogenin mRNA levels were not found to significantly change except at 3WF where they were higher than FC (Fig. 6 D).

The myostatin mRNA levels, after fasting and refeeding, did not differ among fasted fish and FC treatment (Fig. 7 A). The atrogin-1 and MuRF-1 gene expression, at 1WF, 2WF and 3WF, increased significantly ($P < 0.05$) in all fasted fish in comparison to the FC. However, after 10WR, the atrogin-1 and MuRF-1 levels in all fasted fish were similar to the FC treatment (Fig. 7 B, C). Atrogin-1 expression was even higher at 10WR and significantly different among F1, F2 and F3 treatments, but not different than FC (Fig. 7 B).

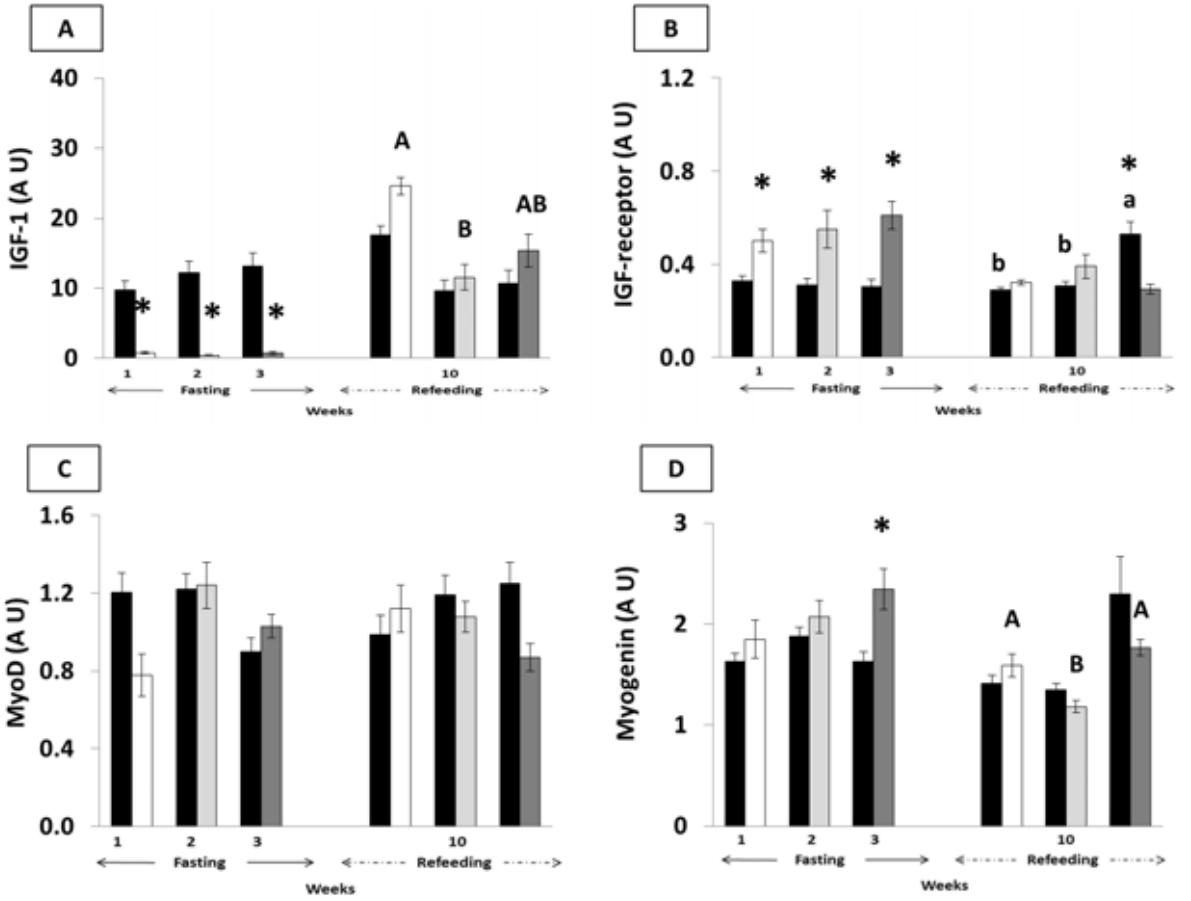

Figure 6 - Relative gene expression of IGF-1, IGF-1 receptor, MyoD and myogenin in white skeletal muscle of Nile tilapia (*Oreochromis niloticus*) juveniles during fasting (1WF, 2WF and 3WF) and after (10WR): (FC) control, feeding continuously during the 13 weeks; (F1) one week of fasting and 10WR; (F2) two weeks of fasting and 10WR; (F3) three weeks of fasting and 10WR. Lowercase letters compare FC during fasting and refeeding, uppercase letters compare F1, F2, and F3 treatments after fasting and refeeding, and (*) denotes significant difference ($P < 0.05$) between control and fasted treatments. Data are means ± SEM (n=8).

