TAMIRA MARIA ORLANDO

EFFECTS OF THE RATION OF LINOLEIC ACID TO LINOLENIC ACID DIETARY TO TILAPIA FEMALES (*Oreochromis niloticus*)

LAVRAS – MG
2018

TAMIRA MARIA ORLANDO

EFFECTS OF THE RATION OF LINOLEIC ACID TO LINOLENIC ACID DIETARY TO TILAPIA FEMALES (*OREOCHROMIS NILOTICUS*)

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Não Ruminantes, para a obtenção do título de Doutor.

Orientadora
Dra. Priscila Vieira Rosa

LAVRAS – MG
2018

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Orlando, Tamira Maria.
Effects ofthe ration of linoleic acid to linolenic acid dietary to tilapia females (*Oreochromis niloticus*) / Tamira Maria Orlando. - 2018.
71 p. : il.

Orientador(a): Priscila Vieira e Rosa.

Tese (doutorado) - Universidade Federal de Lavras, 2018.
Bibliografia.

1. Peixes. 2. Nutrição de reprodutores. 3. Lipídeos. I. Rosa, Priscila Vieira e. . II. Título.

TAMIRA MARIA ORLANDO

EFFECTS OF THE RATION OF LINOLEIC ACID TO LINOLENIC ACID DIETARY TO TILAPIA FEMALES (*OREOCHROMIS NILOTICUS*)

Tese apresentada à Universidade Federal de Lavras, como Parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Não Ruminantes, para a obtenção do título de Doutor.

APROVADA em 12 de março de 2018.

Dr. Jose Fernando Lopes Olmeda | Universidad de Murcia
Dr. Luis David Solis Murgas | UFLA
Dr. Márvio Lobão Teixeira de Abreu | UFLA
Dr. Ronald Kennedy Luz | UFMG

Prof[a]. Dr[a]. Priscila Vieira Rosa
Orientadora

LAVRAS – MG
2018

À minha mãe Cláudia, pelo amor incondicional e por ser meu maior exemplo. À minha família por sempre me apoiar.

Dedico

AGRADECIMENTOS

A Deus, o Provedor de tudo, pela bênção de cada dia.

À Universidade Federal de Lavras e ao Departamento de Zootecnia, pela oportunidade concedida para a realização do Doutorado.

À minha orientadora, Prof.ª Dra. Priscila Vieira Rosa, pelas tantas oportunidades e pela confiança concedidas a mim. Muito obrigada, pelos ensinamentos e amizade em todos esses anos de orientação.

À Marinez Moraes de Oliveira que, ao longo de tantos anos, esteve presente, a minha eterna gratidão.

Ao Prof. Dr. David Solis Murgas, pela disponibilidade e atenção sempre que foi solicitado.

Ao pesquisador Jose Fernando Olmeda, agradeço a disponibilidade e todo auxílio nas análises de biologia molecular.

Aos professores do Departamento de Pós-graduação em Zootecnia, pelos ensinamentos.

Aos colegas do núcleo de estudos NAQUA, pela parceria nos trabalhos desenvolvidos.

Aos colegas de trabalho, que também são amigos e que tornam a tarefa mais leve e são fonte de inspiração: Tafanie Fontes, Renan Paulino, Natalia Mourad, Katia Rodrigues, Raquel Pereira, Leandro Santos, Ariane Franco e Nayara Pereira. Muito obrigada, pela colaboração na execução do trabalho e pelos vários momentos de diversão.

Ao funcionário da Estação de Piscicultura da UFLA Eleci Pereira, por todo auxílio, amizade e bons momentos de convivência no setor.

Às amizades de tantos anos, Carolina Teixeira e Marielle Baenna e às amizades recentes, Tássia Estevão e Desirré Jouan, obrigada pelo amparo. Aos amigos José Willer, Mírian Rosa, Valderí Alcântara, Kelly Vieira, André Paiva,

André Spuri e Marcello Cunha, obrigada pelos tantos cafés, conversas e confraternizações. Ao Luís Fernando, pelo companheirismo ao longo de tanto tempo. Sou imensamente grata pela parceria, apoio, auxílio e paciência ao longo desta jornada.

Aos companheiros Alexandre Diógenes, Carolina Castro e Ana Basto pelo auxílio no ensaio realizado na Universidade do Porto. Minha gratidão por tornarem minha estada na cidade umas das melhores experiências da minha vida.

À minha mãe, Cláudia, pelo amor e apoio incondicional, por não ter poupado esforços para que este objetivo fosse alcançado. Aos meus familiares, em especial aos meus irmãos Otávio, Talita e Thiago, pela paciência e companheirismo, ao meu pai, Vítor e aos meus tios por todo incentivo. Aos meus sobrinhos, Túlio e Fátima, que nos proporcionam tantas alegrias.

Muito obrigada!

RESUMO

Ácidos graxos poli-insaturados (PUFA) e seus derivados de cadeia longa (LC-PUFA) estão envolvidos em diversas funções no organismo. No entanto, um balanço adequado entre os ácidos graxos (AG) dietéticos provenientes de fontes vegetais, pode prevenir possível competição em suas rotas de biossíntese. Objetivou-se, neste trabalho, avaliar o efeito de diferentes relações dos ácidos graxos ácido linoleico/ linolênico (LA/LNA) nas dietas de fêmeas de tilápia Nilo (*Oreochromis niloticus*). Realizou-se um ensaio experimental, utilizando-se 24 machos e 72 fêmeas, com peso inicial médio de 131,3g e 124,4g, respectivamente. Seis fêmeas e dois machos foram distribuídos em doze tanques de 250L cada. O delineamento foi inteiramente casualisado, com 4 tratamentos (20,1; 4,5; 3,9 e 0,7 LA/ LNA) e três repetições. Diariamente, foram realizadas observações para a ocorrência de desova. As taxas de eclosão e tempo de sobrevivência das larvas foram registradas. No final do ensaio, amostras de sangue e tecidos foram coletados. Os peixes alimentados com as relações 3,9 e 0,7 tiveram maior número de ovos produzidos ($p<0,05$). A taxa de eclosão e a sobrevivência das larvas não foram afetadas pelas dietas ($p>0,05$). A taxa de crescimento específico foi maior na relação 0,7 e o índice hepatossomático, maior na relação 3,9 ($p<0,05$). De modo geral, os AG das gônadas refletiram a composição da dieta, sendo o teor de ácido araquidônico maior na relação 20,1 e os ácidos eicosapentaenoico (EPA) e docosahexaenoico (DHA), maiores na relação 0,7 ($p<0,05$). As dietas não influenciaram o teor de triglicerídeos plasmático ($p>0,05$), no entanto, as dietas com relações 3,9 e 4,5 proporcionaram maiores níveis de colesterol ($p<0,05$). As maiores concentrações de ácido linoleico dietético aumentaram a expressão gênica da enzima *cyp17*, enquanto a expressão da *cyp19a1a* foi maior na relação 4,5 ($p<0,05$). Conclui-se que uma relação dietética de 3,9 LA/LNA deve ser utilizada para fêmeas de tilápia do Nilo.

Palavras-chave: Ácidos graxos. Lipídeos. Nutrição. Peixes. Reprodução.

ABSTRACT

Polyunsaturated fatty acids (PUFAs) and their long chain derivatives (LC-PUFA) are involved in various functions in the body. However, an adequate balance between dietary n-3 and n-6 fatty acids (FA) from plant sources, may prevent possible competition in their biosynthetic routes. The objective of this work, was to evaluate the effect of different dietary linoleic acid/ linolenic acid (LA/ LNA) ratio for Nile tilapia (*Oreochromis niloticus*) breeding females. An experiment was performed using 24 males and 72 females with mean initial weight of 131.3 g and 124.4 g, respectively. Six females and two males were distributed in twelve tanks of 250L each. The design was completely randomized, with four treatments (20.1, 4.5, 3.9 and 0.7 LA / LNA) and three replications. Observations were made for the occurrence of spawning every day. At the end of the test, blood and tissue samples were collected. Fish fed the 3.9 and 0.7 LA/LNA diets had higher number of eggs produced ($p<0.05$). Hatch rates and larval survival were not affected by the treatments ($p>0.05$). Specific growth rate was highest in fish fed the 0.7 LA/LNA ratio and hepatosomatic index was highest in fish fed the 4.5 LA/LNA ratio ($p<0.05$). In general, the FA profile of gonads reflected the composition of the diet. The arachidonic acid content was higher in 20.1 ratio and the eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids concentration were higher in 0.7 ratio ($p<0,05$). Diets did not influence the triglycerides content in plasma ($p>0.05$). However, higher levels of cholesterol were observed in fish fed 4.5 and 3.9 LA/ LNA ratio ($p<0.05$). The highest dietary LA levels increased the gene expression of *cyp17*, while the dietary 3.9 LA/ LNA ratio promoted highest expression of *cyp19a1a* ($p<0.05$). In conclusion, a 3.9 LA/ LNA ratio should be used for Nile tilapia female.

Keywords: Fatty acids. Fishes. Nutrition. Reproduction.