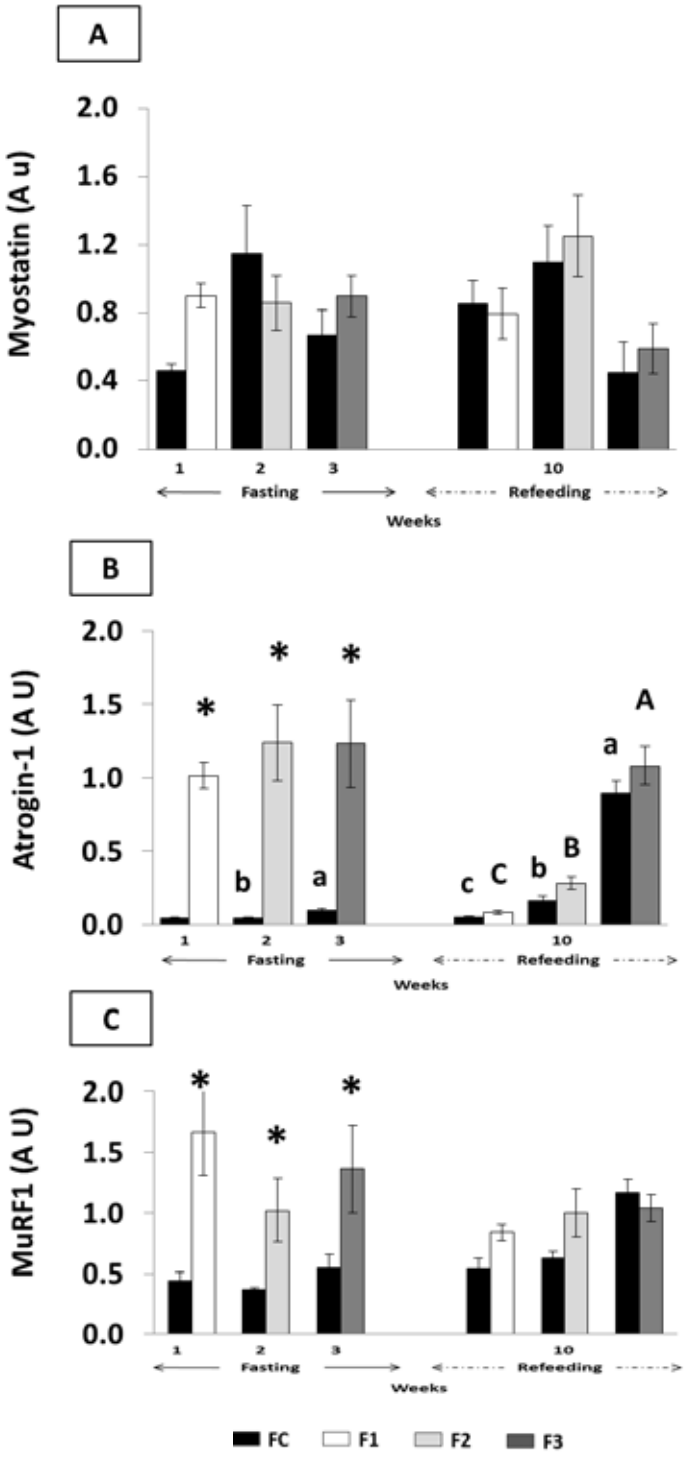

Figure 7- Relative gene expression of myostatin, atrogin-1 and MuRF-1 in white skeletal muscle of Nile tilapia (*Oreochromis niloticus*) juveniles submitted to fasting (1WF, 2WF and 3WF) and after refeeding (10WR): (FC) control, feeding continuously during the 13 weeks; (F1) one week of fasting and 10 weeks of refeeding; (F2) two weeks of fasting and 10WR; (F3) three weeks of fasting and 10WR. Lowercase letters compare FC during fasting and refeeding, uppercase letters compare F1, F2, and F3 treatments after fasting and refeeding, and (*) denotes significant difference ($P < 0.05$) between control and fasted treatments. Data are means ± SEM (n=8).