LISTA DE FIGURAS

Figura 1 - Vias de biossíntese de PUFA em peixes. Todas as atividades foram demonstradas em espécies de peixes teleósteos, mas nem todas as espécies expressam todas as atividades. Δ4 Fad, Δ5 Fad e Δ6 Fad são acil dessaturases; Elovl2, Elovl4 e Elovl5 são alongase. 17
Figura 2 - Vias esteroidogênicas nos folículos de peixes. 24
Figura 3 - Gene expression of key protein related to steroidogenesis in the gonads of female Nile tilapia fed different LA/LNA diets. 58

LISTA DE TABELAS

Table 1 - Ingredient and proximal composition of the experimental diets. 42

Table 2 - Fatty acid composition (% of total fatty acids) of the experimental diets. 44

Table 3 - Primer sequences used for quantitative PCR analyses. 48

Table 4 - Reproductive performance female Nile tilapia fed experimental diets for 105 days. 50

Table 5 - Growth performance and body indices of Nile tilapia fed with different LA/ LNA diets for 105 days. 51

Table 6 - Whole-body (% dry weight) composition of female Nile tilapia fed the experimental diets for 105 days..52

Table 7 - Gonad fatty acid (% total fatty acids) composition of Nile tilapia fed with different n-6/ n-3 fatty acid ratio diets. 54

Table 8 - Liver fatty acid (% total fatty acids) composition of Nile tilapia female fed with different n-6/ n-3 fatty acid ratio diets.....................55

Table 9 - Muscle fatty acid (% total fatty acids) composition of Nile tilapia fed with different n-6/ n-3 fatty acid ratio diets. 56

Table 10 - Plasma Nile tilapia parameters fed different LA/LNA diets for 105 days...57

LISTA DE ABREVIATURAS

ARA	Ácido araquidônico
DHA	Ácido docosaexaenoico
EPA	Ácido eicosapentaenoico
AG	Ácidos graxos
AGE	Ácidos graxos essenciais
PUFA	Ácidos graxos poli-insaturados de cadeia longa
LC-PUFA	Ácidos graxos poli-insaturados de cadeia longa
SAFA	Ácidos graxos saturados
MUFA	Ácidos graxos monoinsaturados
VLDL	Lipoproteína de densidade muito baixa
Vtg	Vitelogenina
E2	Estradiol
StAR	*Steeroidogenic acute regulatory protein*
3β-HSD	3β-*hydroxysteroid dehydrogenase*
FSH	Hormônio folículo estimulante
LH	Hormônio luteinizante
ACTH	Hormônio adrenocorticotrópico
GH	Hormônio do crescimento
IGF	*insulin-like growth factor*
MIS	Maturation inducing steroids
IGS	Índice gonadossomático
IHS	Índice hepatossomático
IVS	Índice viscerossomático
mRNA	RNA mensageiro

SUMÁRIO

PRIMEIRA PARTE 14
1 INTRODUÇÃO 14
2 REFERENCIAL TEÓRICO 16
2.1 LIPÍDEOS NA NUTRIÇÃO DE PEIXES 16
2.1.1 Eicosanoides 19
2.2 LIPÍDEOS E REPRODUÇÃO DE PEIXES 20
2.3 ESTEROIDOGÊNESE 21
2.3.1 Ácidos graxos e esteroidogênese 25
2.4 ESTRADIOL E VITELOGÊNESE 26
3 CONSIDERAÇÕES GERAIS 28
REFERÊNCIAS 29
SEGUNDA PARTE 37
ARTIGO: Effects of the dietary linoleic acid to linolenic acid ratio for Nile tilapia (*Oreochromis niloticus*) breeding females. 37
1 INTRODUCTION 39
2 MATERIAL AND METHODS 41
3 RESULTS 49
4 DISCUSSION 58
5 CONCLUSION 64
REFERENCES 65

PRIMEIRA PARTE

1 INTRODUÇÃO

A produção de tilápias é uma atividade crescente. O cultivo global dessa espécie aumentou de 4,5% da produção total de peixes cultivados em 1990, para 10,2% da produção total em 2012. Atualmente, tilápias representam o segundo maior grupo de peixes de água doce cultivado no mundo (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 2014) e possuem uma combinação de características apreciáveis para a produção. No aspecto reprodutivo, as tilápias são caracterizadas pela maturação sexual precoce e desova parcelada, com curtos períodos vitelogênicos e cuidado parental (COWARD; BROMAGE, 2000). A baixa fecundidade aliada à natureza assíncrona da desova nessa espécie, requer a manutenção de um grande estoque de reprodutores nas pisciculturas (COWARD; BROMAGE, 2000).

Informações sobre as exigências nutricionais de reprodutores de peixes ainda são limitadas. No entanto, a nutrição está diretamente relacionada ao sucesso reprodutivo e subsequente qualidade das larvas em muitas espécies de peixes (IZQUIERDO; FERNANDEZ-PALACIOS; TACON, 2001). Além disso, lipídeos e seus componentes, os ácidos graxos poli-insaturados de cadeia longa, são apontados como um dos principais fatores responsáveis por influenciar o desenvolvimento gonadal, fecundidade, fertilização e a qualidade de ovos e larvas em várias espécies (FERNÁNDEZ-PALACIOS et al., 1995; FURUITA et al., 2003; MAZORRA et al., 2003).

Em geral, peixes de água doce são capazes de alongar e dessaturar ácidos graxos de 18 carbonos, particularmente ácido linoleico [LA (18:2n-6)] e ácido linolênico [LNA (18:3n-3)], em seus respectivos ácidos graxos de 20

carbonos, ácido araquidônico (ARA), ácido eicosapentaenoico (EPA) e ácido docosahexaenoico (DHA) (TOCHER, 2010). Esses ácidos graxos fazem parte da estrutura de membranas celulares e são precursores de moléculas biologicamente ativas, como hormônios e eicosanoides (TOCHER, 2003). Uma vez que ambas as vias metabólicas compartilham as mesmas enzimas, podendo inibir a síntese de determinado ácido graxo, as quantidades relativas dos ácidos graxos das séries n-6 e n-3 devem ser consideradas.

A utilização de óleos vegetais, em substituição ao óleo de peixe nas dietas aquícolas é uma necessidade atual, por permitir a formulação de dietas mais sustentáveis. Entretanto, a compreensão dos eventos que ocorrem durante o período reprodutivo, sob a influência dos ácidos graxos dietéticos, é fundamental para a elaboração de dietas artificiais adequadas, durante o período de pré-desova, que proporcionem maior qualidade na produção de ovos, larvas e alevinos. Na presente tese, aborda-se essa temática, avaliando o efeito de diferentes relações de ácidos graxos n-6/n-3 dietéticos, considerando como parâmetros de resposta variáveis acerca do desempenho reprodutivo, deposição de ácidos graxos nos tecidos, expressão gênica de enzimas esteroidogênicas e níveis plasmáticos de estradiol.

2 REFERENCIAL TEÓRICO

2.1 LIPÍDEOS NA NUTRIÇÃO DE PEIXES

Lipídeos desempenham uma ampla gama de funções no organismo e, junto com proteínas, constituem os principais macronutrientes para peixes (SARGENT; TOCHER; BELL, 2002). Além de fornecerem energia, seus constituintes, os ácidos graxos, têm função estrutural, participando da composição das membranas celulares, e ainda são precursores de moléculas biologicamente ativas, como hormônios e eicosanoides (TOCHER, 2003).

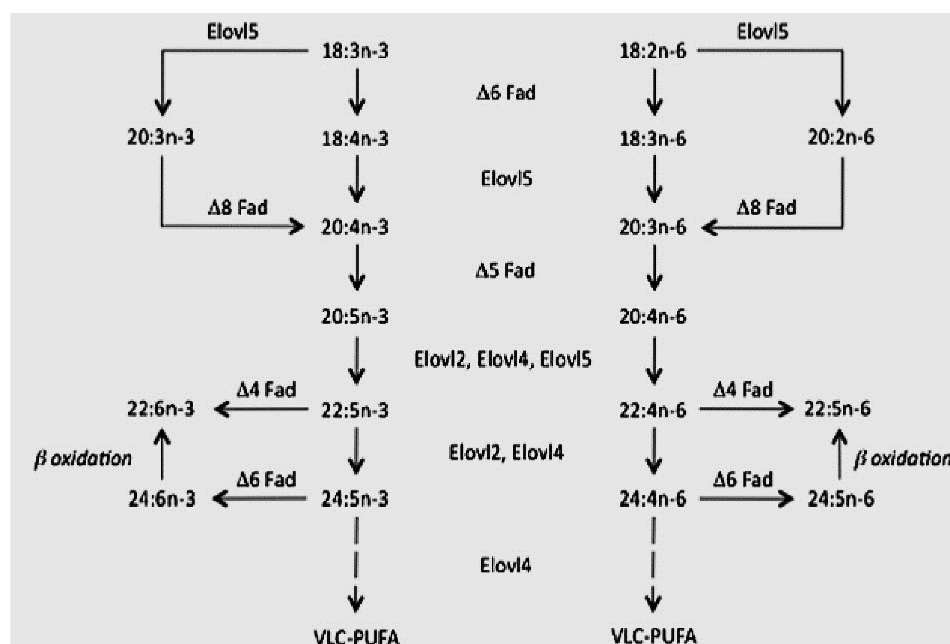
A expansão da aquicultura nos últimos anos e a necessidade de formular dietas que promovam crescimento satisfatório e atendam às necessidades fisiológicas dos animais, têm feito com que muitos trabalhos sejam desenvolvidos para elucidar os mecanismos nos quais os lipídeos estão envolvidos no organismo. Os ácidos graxos denominados essenciais (AGE) são aqueles que devem estar presentes na dieta por não serem sintetizados de forma eficiente pelo animal, para garantir a demanda nos processos fisiológicos ou crescimento, sendo a deficiência dos AGE caracterizada pela redução do crescimento e aumento da mortalidade (GLENCROSS, 2009).