5. Discussion

In the present work, we investigated if fasting followed by ten weeks of refeeding would change muscle gene expression. Concurrently, we also wanted to evaluate if this starve/reefed regime would cause alterations in white muscle fiber plasticity and morphology in Nile tilapia (*Oreochromis niloticus*) juveniles.

The periods of fasting evaluated, one, two and three weeks (1WF, 2WF and 3WF, respectively), were not sufficient to cause a significant decrease in weight in fasted fish compared to weight prior to starvation period. One reason that significant changes were noticed between groups after refeeding is that possibly the fasting periods were not of sufficient length to provoke a strong compensatory growth response (Wang et al., 2000). Another reason may be the period of refeeding, ten weeks, is not enough to equal the feed control growth. In another study with tilapia juveniles subjected to 4 weeks of fasting and 8 weeks of refeeding, body weight in fasted fish was lower in relation to fed control group (Fox et al., 2009). Additionally, in a hybrid tilapia (*O. mossambicus x O. niloticus*) study, only partial compensatory growth after 2-4 weeks of starvation following by refeeding was observed (Wang et al., 2000).Furthermore, in rainbow trout only partial compensatory growth was reported in fish subjected to cycles of 8-day and 14-day fasting and refeeding (Nikki et al., 2004). However, Nile tilapia (chitralada strain) fingerlings (0.6g) submitted to short-term of fasting (5 days) with subsequent 37 days of refeeding, displayed total compensatory weight gain (Nebo et al., 2013). Therefore, the difference in degree of growth compensation achieved depends on fish age and the period of refeeding offered.

As expected, during the fasting periods the SGR of tilapia juvenile decreased proportionally. However, after refeeding the SGR increased in the fasted treatments to a greater level than control fish, suggesting that the fish had a tendency to recover weight and growth rates. A similar tendency was observed in rainbow trout (*Oncorhynchus mykiss*) fry, after one week of fasting and one month refeeding, the SGR increased in fasted treatment, equaling to SGR in fed control treatment (Montserrat et al., 2007).

To comprehend if fasting periods were harmful to fish skeletal muscle and the response of fish refeeding after 10 weeks, it was evaluated the morphology and the diameters of muscle fibers to know the degree of hypertrophy, hyperplasia or atrophy of fibers. High frequency (over 90%) of white muscle fibers in classes 10

and 30 during one 1WF, two 2WF and three 3WF weeks, in both fed control and fasting treatments shows that muscle hyperplasia was occurring in the juvenile tilapia within this study. Hyperplasia was evident within the first week of the experiment in the fish by observation of several small fibers with central nuclei. This morphological feature characterizes skeletal muscle development in mosaic hyperplasia, with small fibers containing central nuclei surrounding big fibers throughout the myotome (Johnston et al., 2003; Johnston and Hall, 2004; Johnston et al., 2008; Steinbacher et al., 2007; Valente et al., 2013). However, after 10 weeks of refeeding the growth pattern changed, increasing the frequencies of muscle fibers in classes 50 and 60 and decreasing the frequencies in classes 10 and 30. This different fiber distribution was demonstrated by the fish in all the fasted and fed treatments as evidence of hypertrophic muscle growth (Veggetti et al., 1993; Valente et al., 1999; Zimmerman and Lowery, 2000; Johnston, 2006). However, such differences seems to be more related to tilapia growth than to the fasting that they suffered, since at the end of the three fasting periods and after 10-weeks refeeding all treatments were similar to the control.

IGF-1 mRNA levels dropped significantly throughout periods of fasting (1WF, 2WF and 3WF), but after subsequent refeeding, the IGF-1 muscle expression in previously fasted fish was identical to the FC. Among fasting treatments, the mRNA level was higher in the F1 treatment. These findings are in agreement with other studies in which reductions in muscle IGF-1 levels as a result of fasting, and subsequent recovery following 2-4 weeks of refeeding, have also been observed in other starvation protocols with tilapia juveniles (*Oreochromis mossambicus*), rainbow trout (*Onchorhynchus mykiss*) fry (Fox et al., 2009; Montserrat et al., 2007), and rainbow trout adults submitted to 10 weeks of food deprivation and 4-34 days of refeeding (Chauvigne et al., 2003).