Vertebrados em geral não são capazes de sintetizar ácidos graxos linoleico [(18:2n-6) LA] e linolênico [(18:3n-3) LNA], a partir ácido oleico (18:1n-9), devido à ausência das enzimas $\Delta 12$ e $\Delta 15$ dessaturases (TOCHER et al., 2006). Portanto, a obtenção dos ácidos graxos poli-insaturados (PUFA) de 18 carbonos, das séries n-6 e n-3, ocorre a partir dos vegetais e, dessa forma, devem ser suplementados na dieta (SARGENT; TOCHER; BELL, 2002).

Os ácidos graxos das séries n-6 e n-3 são exigidos pelos teleósteos, mas os ácidos graxos fisiologicamente essenciais são aqueles de 20 e 22 carbonos na molécula: ácido araquidônico [(ARA) 20:4n-6], ácido eicosapentaenoico [(EPA)

20:5n3] e ácido docosaexaenoico [(DHA) 22:6n-3], sintetizados, a partir dos seus respectivos precursores, LA e LNA (Figura 1). Esses ácidos graxos são geralmente denominados ácidos graxos de cadeia longa ou LC-PUFA.

Figura 1 - Vias de biossíntese de PUFA em peixes. Todas as atividades foram demonstradas em espécies de peixes teleósteos, no entanto, nem todas as espécies expressam todas as atividades. Δ4 Fad, Δ5 Fad e Δ6 Fad são acil dessaturases; Elovl2, Elovl4 e Elovl5 são alongase.



Fonte: Tocher (2015).

As exigências nutricionais de AGE variam entre as espécies e também o nível de desenvolvimento dos animais, sendo o ambiente um fator importante para a determinação das exigências dos peixes (GLENCROSS, 2009). Espécies marinhas, não são capazes de converter LA e LNA em seus metabólitos biologicamente ativos, ARA, EPA e DHA, devido à falta ou baixa expressão de

enzimas nas vias de dessaturação e alongamento da cadeia de carbono. Estudos em espécies de água salgada identificaram baixa atividade das enzimas envolvidas nas rotas de dessaturação e alongamento da cadeia de carbono, alongase e Δ5 dessaturase (TOCHER; GHIONI, 1999). Espécies marinhas têm, portanto, exigência dietética em LC-PUFA para suprirem a demanda fisiológica desses ácidos graxos (SARGENT et al., 1999; TOCHER, 2015). Por outro lado, peixes de água doce podem ter suas exigências atendidas pelos PUFA de 18 carbonos na dieta (TOCHER, 2010).

Os LC-PUFA são componentes dos fosfolipídeos, principais constituintes da camada bilipídica das membranas celulares e, são importantes para a manutenção da estrutura e função celular (TOCHER, 2003). Além disso, o fornecimento de dietas com diferentes fontes de óleo pode promover alterações na composição e nível de lipoproteínas plasmáticas, bem como nos triglicerídeos e outros lipídeos (JORDAL; LIE; TORSTENSEN, 2007; MORAIS et al., 2011; RICHARD et al., 2006a, 2006b; TORSTENSEN; LIE; FRØYLAND, 2000).

É importante destacar as funções específicas dos ácidos graxos, em relação à sua essencialidade como reguladores do metabolismo e como mediadores lipídicos (TOCHER, 2010). LC-PUFA ou seus derivados, que incluem os eicosanoides, prostagladinas, leucotrienos, resolvinas, protectinas e lipoxinas, podem atuar nos tecidos ou como ligantes para fatores de transcrição que controlam a expressão gênica no interior da célula (TOCHER, 2015).

Devido aos efeitos dos LC-PUFA como reguladores do metabolismo e mediadores lipídicos (SARGENT; TOCHER; BELL, 2002), destaca-se que as exigências nutricionais por AGE devem ser consideradas, não somente em quantidades absolutas, mas também em suas quantidades relativas.

2.1.1 Eicosanoides

Eicosanoides são compostos oxigenados que podem ser formados a partir de AG de 20C que contêm de três a cinco *cis*-duplas ligações [ácido dihomo-gamma-linolênico (20:3n-6), ARA e EPA] (CALDER, 2001). Esses ácidos graxos geralmente estão esterificados na posição *sn-2* do glicerol dos fosfolipídios da membrana celular. Os eicosanoides são compostos altamente bioativos, com meia-vida curta, que podem ser sintetizados em quase todos os tecidos e atuam nas respostas imunes e inflamatórias, tônus cardiovascular, funções renais e neural e, também na reprodução (SCHMITZ; ECKER, 2008).

A síntese desses compostos inicia-se pela clivagem e liberação do ácido graxo dos fosfolipídeos, sob a ação da fosfolipase A2 ou fosfolipase C, podendo, então, ser metabolizado (CALDER, 2006). O ARA é reconhecido como o principal precursor de eicosanoides em peixes (TOCHER, 2003), mas podem também serem originados a partir do EPA. Os compostos formados pelo EPA dão origem à série 3 de prostanoides e 5 de leucotrienos, enquanto os prostanoides originados pelo ARA recebem o sufixo 2 e 4 de leucotrienos (SARGENT et al., 1999). Embora percorram as mesmas rotas metabólicas e possuam as mesmas funções, os compostos originados pelo ARA são mais biopotentes que aqueles oriundos do EPA.

EPA pode competir e inibir a atividade das enzimas que atuam nas vias da ciclooxigenase e lipoxigenase, reduzindo a produção dos derivados pro-inflamatórios do ARA com a produção de mediadores inflamatórios que possuem efeitos mais brandos. Dessa forma, EPA tem um efeito modulador na produção desses compostos (GLENCROSS, 2009).

2.2 LIPÍDEOS E REPRODUÇÃO DE PEIXES

Lipídeos dietéticos e sua composição em ácidos graxos, especialmente os LC-PUFA (EPA, DHA e ARA), são apontados como um dos principais fatores para o sucesso do desempenho reprodutivo e qualidade dos ovos e larvas de peixes marinhos (IZQUIERDO; FERNANDEZ-PALACIOS; TACON, 2001). O efeito de PUFA n-3 dietéticos no desempenho reprodutivo das matrizes foi evidenciado em muitos estudos. Sua suplementação pode afetar as taxas de fertilização e eclosão, composição de ácidos graxos dos ovos e qualidade das larvas (FERNÁNDEZ-PALACIOS et al., 1995; FURUITA et al., 2007; LAVENS et al., 1999). No entanto, efeitos negativos foram observados com o excesso ou deficiência de LC-PUFA n-3 dietéticos. Em gilthead sea bream (*Spaurus aurata*), observou-se melhora na fecundidade com suplementação dietética até 1,6%, no entanto, níveis acima de 2,18% diminuíram a quantidade total de ovos (FERNÁNDEZ-PALACIOS et al., 1995). Por outro lado, a deficiência desses ácidos graxos, durante o período de vitelogênese, foi relacionada à baixa qualidade dos ovos em reprodutores de European sea bass (*Dicentrarchus labrax*), alimentados com dietas com alto teor de triglicerídeos (NAVAS et al., 1997).

Em espécie de água doce, como a tilápia do Nilo (*Oreochromis niloticus*), observou-se desempenho inferior nos peixes alimentados com dietas suplementadas com 5% de óleo de bacalhau, rico em AG’s n-3 (SANTIAGO; REYES, 1993), enquanto as dietas contendo óleo de soja proporcionaram melhor desempenho reprodutivo nos peixes. Em outro trabalho, avaliou-se o efeito de fontes de óleo em diferentes salinidades e foi relatado que uma fonte de LC-PUFA n-3 deve ser utilizada, quando esses animais são cultivados em água salobra; no entanto, uma fonte de óleo vegetal pode ser fornecida para atender às

exigências nutricionais dessa espécie em água doce (EL-SAYED; MANSOUR; EZZAT, 2005).

A comparação do perfil de ácidos graxos dos tecidos e ovos entre peixes cultivados e selvagens, tem demonstrado um fornecimento inadequado de LC-PUFA na dieta dos animais em cativeiro. A comparação da composição dos ácidos graxos nos tecidos desses animais, permite a identificação de possíveis deficiências nutricionais que poderiam ser supridas pela elaboração de dietas adequadas (CEJAS et al., 2004). Um imbalanço de lipídeos e ácidos graxos essenciais em Senegalese sole cultivados (*Solea senegalensis*) foi atribuído ao fornecimento de dietas extrusadas aos animais (NORAMBUENA et al., 2012). A menor concentração de ARA e menor relação EPA/DHA em ovos de sea bass (*Centropristis striata*) cultivados, comparados aos animais selvagens, foi apontado como um fator para a diminuição da qualidade dos ovos (SEABORN et al., 2009).

2.3 ESTEROIDOGÊNESE GONADAL EM PEIXES

Esteroidogênese é o processo em que células especializadas de alguns tecidos sintetizam hormônios esteroides, pequenas moléculas derivadas do colesterol (STOCCO, 2001). Em peixes, esteroides podem ser produzidos nas células do cérebro, inter-renais, testículos e ovários. Essas células expressam uma variedade de genes que codificam enzimas esteroidogênicas, as quais atuam sobre o colesterol e seus derivados (YOUNG et al., 2005).

A regulação da esteroidogênese ocorre principalmente pelos hormônios tróficos da pituitária, hormônio luteinizante (LH), hormônio folículo estimulante (FSH) e hormônio adrenocorticotrópico (ACTH) (STOCCO, 2001). Em todos os

tecidos, a regulação ocorre em duas fases: a fase aguda ocorre na ordem de minutos e é caracterizada pela liberação de esteroides sintetizados previamente, devido a uma necessidade imediata, enquanto a regulação crônica envolve a síntese de RNAm e enzimas para assegurar a capacidade das células na produção de esteroides (STOCCO; CLARCK, 1996).

Colesterol é o substrato comum para a síntese de todos os hormônios esteroides e sua passagem do citoplasma para o interior da membrana da mitocôndria é apontado como o passo limitante para a síntese desses hormônios (MANNA; DYSON; STOCCO, 2009; STOCCO; CLARCK, 1996). A disponibilidade de colesterol como substrato para a síntese de esteroides é crítica e sua obtenção pode ocorrer de várias formas: síntese *de novo* a partir do acetato; por meio de lipoproteínas LDL (lipoproteína de baixa densidade) e HDL (lipoproteína de alta densidade); e também pela clivagem de ésteres de colesterol armazenados em gotículas de lipídeos (MANNA; DYSON; STOCCO, 2009).

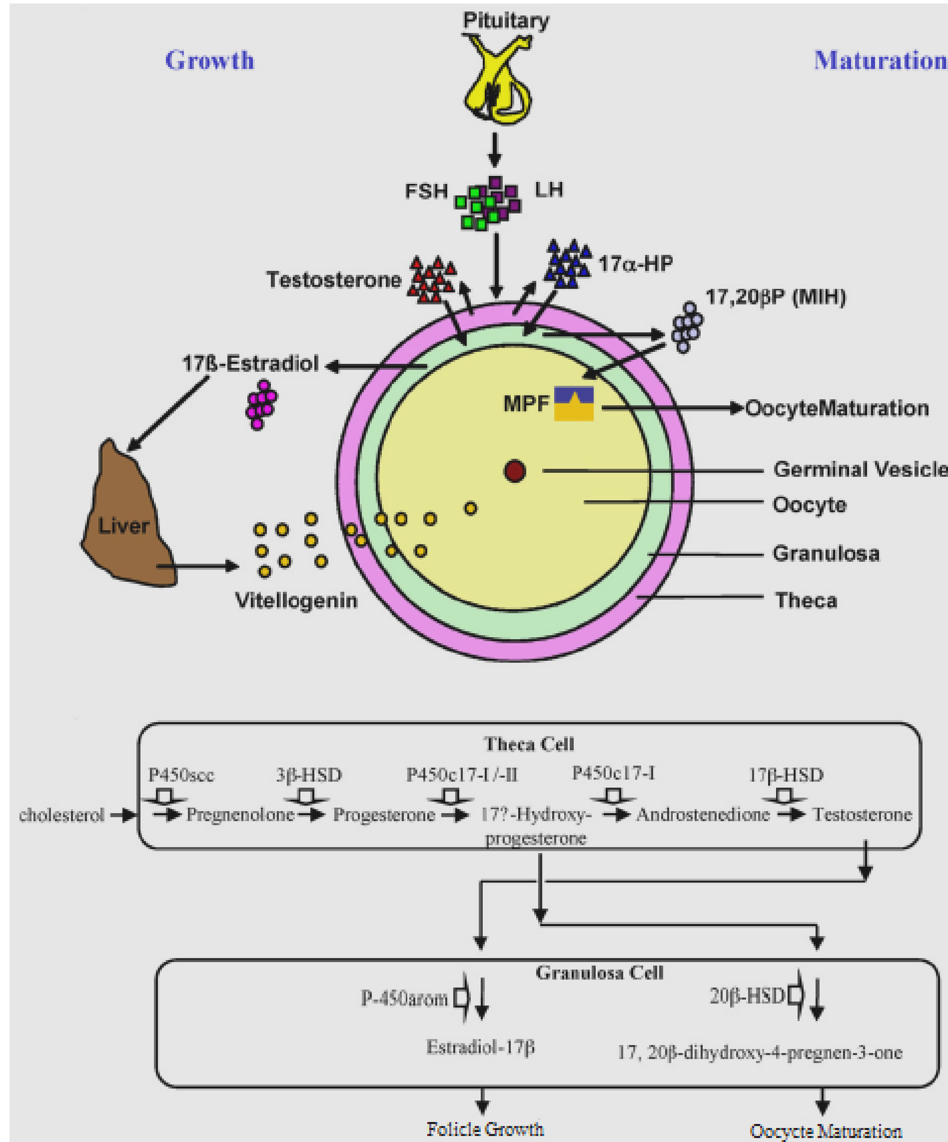
Devido à característica hidrofóbica da molécula do colesterol, é necessário um transportador para atravessar o espaço inter-membrana aquoso da mitocôndria e chegar à enzima de clivagem da cadeia lateral (P450scc), que o converterá à pregnenolona (hidrofílica), em velocidade suficiente para a ocorrência da esteroidogênese aguda (STOCCO, 2001). A StAR (*steroidogenic acute regulatory protein*) é uma proteína de 30kDa sintetizada inicialmente no citoplasma como um precursor de 37kDa, cuja ativação é estimulada pela rota de sinalização celular da cAMP-proteinaquinase A. Essa proteína regula a transferência do colesterol para o interior da membrana da mitocôndria (NAGAHAMA; YAMASHITA, 2008) e é reconhecida como a principal proteína reguladora da esteroidogênese em mamíferos (MANNA; DYSON; STOCCO, 2009; MILLER, 2007). A estreita correlação entre a expressão gênica da StAR em tecidos específicos relacionados à síntese de esteroides, evidencia sua função na esteroidogênese em vertebrados (MANNA; DYSON; STOCCO,

2009; STOCCO, 2001). Além disso, a inibição da transcrição ou tradução da StAR resulta em diminuição da biossíntese de esteroides nos tecidos (STOCCO; CLARK, 1996).

Hormônios esteroides gonadais produzidos por peixes teleósteos, incluem os estrógenos, progestágenos, andrógenos dentre outros. A produção desses hormônios ocorre nos folículos ovarianos (células da teca e da granulosa) nas fêmeas e nos testículos (células de Leydig) nos machos (YOUNG et al., 2005). Após a transferência do colesterol para o interior da membrana da mitocôndria, inicia-se uma série de eventos enzimáticos para a síntese de esteroides. Durante a vitelogênese, a via de biossíntese de esteroides é direcionada para a produção de 17β-estradiol (E2), o qual tem seu papel bem estabelecido no fígado, onde estimula a síntese de vitelogenina (OKUMURA et al., 2002).

O primeiro passo para a biossíntese de hormônios esteroides é a conversão do colesterol a pregnenolona, por meio da enzima de clivagem da cadeia lateral (P450scc), codificada pelo gene *cyp11a* (NAGAHAMA; YAMASHITA, 2008). A pregnenolona, por sua vez, é convertida a E2 em folículos vitelogênicos, pela ação de uma série de enzimas, codificadas por diversos genes, incluindo 3β-hsd (3β-*hydroxysteroid dehydrogenase*), 17α-hidroxilase/ citocromo P450 c17,20-liase e P450 aromatase (CLELLAND; PENG, 2009) (Figura 2).

Figura 2 - Vias esteroidogênicas nos folículos de peixes.



Fonte: Adaptado de Clelland e Peng (2009).

2.3.1 Ácidos graxos e esteroidogênese em peixes

Estudos demonstraram que os LC-PUFA, especialmente o ARA, podem agir nas células para modular a síntese de esteroides em peixes, assim como ocorre em mamíferos (SORBERA et al., 2001). ARA tem diferentes efeitos na biossíntese de esteroides, podendo estar relacionado na produção de testosterona, com elevação do AMPc de forma dose dependente, por exemplo (MERCURE; VAN DER KRAAK, 1995). No entanto, demonstrou-se que em altos níveis, ARA inibiu a biossíntese de hormônios esteroides, por afetar a disponibilidade de colesterol e a expressão da StAR em folículos ovarianos pré-maturacionais de truta arco-íris (*Oncorhynchus mykiss*) e goldfish (*Carassius auratus*) (MERCURE; VAN DER KRAAK, 1995, 1996).

Os efeitos de metabólitos derivados do EPA foram mais modestos na produção basal de testosterona nos testículos de goldfish. No entanto, em razão da competição pela Cox-2, esses produtos bloquearam a produção de esteroides produzidos a partir do ARA e a formação de AMPc (MERCURE; VAN DER KRAAK, 1995).

Em Senegalese sole, os níveis de testosterona e 11-cetotestosterona nos machos aumentaram de acordo com o aumento de ARA na dieta, enquanto que os níveis de estradiol nas fêmeas não foi afetado pelo incremento de ARA dietético (NORAMBUENA et al., 2013). A suplementação de ARA reduziu a expressão gênica da aromatase, tanto em ovários maduros quanto em ovários imaturos de tongue sole (*Cynoglossus semilaevis*) (XU et al., 2017). Esses trabalhos sugerem que o ARA é metabolizado de forma diferente em machos e fêmeas e que esse AG pode ser mais relevante para o sucesso reprodutivo de machos. No entanto, é importante notar que ácidos graxos n-3 também podem influenciar as vias de biossíntese de prostaglandinas e esteroides (WATHES;

ABAYASEKARA; AITKEN, 2007), envolvidas na regulação da função reprodutiva .