In channel catfish (*Ictalurus punctatus*) the IGF-1 expression decreased after 30 days of fasting, although after refeeding, the levels of expression were similar to the fed control group (Peterson and Waldbieser, 2009). In rainbow trout treated with IGF-1 during feed deprivation, inhibition of atrogin-1 expression was observed, along with a reduction of proteolytic activity (Cleaveland et al., 2009). In the present study, IGF-1 receptor was upregulated in tilapia during 1-3 weeks of fasting in F1, F2 and F3, significantly higher than in FC. Maybe the high level in IGF-1 receptor,

during fasting periods, could be related by downregulation in IGF-1, being a fish biological response to compensate the lack of IGF-1.

Other studies reported diverse results with different fish species and experimental conditions and, in general, fasting cause significantly changes in MRF gene expression (Chauvigné et al., 2003; Valente et al., 2012). However, in our results there was no change in the expression of MyoD between FC and fasted treatments (F1, F2 and F3) during the entire experiment ($P > 0.05$). Similar results were found in rainbow trout, no significant change in MyoD expression was found in response to starvation and refeeding (Johansen and Overturf, 2006). And in another study with rainbow trout fasted for 1, 2 and 4 weeks, the MyoD levels in white muscle were not significantly affected by fasting (Montserrat et al., 2007). Furthermore, myogenin levels at the first two weeks of fasting (2WF) and after ten weeks of refeeding (10WR) did not differ in relation to FC. In cell culture of gilthead sea bream muscle, the myogenin gene expression was not affected after 30 days of food deprivation (Garcia et al., 2014). We would expect that the expression of MyoD in the fasted fish would be lower than the control, since there is no energy or nutrients for growth and the catabolism of muscle for energy would be stimulating the expression of atrophy genes (MuRF-1 and atrogin-1).

If the expression of myostatin was high, it would explain the relatively low expression of MyoD in fasted treatments. However, in our study, skeletal muscle myostatin levels were not different ($P > 0.05$) in Nile tilapia juveniles between fasted and control fish throughout the experiment. A long-term study of fasting in adult tilapia also showed that myostatin expression was unaffected by fasting. It is possible that the degree of starvation to which the fish were imposed was not enough to stress the fish and modify myostatin levels (Rodgers et al., 2003). In rainbow trout deprived of food for 10 weeks and refed for 34 days, the myostatin expression, similar our findings, was not significantly different during the experiment (Chauvigné et al., 2003). On the other hand, in tilapia fingerlings (0.6g) the myostatin level was upregulated after 5 days of fasting in comparison feed control; nonetheless, after refeeding no differences were found between feed control group (Nebo et al., 2013). Still with tilapia, in larval phase, the myostatin mRNA levels was also altered by fasting and then reduced during refeeding (Rodgers et al., 2003). Taken together, these results suggest that the degree of catabolism induced by fasting in tilapia is greater in larvae and fingerlings, likely due to lower body

reserves, than in juvenile and adult fish that have more energy storage, and thus a higher capacity to tolerate fasting conditions.

The levels of atrogin-1 and MuRF1 in white skeletal muscle after 1-3 weeks of fasting increased rapidly, suggesting that an increase in protein degradation after one, two and three weeks of fasting. However, the atrogin-1 and MuRF1 expressions after 10 weeks refeeding dropped to basal levels, and no significant differences were found among all treatments ($P>0.05$). Our results corroborated with other reports observed in different fish species submitted to different fasting protocols. For instance, in rainbow trout juveniles, after 14 days of food deprivation the atrogin-1 levels increased and 12h after refeeding decreased significantly (Seiliez et al., 2008); in salmon white muscle, the expression of MuRF1 rose in fasted fish compared to fed control (Tacchi and Bicherdike, 2012); and in Atlantic salmon and zebrafish, the genes regulated by atrophy, such as E3 ligases (atrogin-1 and MuRF1) were also upregulated during fasting and downregulated after refeeding (Bower et al., 2009; Bower and Johnston, 2010; Amaral and Johnston, 2011).