2.4 ESTRADIOL E VITELOGÊNESE

O estradiol está relacionado com os processos de desenvolvimento e a manutenção do sistema reprodutivo dos vertebrados em geral. Em peixes, o E2 atua principalmente na vitelogênese no fígado, enquanto nas gônadas se relaciona com a diferenciação sexual e esteroidogênese durante o ciclo reprodutivo e desenvolvimento ovariano (DORRINGTON; BENDELL; KHAN, 1993). A vitelogênese é o processo de desenvolvimento do ovócito, no qual ocorre a incorporação de vitelogenina (Vtg) e seu processamento em proteínas para a formação do vitelo. Além da Vtg, esse processo engloba também a incorporação de outros nutrientes, como vitaminas e lipídeos.

A Vtg é uma fosfolipoglicoproteína, de alto peso molecular com a função de carrear nutrientes pelo plasma até os ovócitos. O início da vitelogênese é caracterizado pelo aumento de FSH e de E2 no plasma, acompanhado pela maior expressão de receptores de FSH nos ovários (KOBAYASHI et al., 2009). Por outro lado, os níveis de LH atingem um pico no período pós-vitelogênico, em que se observa uma mudança na via de biossíntese de esteroides, com o aumento da produção de esteroides que induzem a maturação (*Maturation inducing steroids*, MIS) (MONTSERRAT et al., 2004).

Durante a vitelogênese, o FSH estimula a síntese da enzima P450 aromatase nas células da granulosa, que utilizará como substrato a testosterona, fornecida pelas células da teca ovariana para a síntese de E2. Por sua vez, o E2 promoverá a síntese de vitelogenina no fígado que, posteriormente. será

incorporada nos ovócitos. As gotículas de lipídeos formadas nos ovócitos são constituídas, principalmente, por lipídeos neutros (triglicerídeos). Os lipídeos são absorvidos e acumulados nos ovócitos a partir da Vtg e também por meio de lipoproteínas plasmáticas (VLDL, *very low density lipoprotein*) (LUBZENS et al., 2010), os quais se ligam a receptores específicos que se encontram ancorados na superfície dos ovócitos (LE MENN; CERDÀ; BABIN, 2007). O E2 também é importante no feedback da regulação da síntese e secreção de gonadotropinas da pituitária. Ao final do processo de vitelogênese, o ovócito conterá RNA materno, nutrientes e hormônios essenciais para o desenvolvimento do embrião, tornando-se apto à fertilização (LUBZENS et al., 2010).

Vários trabalhos demonstraram que o estímulo da produção de E2 promovido por gonadotropinas é acompanhado pelo aumento da expressão de um ou mais genes relacionados à esteroidogênese ovariana, dentre eles a StAR, 3β-HSD e P450 aromatase (INGS; VAN DER KRAAK, 2006; KAGAWA et al., 2003; MONTSERRAT et al., 2004).

Em cultivos primários de hepatócitos de silver eel (*Anguilla Anguilla*), foi relatado que o hormônio do crescimento (GH) potencializa o efeito do estradiol na síntese de vitelogenina nos hepatócitos (PEYON; BALOCHE; BURZAWA-GERARD, 1996), além de estimular a síntese de E2 nos ovários de spotted seatrout (*Cynoscion nebulosus*) (SINGH; THOMAS, 1993).

Os IGF’s (*insulin-like growth factor*) também têm sido relacionados à esteroidogênese ovariana. Em peixes, os IGF’s são expressos principalmente no fígado, mas são encontrados também em vários tecidos extra-hepáticos, inclusive nas células da granulosa ovariana (KAGAWA, 2013). Esses fatores de crescimento, possivelmente, estimulam as diferentes funções das células, nos vários órgãos, via autócrina, parácrina e endócrina (REINDL; SHERIDAN, 2012; WOOD; DUAN; BERN, 2005), sendo o estímulo primário para a síntese

de IGF's no fígado e sua liberação na circulação o GH, produzido na pituitária (REINECKE, 2010). A expressão de IGF-1 e de seus receptores foi identificada nos ovários de starlet (*Acipenser ruthenus*), com maiores níveis de expressão no início da vitelogênese (WUERTZ et al., 2007). Verificou-se também, aumento da expressão da P450aromatase nos folículos ovarianos de red seabream (*Pagrus major*) e de truta arco-íris pelo estímulo do IGF-1 (KAGAWA et al., 2003; NAKAMURA; KUSAKABE; YOUNG, 2003)

3 CONSIDERAÇÕES GERAIS

A relação entre a nutrição e a fisiologia reprodutiva é altamente complexa e um dos desafios da produção de peixes em sistemas de cultivo é compreender esse mecanismo. Estudos com reprodutores de peixes demonstram a necessidade do fornecimento de dietas adequadas para ótimo desempenho, sendo os lipídeos apontados como nutrientes-chave para o sucesso reprodutivo. O perfil de ácidos graxos dos lipídeos tem influência em vários parâmetros reprodutivos, incluindo o processo de desova, esteroidogênese e qualidade das larvas.

A tilápia do Nilo é uma espécie de água doce de grande importância no cenário da produção aquícola nacional, capaz de utilizar com eficiência dietas a base de ingredientes vegetais. No entanto, há a necessidade de avaliar a relação entre os ácidos graxos LA e LNA dietéticos, bem como seus potenciais efeitos na reprodução dessa espécie.

REFERÊNCIAS

BRASH, A. R. Arachidonic acid as a bioactive molecule. **The Journal of Clinical Investigation**, New York, v. 107, n. 11, p. 1339-1345, June 2001.

CALDER, P. C. Polyunsaturated fatty acids and inflammation. **Prostaglandins, Leukotrienes and Essential Fatty Acids**, Edinburgh, v. 75, n. 3, p. 197-202, Sept. 2006.

______. Polyunsaturated fatty acids, inflammation, and immunity. **Lipids**, Champaign, v. 36, n. 9, p. 1007-1024, Sept. 2001.

CEJAS, J. R. et al. Lipid and fatty acid composition of muscle and liver from wild and captive mature female broodstocks of white seabream, *Diplodus sargus*. **Comparative Biochemistry and Physiology. Part B:** biochemistry and molecular biology, New York, v. 138, n. 1, p. 91–102, May 2004.

CLELLAND, E.; PENG, C. Endocrine/paracrine control of zebrafish ovarian development. **Molecular and Cellular Endocrinology**, New York, v. 312, n. 1/2, p. 42–52, Nov. 2009.

COWARD, K.; BROMAGE, N. R. Reproductive physiology of female tilapia broodstock. **Reviews in Fish Biology and Fisheries**, London, v. 10, n. 1, p. 1-25, Mar. 2000.

DORRINGTON, J. H.; BENDELL, J. J.; KHAN, S. A. Interactions between FSH, estradiol-17β and transforming growth factor-β regulate growth and differentiation in the rat gonad. **The Journal of Steroid Biochemistry and Molecular Biology**, Oxford, v. 44, n. 4, p. 441-447, Mar. 1993.

EL-SAYED, A.-F. M.; KAWANNA, M. Effects of dietary protein and energy levels on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock in a recycling system. **Aquaculture**, Amsterdam, v. 280, n. 1/4, p. 179–184, Aug. 2008.

EL-SAYED, A.-F. M.; MANSOUR, C. R.; EZZAT, A. A. Effects of dietary lipid source on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. **Aquaculture**, Amsterdam, v. 248, n. 1/4, p. 187-196, July 2005.

______. Effects of dietary protein level on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. **Aquaculture**, Amsterdam, v. 220, n. 1/4, p. 619–632, Apr. 2003.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO. **Global aquaculture production 1950-2012**. Rome: FAO, 2014.

FERNÁNDEZ-PALACIOS, H. et al. Effect of n - 3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). **Aquaculture**, Amsterdam, v. 132, n. 3/4, p. 325-337, May 1995.

FURUITA, H. et al. Effect of arachidonic acid levels in broodstock diet on larval and egg quality of Japanese flounder *Paralichthys olivaceus*. **Aquaculture**, Amsterdam, v. 220, n. 1/4, p. 725-735, Apr. 2003.

______. Effect of n-3 and n-6 fatty acids in broodstock diet on reproduction and fatty acid composition of broodstock and eggs in the Japanese eel *Anguilla japonica*. **Aquaculture**, Amsterdam, v. 267, n. 1/4, p. 55-61, July 2007.

GLENCROSS, B. D. Exploring the nutritional demand for essential fatty acids by aquaculture species. **Reviews in Aquaculture**, Richmond, v. 1, n. 2, p. 71-124, May 2009.

GUNASEKERA, R. M.; LAM, T. J. Influence of dietary protein level on ovarian recrudescence in Nile tilapia, *Oreochromis niloticus*. **Aquaculture**, Amsterdam, v. 149, n. 1/2, p. 57-69, Mar. 1997.

INGS, J. S.; VAN DER KRAAK, G. Characterization of the mRNA Expression of StAR and Steroidogenic Enzymes in Zebrafish Ovarian Follicles. **Molecular reproduction and development**, New York, v. 73, n. 8, p. 943-954, Aug. 2006.

IZQUIERDO, M. S.; FERNANDEZ-PALACIOS, H.; TACON, A. G. J. Effect of broodtsock nutrition on reproductive performance of fish. **Aquaculture**, Amsterdam, v. 197, n. 1, p. 25-42, June 2001.

JORDAL, A.-E. O.; LIE, Ø.; TORSTENSEN, B. E. Complete replacement of dietary fish oil with a vegetable oil blend affect liver lipid and plasma lipoprotein levels in Atlantic salmon (*Salmo salar* L.). **Aquaculture Nutrition**, Oxford, v. 13, n. 2, p. 114-130, Mar. 2007.

KAGAWA, H. et al. Effects of luteinizing hormone and follicle-stimulating hormone and insulin-like growth factor-i on aromatase activity and P450 aromatase gene expression in the ovarian follicles of red seabream, *Pagrus major*. **Biology of Reproduction**, Oxford, v. 68, n. 5, p. 1562-1568, May 2003.

KAGAWA, H. Oogenesis in teleost fish. **Aqua-BioScience Monographs**, Tokyo, v. 6, n. 4, p. 99-127, 2013.

KOBAYASHI, Y. et al. Sex change in the gobiid fish is mediated through rapid switching of gonadotropin receptors from ovarian to testicular portion or vice versa. **Endocrinology**, San Diego, v. 150, n. 3, p. 1503-1511, Mar. 2009.

LAVENS, P. et al. Effect of dietary essential fatty acids and vitamins on egg quality in turbot broodstocks. **Aquaculture International**, Sorrey, v. 7, n. 4, p. 225-240, July 1999.

LE MENN, F.; CERDÀ, J.; BABIN, P. J. Ultrastructural aspects of the ontogeny and differentiation of ray-finned fish ovarian follicles. In: BABIN, P. J. (Ed.). **The fish oocyte:** from basic studies to biotechnological applications. Wageningen: Springer, 2007. p. 1-37.

LUBZENS, E. et al. Oogenesis in teleosts: how fish eggs are formed. **General and Comparative Endocrinology**, San Diego, v. 165, n. 3, p. 367-389, Feb. 2010.

MANNA, P. R.; DYSON, M. T.; STOCCO, D. M. Regulation of the steroidogenic acute regulatory protein gene expression: Present and future perspectives. **Molecular Human Reproduction**, Oxford, v. 15, n. 6, p. 321-333, June 2009.

MAZORRA, C. et al. Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hipoglossus*). **Aquaculture**, Amsterdam, v. 227, n. 1/4, p. 21-33, Nov. 2003.

MERCURE, F.; VAN DER KRAAK, G. Inhibition of gonadotropin-stimulated ovarian steroid production by polyunsaturated fatty acids in teleost fish. **Lipids**, Champaign, v. 30, n. 6, p. 547-554, June 1995.

______. Mechanisms of action of free arachidonic acid on ovarian steroid production in the goldfish. **General and Comparative Endocrinology**, San Diego, v. 102, n. 1, p. 130-140, Apr. 1996.

MILLER, W. L. Steroidogenic acute regulatory protein (StAR), a novel mitochondrial cholesterol transporter. **Biochimica et Biophysica Acta**, Amsterdam, v. 1771, n. 6, p. 663–676, June 2007.

MONTSERRAT, N. et al. Effects of follicle stimulating hormone on estradiol-17β production and P-450 aromatase (CYP19) activity and mRNA expression in brown trout vitellogenic ovarian follicles in vitro. **General and Comparative Endocrinology**, San Diego, v. 137, n. 2, p. 123-131, June 2004.

MORAIS, S. et al. Diet x genotype interactions in hepatic cholesterol and lipoprotein metabolism in Atlantic salmon (*Salmo salar*) in response to replacement of dietary fish oil with vegetable oil. **British Journal of Nutrition**, Cambridge, v. 106, n. 10, p. 1457-1469, Nov. 2011.

NAGAHAMA, Y.; YAMASHITA, M. Regulation of oocyte maturation in fish. **Development, Growth & Differentiation**, Nagoya, v. 50, Suppl. 1, p. 195-219, May 2008.

NAKAMURA, I.; KUSAKABE, M.; YOUNG, G. Regulation of steriodogenic enzyme mRNAs in rainbow trout (*Oncorhynchus mykiss*) ovarian follicles in vitro. **Fish Physiology and Biochemistry**, Amsterdam, v. 28, n. 1/4, p. 355-356, Mar. 2003.

NAVAS, J. M. et al. The impact of seasonal alteration in the lipid composition of broodstock diets on egg quality in the european sea bass. **Journal of Fish Biology**, London, v. 51, n. 4, p. 760-773, Oct. 1997.

NORAMBUENA, F. et al. Effects of graded levels of arachidonic acid on the reproductive physiology of Senegalese sole (*Solea senegalensis*): Fatty acid composition, prostaglandins and steroid levels in the blood of broodstock bred in captivity. **General and Comparative Endocrinology**, San Diego, v. 191, p. 92-101, Sept. 2013.

______. Proximate and fatty acid compositions in muscle, liver and gonads of wild versus cultured broodstock of Senegalese sole (*Solea senegalensis*). **Aquaculture**, Amsterdam, v. 356/357, p. 176-185, Aug. 2012.

OKUMURA, H. et al. Changes in hepatic vitellogenin mRNA levels during oocyte development in the Japanese eel, *Anguilla japonica*. **General and Comparative Endocrinology**, San Diego, v. 125, n. 1, p. 9-16, Jan. 2002.

PEYON, P.; BALOCHE, S.; BURZAWA-GERARD, E. Potentiating effect of growth hormone on vitellogenin synthesis induced by 17 beta-estradiol in primary culture of female silver eel (*Anguilla anguilla* L.) hepatocytes. **General and Comparative Endocrinology**, San Diego, v. 102, n. 2, p. 263-73, May 1996.

REINDL, K. M.; SHERIDAN, M. A. Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. **Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology**, New York, v. 163, n. 3/4, p. 231-245, Nov. 2012.

REINECKE, M. Insulin-like Growth Factors and Fish Reproduction. **Biology of Reproduction**, Champaign, v. 82, n. 4, p. 656-661, Apr. 2010.

RICHARD, N. et al. Replacement of a large portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.). **Aquaculture**, Amsterdam, v. 261, n. 3, p. 1077-1087, Dec. 2006a.

______. Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). **British Journal of Nutrition**, Cambridge, v. 96, n. 2, p. 299-309, Aug. 2006b.

SANTIAGO, C. B.; REYES, O. S. Effects of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia *Oreochromis niloticus* (Linnaeus) broodstock. **Journal of Applied Ichthyology**, Berlin, v. 9, n. 1, p. 33-40, Apr. 1993.

SARGENT, J. et al. Lipid nutrition of marine fish during early development: current status and future directions. **Aquaculture**, Amsterdam, v. 179, n. 1/4, p. 217-229, Sept. 1999.

SARGENT, J. R.; TOCHER, D. R.; BELL, J. G. The lipids. In: HALVER, J. E.; HARDY, R. W. (Ed.). **Fish nutrition** . 3rd ed. San Diego: Elsevier Science, 2002. p. 181-257.

SCHMITZ, G.; ECKER, J. The opposing effects of n-3 and n-6 fatty acids. **Progress in Lipid Research**, Oxford, v. 47, n. 2, p. 147–155, Mar. 2008.

SEABORN, G. T. et al. Comparative fatty acid composition of eggs from wild and captive black sea bass, *Centropristis striata* L. **Aquaculture Research**, Oxford, v. 40, n. 6, p. 656-668, Mar. 2009.

SINGH, H.; THOMAS, P. Mechanism of Stimulatory Action of Growth Hormone on Ovarian Steroidogenesis in Spotted Seatrout, Cynoscion nebulosus. **General and Comparative Endocrinology**, San Diego, v. 89, n. 3, p. 341–353, Mar. 1993.

SORBERA, L. A et al. Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). **Biology of Reproduction**, Champaign, v. 64, n. 1, p. 382-389, Jan. 2001.

STOCCO, D. M.; CLARK, B. J. Regulation of the acute production of steroids in steroidogenic cells. **Endocrine Reviews**, Baltimore, v. 17, n. 3, p. 221-244, June 1996.

STOCCO, D. M.; CLARCK, B. J. Role of the steroidogenic acute regulatory protein (StAR) in steroidogenesis. **Biochemical Pharmacology**, Oxford, v. 51, n. 806, p. 197-205, Feb. 1996.

STOCCO, D. M. StAR protein and the regulation of steroid hormone biosynthesis. **Annual Review of Psychology**, Palo Alto, v. 63, p. 193-213, 2001.

TOCHER, D. R. et al. Highly unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and nutritional regulation of fatty acyl delta 6 desaturase of Atlantic cod (*Gadus morhua* L.). **Lipids**, Champaign, v. 41, n. 11, p. 1003-1016, Nov. 2006.

TOCHER, D. R. Fatty acid requirements in ontogeny of marine and freshwater fish. **Aquaculture Research**, Oxford, v. 41, n. 5, p. 717-732, Apr. 2010.

TOCHER, D. R.; GHIONI, C. Fatty acid metabolism in marine fish: Low activity of fatty acyl Δ5 desaturation in gilthead sea bream (*Sparus aurata*) cells. **Lipids**, Champaign, v. 34, n. 5, p. 433-40, Jan. 1999.

TOCHER, D. R. Metabolism and functions of lipids and fatty acids in teleost fish. **Rewies in Fisheries Science**, Boca Raton, v. 11, n. 2, p. 107-184, Feb. 2003.

______. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. **Aquaculture**, Amsterdam, v. 449, p. 94-107, Dec. 2015.

TORSTENSEN, B. E.; LIE, Ø.; FRØYLAND, L. Lipid metabolism and tissue composition in Atlantic Salmon (*Salmo salar* L.)--effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. **Lipids**, Champaign, v. 35, n. 6, p. 653-664, June 2000.

WATHES, D. C.; ABAYASEKARA, D. R. E.; AITKEN, R. J. Polyunsaturated fatty acids in male and female reproduction. **Biology of Reproduction**, Champaign, v. 77, n. 2, p. 190-201, Aug. 2007.

WOOD, A. W.; DUAN, C.; BERN, H. A. Insulin-like growth factor signaling in fish. **International Review of Cytology**, New York, v. 243, p. 215-285, 2005.

WUERTZ, S. et al. Expression of IGF-I and IGF-I receptor in male and female sterlet, Acipenser ruthenus - Evidence for an important role in gonad maturation. **Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology**, New York, v. 147, n. 1, p. 223-230, May 2007.

XU, H. et al. Dietary arachidonic acid differentially regulates the gonadal steroidogenesis in the marine teleost, tongue sole (*Cynoglossus semilaevis*), depending on fish gender and maturation stage. **Aquaculture**, Amsterdam, v. 468, p. 378-385, Feb. 2017.

YOUNG, G. et al. Gonadal steroidogenesis in teleost fish. **Molecular Aspects of Fish & Marine Biology**, Oxfor, v. 4, p. 155-223, 2005.

SEGUNDA PARTE

ARTIGO

Effects of the dietary linoleic acid to linolenic acid ratio for Nile tilapia (*Oreochromis niloticus*) breeding females.

Tamira M. Orlando[a]; Tafanie Valácio Fontes[a]; Renan Rosa Paulino[b]; Jose Fernando Lopes Olmeda[c]; Priscila Vieira Rosa[a].

[a] *Department of Animal Science, Federal University of Lavras UFLA, Lavras, Minas Gerais, 37200-000, Brazil.*

[b] *Department of Animal Science, Federal University of Uberlândia UFU, Uberlândia, Minas Gerais, 38400-902, Brazil.*

[c] *Department of Physiology, Faculty of Biology, Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia, 30100 Murcia, Spain*

Correspondence: Tamira M. Orlando. Departamento de Zootecnia, Department of Animal Science, Federal University of Lavras, Lavras, Minas Gerais, Brasil. E-mail address: tamira_maria@yahoo.com.br

Preliminary version

ABSTRACT

The present study was conducted to evaluate the effect of dietary linoleic acid/ linolenic acid (LA/ LNA) ratio for Nile tilapia (*Oreochromis niloticus*) breeding females. Four isonitrogenous (38% protein) and isolipidic diets were formulated to contain different dietary LA/LNA ratios (20.1; 4.5; 3.9 and 0.7) by increasing of linseed oil at the expense of corn oil. Six females and two males were stocked in twelve 250-L each tank and their reproductive performance assessed over 105 days. At the end of the experiment, fish fed the 3.9 and 0.7 LA/LNA diets produced higher number of eggs. Hatch rates and larval survival did not vary among dietary treatments. In general, the fatty acid profile of gonads reflected the composition of the diet. The arachidonic acid content was higher in the 20.1 ratio and the eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids concentration, higher in the 0.7 ratio. Diets did not influence the triglycerides content in plasma. However, higher levels of cholesterol were observed in fish fed 4.5 and 3.9 LA/ LNA diets. The highest dietary LA levels increased the gene expression of *cyp17*, while the dietary 3.9 LA/ LNA ratio promoted higher expression of *cyp19a1a*. In conclusion, this study suggests that dietary n-3 and n-6 PUFA ratio for Nile tilapia female should be around 3.9 to highest expression of ovarian aromatase and better spawning performance

Keywords: Fishes. Broodstock nutrition. Reproduction. Fatty acids.

1 INTRODUCTION

For a long time, fish oil was used as the main lipid source in aquafeeds. Because the shortage supplies and increasing of prices worldwide (Sprague et al., 2016), the use of vegetable oils as a substitute to fish oil is a current issue to allow the formulation of more sustainable and cheaper diets. However, the use of vegetable understanding of the events that occur during the reproductive period, due the influence of dietary fatty acids, is fundamental for elaboration of adequate artificial diets during the pre-spawning period, which can provide higher quality of eggs, larvae and fingerlings.

Lipids are recognized as the major dietary factor in broodstock nutrition by influencing the reproductive success (Bell and Sargent, 2003), and the long chain polyunsaturated fatty acids (LC-PUFA) n-3 and n-6 series are pointed as one of the main factors by the improvement in the quality of fish eggs and larvae (Izquierdo, M. S., Fernandez-Palacios, H., & Tacon, 2001; Lavens et al., 1999; Tocher, 2010). Contrary to marine fishes, freshwater species can convert 18C-polyunsaturated fatty acids (PUFA), linoleic acid (18:2n-6, LA) and linolenic acid (18:3n-3, LNA), in their respective LC-PUFA, namely arachidonic acid (ARA), eicosapentaeneoic acid (EPA) and docosahexaenoic acid (DHA) (Tocher, 2015), through the activities of desaturase and elongase enzymes. However, n-3 and n-6 PUFA share the same bioconversion pathways and a competition between them may occur, compromising LA or LNA metabolism (Glencross, 2009).

Dietary n-3 and n-6 fatty acids have received attention in several studies of fish reproduction (Bell and Sargent, 2003; Bruce et al., 1999; Furuita et al., 2007; Liang et al., 2014). ARA and EPA have a major role in the synthesis of eicosanoids, a group of biologically active products. ARA is recognized to produce series-2 prostaglandins and leukotrienes, which have more potent

effects than series-3, produced from EPA. Prostaglandins mediate oocyte maturation process and affects steroidogenesis. Furthermore, LC-PUFA modulate gonadal steroid synthesis (Sorbera et al., 2001) and may act in the tissues or intracellularly as ligands for transcription factors controlling gene expression (Tocher, 2015). Furuita et al. (2007) suggested that the inclusion of both dietary n-3 and n-6 fatty acids for reproduction is necessary in Japanese eel (*Anguila japonica*). Liang et al. (2014) related that dietary fatty acids n-3 and n-6 ratio affected the spawning performance, egg and larval quality of tongue sole (*Cynoglossus semilaevis*). Spawning performance and larval quality were better in Atlantic halibut (*Hippoglossus hippoglossus*) fed 1.8% of dietary ARA than fish fed a level of 0.4% (Mazorra et al., 2003).

Cholesterol transport from the outer to the inner of the mitochondrial membrane, is the rate limiting step of steroidogenesis. The first reaction in the biosynthetic pathway is the conversion of cholesterol to pregnenolone by the cytochrome P450 cholesterol side-chain cleavage (P450scc). A series of enzymes, encoded by numerous genes, act in pregnenolone to synthesize steroids hormones. The final product of the steroid biosynthetic pathway, will depend on the stage of gonadal development.

Nile tilapia female are multiple spawners, with early sexual maturation and short vitellogenic period (Izquierdo, M. S., Fernandez-Palacios, H., & Tacon, 2001). Furthermore, they are mouth-brooders and have parental care. The present study, aimed to evaluate the effects of dietary linoleic/ linolenic ratios on reproductive performance, fatty acid profile and gene expression of proteins related to steroidogenesis in Nile tilapia female.

2 MATERIAL AND METHODS

2.1 Fish and culture facilities

All procedures adopted were in accordance with ethical standards and approved by the Ethics Committee of Animal Welfare of the Universidade Federal de Lavras, protocol number 006/2015. The trial was conducted at the fish farming Station of Federal University of Lavras, UFLA, Brazil. A total number of 96 Nile tilapia UFLA strain were acclimatized to experimental conditions. UFLA strain underwent genetic improvement programs at the fish farming sector of the Federal University of Lavras over the last 25 years (Dias et al., 2016). Triplicate groups were randomly distributed into 12 tanks (250-L capacity). Each tank was stocked with 6 female (mean initial weight, 124.5g) and 2 male (mean initial weight, 131.3g), in a male/ female sex ratio of 1/3 as recommended by Litlle and Hulata (2000) under aquarium conditions. All fishes were implanted with microchips before beginning the experiment. The culture system consisted of an indoor thermoregulated recirculation water system supplied with a continue flow of aeration and sand filter. Water temperature was kept at 28 ± 0.5°C and the photoperiod was set at 12h light:12h dark cycle.

2.2 Experimental diets

Four isonitrogenous (38% crude protein), according to Oliveira et al. (2014), and isolipidic (7% lipids) diets were formulated (Table 1).

Table 1 - Ingredient and proximal composition of the experimental diets.