It is perfectly established in vertebrates that insulin-like growth factor-1 (IGF-1) stimulates activation of the PI3K/Akt/mTOR cascade, increasing protein synthesis and hypertrophic muscle growth (Engert et al., 1996; Zhang et al., 2007; Duan et al., 2010), and inactivates FOXO transcription factors, decreasing the atrogin-1/MAFbx and MuRF1 expression (Sacheck et al., 2004; Edstron et al., 2006; Cleaveland et al., 2010; Fuentes et al., 2013). In this context, the lower IGF-1 expression observed in fasted tilapia juveniles is likely linked to the higher expression of MuRF1 and atrogin-1, consequently inducing muscle atrophy and blocking somatic growth. On the other hand, after refeeding, the expression of IGF-1 increased to the basal level, equal to the control, and resulted in growth recovery.

In conclusion, results from the study showed that the corresponding upregulation of atrogin-1 and MuRF1 and the drop IGF-1 expression during fasting periods correlate with an initial stage of muscle atrophy. No signs of atrophy were observed in muscle morphology, but the expression of atrophy genes changed during fasting. Nevertheless, the increase of IGF-1 levels after 10 weeks of refeeding activated the Akt pathway, decreasing the expression of protein in the ubiquitin proteasome pathway, thus indicating reduced rates of protein degradation and contributing to growth recovery. Furthermore, we found a partial compensatory

growth, after refeeding in Nile tilapia juveniles. These results from our study should provide a better understanding of molecular control related to skeletal muscle growth, and it will contribute to the fish development of feeding strategies providing optimal nutrition regimes to economically maximize growth of fish in culture.

Acknowlegment

The authors are grateful to the Sao Paulo Research Foundation/FAPESP for the financial support by means of scholarships (2011/08426-3 and 2011/13100-3) and research grant (2011/22326-0), and to the facilities and team of The University of Idaho – Hagerman Fish Culture Experiment Station. We thank technician Karen Frank at Hagerman Fish Culture Experiment Station for help in gene expression analyses.

6. References

ABDEL-HAKIM., N.F., ABO-STATE., H.A., AL-AZAB., A.A., EL-KHOLY, KH.F. Effect of feeding regimes on growth performance of juvenile hybrid tilapia (Oreochromis niloticus x Oreochromis aureus). **World Journal Agricultural Science,** v.5 n.1, p.49-54, 2009.

ACOSTA, J., CARPIO, Y., BORROTO, I., GONZÁLEZ, O., ESTRADA, M.P. Myostatin gene silenced by RNAi show a zebrafish giant phenotype. **Journal of Biotechnology,** v.119, p.324-331, 2005.

ALI M, NICIEZA A, R.J, W. Compensatory growth in fishes: a response to growth depression. **Fish and Fisheries,** v.4, p.147-190, 2003.

AMARAL, I.P.G., JOHNSTON, I.A. Insulin-like growth factor (IGF) signaling and genome-wide transcriptional regulation in fast muscle of zebrafish following a single-satiating meal. **Journal of Experimental Biology,** v.214, p.2125-2139, 2011.

BOWER, N.I., JOHNSTON, I.A. Transcriptional regulation of the IGF signaling pathway by amino acids and insulin-like growth factors during myogenesis in Atlantic salmon. **Plos One,** v.5, n.6, p.1-14, 2010.

BOWER, N.I., TAYLOR, R.G., JOHNSTON, I.A. Phasing of muscle gene expression with fasting-induced recovery growth in Atlantic salmon. **Frontier Zoology,** v.6, n.18, p.1-13, 2009.

CHAUVIGNE, F., GABILLARD, J.C., WEIL, C., RESCAN, P.Y. Effect of refeeding on IGF-1, IGF-II, IGF receptors, FGF2, FGF6, and myostatin mRNA expression in rainbow trout myotomal muscle. **General Comparative Endocrinology,** v.132, p.209-215, 2003.

CLEVELAND, B.M., EVENHUIS, J.P. Molecular characterization of atrogin-1/F-box protein-32 (FBXO32) and F-box protein-25 (FBXO25) in rainbow trout (*Oncorhynchus mykiss*): Expression across tissues in response to feed deprivation**. Comparative Biochemistry Physiology, Part B,** v.157, p.248-257, 2010.

CLEVELAND, B.M., WEBER, G.M., BLEMINGS, K.P., SILVERSTEIN, J.T. Insulin-like growth factor-I and genetic effects on indexes of protein degradation in response to feed deprivation in rainbow trout (Oncorhynchus mykiss). **American Journal of Physiology. Regulatory Integrative and Comparative Physiology,** v.297, p.1332-1342, 2009.

DE SANTIS, C., GOMES, G.B., JERRY, D.R. Abundande of myostatin gene transcripts and their correlation with muscle hypertrophy during the development of barramundi, *Lates calcarifer*. **Comparative Biochemistry and Physiology, Part B,** v.163, p.101-107, 2012.

DUAN, C., REN, H., GAO, S. Insulin-like growth factors (IGFs), IGF receptors and IGF-binding proteins: Roles in skeletal muscle growth and differentiation. **General Comparative Physiology,** v.167, p.344-351, 2010.

EDSTROM, E., ALTUN, M., HAGGLUND, M., ULFHAKE, B. Atrogin-1/MAFbx and MuRF1 are downregulated in aging-related loss of skeletal muscle. **Journal of Gerontology,** v.61, n.7, p.663-674, 2006.

ENGERT, C., BERGLUND, E.B., ROSENTHAL, N. Proliferation precedes differentiation in IGF-I stimulated myogenesis. **Journal of Cell Biology,** v.135, p.431-440, 1996.

FOX., B.K., BREVES, J.P., DAVIS, L.K., PIERCE., A.L. Tissue-specific regulation of the growth hormone/insulin-like growth factor axis during fasting and re-feeding: Importance of muscle expression of IGF-I AND IGF-II mRNA in the tilapia. **General Comparative Endocrinology,** v.166, p.573-580, 2010.

FUENTES, E.N., VALDES, J.A., MOLINA, A., BJORNSSON, B.T. Regulation of skeletal muscle growth in fish by the growth hormone – Insulin-like growth factor system. **General Comparative Endocrinoly,** v.192, p.136-148, 2013.

GARCIA DE LA SERRANA, D., CODINA, M., CAPILLA, E., JIMENEZ-AMILBURU, V., NAVARRO, I., DU, S.J., JOHNSTON, I.A., GUTIERREZ, J. Characterisation and expression of myogenesis regulatory factors during in vitro myoblast development and in vivo fasting in the gilthead sea bream (*Sparus aurata*). **Comparative Biochemistry Physiology, Part B,** v.167, p.90-99, 2014.

GLASS, D.J. Molecular mechanisms modulating muscle mass. **Trends in Molecular Medicine,** v.9, p.344-350, 2003.

GLASS, D.J. Skeletal muscle hypertrophy and atrophy signaling pathways. **The International Journal of Biochemistry & Cell Biology,** v.37, p.1974-1984, 2005.

JOHANSEN, K.A., OVERTURF., K. Alterations in expression of genes associated with muscle metabolism and growth during nutritional restriction and refeeding in rainbow trout. **Comparative Biochemistry Physiology,** v.144, p.119-127, 2006.

JOHNSTON, I.A. Environment and plasticity of myogenesis in teleost fish. **The Journal of Experimental Biology,** v.209, p.2249-2264, 2006.

JOHNSTON, I.A., BOWER, N.I., MACQUEEN, D.J. Growth and regulation of myotomal muscle mass in teleost fish. **The Journal of Experimental Biology,** 214, p.1617-1628, 2011.

JOHNSTON, I.A., FERNANDEZ, D.A., CALVO, J., VIEIRA, V.L.A., NORTH, A.W., ABERCROMBY, M., GARLAND, T. Reduction in muscle fibre number during the adaptavie radiation of notothenioid fishes: a phylogenetic perspective. **The Journal of Experimental Biology,** v.206, p.2595-2609, 2003.

JOHNSTON, I.A., MACQUEEN, D.J., WATABE, S.. Molecular biotechnology of development and growth in fish muscle. Fisheries for global welfare and environment, **5th World Fish Congr**. 241-262, 2008.

JOHNSTON, I.A., HALL, T.E. Mechanisms of muscle development and responses to temperature change in fish larvae. **American Fisheries Society Symposium,** 40, p.85-116, 2004.

KANDARIAN, S.C., JACKMAN, R.W. Intracellular signaling during skeletal muscle atrophy. **Muscle Nerve**, v.33, p.155-165, 2006.

LOVE, R.M. **The chemical biology of fishes,** vol.I. New York: Academic Press, 1970.

MACCATROZZO, L., BARGELLONI, L., RODAELLI, G., MASCARELLO, F., PATARNELO, T. Characterization of the Myostatin gene in the giltherdseabrean (*Sparus aurata*): sequence, genomic structure andexpression pattern. **Marine Biotechnology,** v.3, p.224-230, 2001.

MONTSERRAT, N., GABILLARD, J.C., CAPILLA, E.; NAVARRO, M.I.; GUTIÉRREZ, J. Role of insulin, insulin-like growth factors, and muscle regulatory

factors in the compensatory growth of the trout (Oncorhynchus mykiss). **General Comparative Endocrinology,** v.150, p.462-472, 2007.

NEBO, C., PORTELLA, M.C., CARANI, F.R., ALMEIDA, F.L.A., PADOVANI, C.R., CARVALHO, R.F., DAL-PAI-SILVA, M. Short periods of fasting followed by refeeding change the expression of muscle growth-related genes in juvenile Nile tilapia (Oreochromis niloticus). **Comparative Biochemistry and Physiology, Part B,** v.164, p.268-274, 2013.

NIKKI, J., PIRHONEN, J., JOBLING, M., KARJALAINEN, J. Compensatory growth in juvenile rainbow trout, Oncorhynchus mykiss (Walbaum), held individually. **Aquaculture,** v.235, p.285-296, 2004.

PETERSON, B.C., SMALL, B.C., WALDBIESER, G.C., BOSWORTH, B.G. Endocrine response of fast and slow-growing families of channel catfish. **North American Journal of Aquaculture,** v.70, p.240-250, 2009.

RIOS, R.; CARNEIRO, I.; ARCE, V.M.; DEVESA, J. Myostatin is an inhibitor of myogenic differentiation. **American Journal of Physiology & Cell Physiology,** v.282, p.993-999, 2002.

RODGERS, B.D., WEBER, G.M., KELLEY, K.M., LEVINE, M.A. Prolonged fasting and cortisol reduce myostatin mRNA levels in tilapia larvae; short-term fasting elevates. **American Journal of Physiology. Regulatory, Integrative and Comparative physiology,** v.284, p.1277-1286, 2003.

RUDNICKI, M.A., SCHNEGEISBERG, P.N.J., STEAD, R.H., BRAUN, T., ARNOLD, H.H., JAENISH, R. MyoD or myf5 is required for the formation of skeletal muscle. **Cell,** v.75, p.1351-1359, 1993.

SACHECK, J.M., OHTSUKA, A., MCLARY, S.C., GOLDBERG, A.L. IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. **American Journal Physiology Endocrinology Metabolism,** v.287, p.591-601, 2004.

SEILIEZ, I., PANSERAT, S., SKIBA-CASSY, S., FRICOT, A., VACHOT, C., KAUSHIK, S., TESSERAUD, S. Feeding status regulates the polyubiquitination step of the ubiquitin-proteasome-dependent proteolysis in rainbow trout (*Oncorhynchus mykiss*) muscle. **Journal of Nutrition,** v.138, p.487-491, 2008.

STAINBACHER, P., HASLETT, J.R., OBERMAYER, A., MARSCHALLINGER, J., BAUER, H.C., SANGER, A.M., STOIBER, W.MyoD and miogenina expression during myogenic phases in brown trout: a precocious onset of masaic hyperplasia is a prerequisite for fast somatic growth. **Developmental Dynamics,** v.236, p.1106-1114, 2007.

TACCHI, L., BICHERDIKE, R. Muscle-specific RING finger (MuRF) cDNAs in atlantic salmon (*Salmo salar*) and their role as regulators of muscle protein degradation. **Marine Biotechnology,** v.14, p.35-45, 2012.

VALENTE, L.M., BOWER, N.I., JOHNSTON, I.A. Postprandial expression of growth-related genes in Atlantic salmon (Salmo salar L.) juveniles fasted for 1 week and fed a single meal to satiation. **British Journal of Nutrition,** v.108, p.2148-2157, 2012.

VALENTE, L.M., MOUTOU, K.A., CONCEICÃO, L.E.C., ENGROLA, S., FERNANDES, J.M.O., JOHNSTON, I.A. What determines growth potential and juvenile quality of farmed fish species? **Reviews Aquaculture,** v.5, n.1, p.5168-5193, 2013.

VALENTE, L.M.P., ROCHA, E., GOMES, E.F.S., SILVA, M.W., OLIVEIRA, M.H., MONTEIRO, R.A.F., FAUCONNEAU, B. Growth dynamics of White and red muscle fibres in fast and slow growing strains of rainbow trout. **Journal of Fish Biology,** v.55, p.675-691,1999.

VEGGETTI, A., MASCARELLO, F., SCAPOLO, P.A.; ROWLERSON, M.D.; CARNEVALI, C.M.D. Muscle growth and myosin isoform transitions during development of a small teleost fish (*Poecilia reticulate)* (Atheriniformes,

Poeciliidae): a histochemical, immunohistochemical, ultrastructural and morphometric study. **Anatomy and Embryology,** v.187, p.353-361, 1993.

WANG, Y., CUI, Y., YANG, Y., CAI, F.. Compensatory growth in hybrid tilapia, *Oreochromis mossambicus X O. niloticus*, reared in seawater. **Aquaculture,** v.189, p.101-108, 2000.

CAPÍTULO 4 – CONSIDERAÇÕES FINAIS

Neste trabalho nós concluímos que o período 1 a 3 semanas de jejum, em juvenis de tilápia-do-nilo, foram suficientes para provocar menor taxa de crescimento nos peixes jejum em relação aos do tratamento controle. Entretanto, após 10 semanas de realimentação, a taxa de crescimento específico dos peixes aumentaram, sendo semelhante ao crescimento dos animais que foram alimentados continuamente, apresentando crescimento compensatório parcial. Porém, o crescimento específico, bem como o consumo alimentar, conversão alimentar e ganho em peso dos peixes dos tratamentos jejum, não foram superiores ao tratamento alimentado para induzir o crescimento compensatório total ou uma sobre-compensação nos peixes, rejeitando-se assim, a primeira hipótese testada.

Os períodos de jejum, testados no experimento não são tão severos para os juvenis de tilapia a ponto de prejudicar a taxa de sobrevivência, que foram superiores a 79%.

Durante os períodos de jejum, os animais utilizam gordura visceral e da carcaça e o glicogênio hepático para manter o metabolismo basal, poupando assim a utilização de proteína tecidual. Os índices de triglicérides, colesterol, proteína total e glicose não diferem entre os peixes dos tratamentos jejum e controle. Provavelmente, a glicose sanguínea nos peixes em jejum não diminui devido à gliconeogênese.

Não foi evidenciado padrão de atrofia muscular durante o jejum pelas análises morfológicas das fibras musculares. A morfometria das fibras, revelou que a grande quantidade de fibras de menor diâmetro encontrada nos períodos de jejum, foi devido ao crescimento das fibras, mostrando crescimento das fibras por hiperplasia. Porém, após o período de realimentação, os peixes dos tratamentos jejum e controle apresentaram maior quantidade de fibras de maior diâmetro, sendo o crescimento das fibras por hipertrofia.

De acordo com a segunda hipótese testada, os períodos de jejum avaliados, foram suficientes para ativação atrofia muscular, uma vez que a expressão dos genes MuRF-1 e atrogina estavam significativamente maiores que a expressão do tratamento controle. Entretanto, na análise morfológica das fibras musculares não foram observadas alterações na matriz extracelular e perda do formato poligonal das fibras. Durante o jejum, os níveis de expressão do IGF-1 muscular estavam

menores nos tratamentos jejum em relação ao controle. Entretanto, a expressão do receptor de IGF-1 durante o jejum foi maior nos peixes submetidos ao jejum em relação ao controle, mostrando assim uma resposta fisiológica para compensar a diminuição da expressão do IGF-1. Após a realimentação a expressão dos genes relacionados a atrofia, bem como o IGF-1 não difere entre os peixes dos tratamento controle e jejum. A MyoD, miogenina e miostatina não apresentaram grandes alterações de expressão durante o jejum e após 10 semanas de realimentação em todos os tratamentos. Portanto, concluímos que, de acordo com os resultados da análise de expressão gênica, foi aceita a segunda hipótese testada.

Nossos resultados mostram que diferentemente de outros protocolos, com outras espécies que apresentaram crescimento compensatório total , a estratégia utilizada com juvenis de tilápia-do-nilo (1-3 semanas de jejum seguido por 10 semanas de realimentação) são capazes de induzir somente crescimento compensatório parcial nos peixes. Entretanto, o nosso trabalho indica que a estratégia utilizada não inibe o crescimento dos peixes durante a realimentação, mas ao mesmo tempo, os peixes que foram submetidos ao jejum, não foram capazes de atingir crescimento compensatório total ou sobrecompensação do peso como nos peixes do tratamento controle. O aumento da expressão dos genes relacionados a atrofia muscular (MuRF1 e atrogina-1) durante o jejum foi capaz de ativar a via da atrofia muscular, inibindo assim, a via de crescimento e desenvolvimento muscular dos peixes.