	Diets			
Ingredients	**20.1**	**4.5**	**3.9**	**0.7**
Texturized soy protein[1]	55.00	55.00	55.00	55.00
Fish meal	8.00	8.00	8.00	8.00
Corn starch	17.00	17.00	17.00	17.00
Gelatin	5.74	5.74	5.74	5.74
Cellulose	2.21	2.21	2.21	2.21
Corn oil	3.00	2.01	1.02	0.00
Linseed oil	0.00	0.99	1.98	3.00
Palm oil	4.00	4.00	4.00	4.00
Dicalcium phosphate[2]	1.50	1.50	1.50	1.50
Kaolim	2.18	2.18	2.18	2.18
Sodium chloride	0.5	0.5	0.5	0.5
Mineral and Vitamin mix[3]	0.5	0.5	0.5	0.5
DL-Methionine	0.19	0.19	0.19	0.19
Threonine	0.1	0.1	0.1	0.1
Vitamin C	0.06	0.06	0.06	0.06
Antioxidant BHT	0.02	0.02	0.02	0.02
Proximate composition (% dry weight)				
Dry Matter	93.87	93.51	94.47	93.16
Crude protein	38.3	38.6	37.9	38.0
Crude lipid	7.4	7.2	7.4	7.3
Ash	9.5	9.3	9.2	9.9
Energy (MJ/ Kg)[4]	16.73	16.73	16.73	16.73

[1] Crude protein 50%, Crude lipid 1.3% [2]Nutrimix, MS, Brazil Mix vita/min omnivorous fish Cargill, SP, Brazil. [3]Composition (mg kg−1 diet): iron sulfate, 196; copper sulfate, 28; zinc oxide, 280; manganese oxide, 52; sodium selenite, 1.2; cobalt sulfate, 0.4; potassium iodide, 1.2; vitamin A, 19,950 (UI kg−1 diet); vitamin D3, 7980 (UI kg−1 diet); vitamin E, 199; vitamin K3, 10; vitamin C, 700; thiamin, 50; riboflavin, 50; pyridoxine, 50; cyanocobalamin, 0.1; niacin, 200; calcium pantothenate, 100; folic acid, 10; biotin, 1.6; inositol, 100; ethoxyquin, 247. [4]Calculated values.

The diets were formulated to have different n-6/ n-3 ratio obtained from corn oil (CO) and linseed oil (LO), rich in linoleic acid (LA) and linolenic acid (LNA), respectively. The ingredient composition of all diets was similar, except the amount of CO and LO, used to provide different n-6/ n-3 ratio by increase the percentage of LO and decrease of corn oil. Palm oil was added in constant amount to provide short and medium chain fatty acids (Table 2). The residual oil from fishmeal was minimal, about 0.74%. The ingredients were finally ground and thoroughly mixed. Pellets were dried in an oven at 40 °C for 48 h and stored at -20 °C until use. Experimental diets were hand-fed to apparent visual satiation, twice a day (9:00h and 15:00h), during 105 days.

Table 2 - Fatty acid composition (% of total fatty acids) of the experimental diets.

	Dietary LA/ LNA ratio			
Fatty acid	**20.1**	**4.5**	**3.9**	**0.7**
C14:0	7.06	0.7	7.7	7.7
C16:0	18.3	27.2	12.7	10.5
C16:1	0.5	0.6	0.7	0.6
C17:0	0.0	0.1	0.1	0.1
C18:0	3.3	4.2	3.5	4.3
C18:1n9	25.4	34.5	22.7	21.0
C18:2n6	26.7	24.1	23.2	13.6
C18:3n3	1.3	5.4	5.9	18.4
C20:0	0.3	0.4	0.2	0.1
C20:1n9	0.3	0.3	0.3	0.3
C20:2	0.1	0.1	0.1	0.1
C20:4n6	0.1	0.1	0.2	0.1
C20:5n3	0.6	0.5	0.5	0.5
C22:0	0.1	0.0	0.0	0.1
C22:6n3	0.7	0.7	0.8	0.6
Σ SAFA	29.0	32.7	24.2	22.9
Σ MUFA	26.2	35.4	23.8	22.0
Σ PUFA	29.3	31.3	30.3	33.4
Σ LC-PUFA	1.4	1.3	1.6	1.3
Σ n-6	26.7	24.2	23.4	13.7
Σ n-3	2.6	6.5	7.4	19.6
LA/LNA	20.1	3.9	4.5	0.7

Σ SAFA: sum of saturated fatty acids; Σ MUFA: sum of monounsaturated fatty acids; Σ PUFA: sum of polyunsaturated fatty acids; LC-PUFA: sum of long chain polyunsaturated fatty acids.

2.3 Reproductive management

The reproductive state of all female was verified on a daily basis by examining the oral cavity to check the presence of eggs. When present, eggs were gently removed from their buccal cavity, counted and weighted on a digital precision scale (0.001 g). The weight of females was registered before returning them to their respective tank. The following reproductive parameters were determined:

Absolute fecundity = mean number of eggs at each spawning per fish.

Relative fecundity = mean number of eggs at each spawning/ fish body weight.

Egg weight

Total spawning per fish = total number of spawning/ number of spawned female

Total spawning per tank

Total eggs spawned per fish = total number of eggs per tank per number of spawned female

Hatchability = number of hatched larvae/ number of eggs *100

The collected eggs were counted and submitted to artificial incubation in 3-L capacity plastic incubators with rounded bottom in a thermoregulated (28 °C) recirculation system. A gently flow of water was maintained to ensure a slow movement of the eggs, as occur on female's tilapia mouth. Percentage of hatching was recorded. After the eggs hatched, 100 3-day-old larvae per treatment were submitted to a fasting survival test according to an adaptation by Lavens et al. (1999). Larvae were distributed in 16 30-L capacity aquaria in a completely randomized design with four treatments (experimental broodstock diets) and four replicates. Larvae remained in the aquariums until detect the time

of 50% mortality. Larvae mortality was verified twice a day, when dead were removed and counted.

2.4 Sample collection

At the end of the experiment, sampling of all females occurred after 24h of fasting. Blood was collected from caudal vein with syringe containing EDTA (4%), immediately centrifuged at 1000g for 10 min, and plasma collected and stored at -80 °C until analysis. Then, fishes were euthanized by an overdose of 2-phenoxyethanol solution (1:500 v/v, Fluka; Sigma-Aldrich, Madrid, Spain). Fishes were dissected, and the weights of the liver, gonads and viscera were recorded to determine the hepatosomatic index (HSI), gonadosomatic index (GSI), and viscerosomatic index (VSI), respectively, as a percentage of the tissue in relation to the body weight. Liver, ovaries and muscle samples were collected and stored at -80 °C to further analysis.

2.5 Chemical analysis

2.5.6 Proximate analysis

Chemical composition of diets and carcass were analyzed according to AOAC methods (AOAC, 2005). Briefly, dry matter content was determined in an oven for 24 h at 105 °C until constant weight. Ash was determined by incineration of the organic components for 5 h at 550 °C using a furnace (LF 0213, JUNG, São Paulo, Brazil); Kjeldahl method was used for the evaluation of crude protein content (N×6.25) after acid digestion; crude lipids determined by extraction with ethyl ether in a Soxhlet extraction system.

2.5.7 Fatty acid analysis

Lipid extraction and FA profile of diet, liver, muscle, gonads, and eggs were analyzed according to Araújo et al. (2017). Briefly, total lipid was extracted with chloroform and methanol using a modification of the method from Folch et al. (1957). FA profile was determined using a GC2010 gas chromatograph (GC) (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and a SP-2560 fused silica capillary column (100.0 m 9 0.25 mm, 0.20 lm film; Supleco, Sigma-Aldrich, St. Louis, MO, USA). Fatty acid peaks were integrated using GC solution chromatography software (version 4.02), and peaks were identified by comparison to known standards (37 Component FAME Mix; Supelco, Sigma-Aldrich)

2.5.8 Plasma

Commercial kits were used to perform triglycerides (TRI - Triglycerides Liquiform, Cat. 87), total cholesterol (CT - Cholesterol Liquiform, Cat. 76) and oestradiol (OE – Biomedix, Cat. EIA2693) analysis. Samples were quantified in triplicate in a 96-well plate and read in a spectrophotometer (Multskan GO, Thermo Scientific, USA).

2.5.9 Gene expression

Total RNA was extracted from ovary of each fish using Trizol reagent in sterile tubes, according to the manufacturer's recommendations (Invitrogen, CA, EUA). RNA concentration was determined by spectrophotometry (DeNovix DS-11). Complementary DNA (cDNA) synthesis was realized with 2µg of the resulting total using the High-Capacity RNA-to-cDNA kit (Thermo Fisher

Scientific), following manufacturer's instructions. cDNA was submitted to real-time quantitative PCR (q-PCR) analysis in a light thermocycler (7500 Real-Time PCR system, Applied Biosystems, CA, USA). q-PCR reactions were performed using SYBR-green primer master mix (Applied Biosystems) in a final volume of 20 μL. All samples were tested in triplicate and every amplification experiment also included non-template controls (NTC). Primers for tilapia steroidogenic genes were designed with the Primer3 software (Untergasser et al. 2012) using the sequence obtained for each gene from the Ensembl database (www.ensembl.org) (Table 3).

Two housekeeping genes, *18 s* and *Actin*, were selected after checking the coefficient of variation (CV) for each gene within each tissue, and it was lower than 5%. The primers of *18 s*, Actin, *cyp11a* and *cyp19a1a* were added to a final concentration of 200 nM, and the *cyp11a* and *hsd3β1* primers were added to a final concentration of 400 nM in the reaction. The relative expression levels of the samples were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). The second normalization in the $2^{-\Delta\Delta CT}$ calculations was performed using the sample with the lowest value within each gene as reference.

Table 3 - Primer sequences used for quantitative PCR analyses.

Gene	Fw/ Rv	Primer sequence	Ensembl/GenBank number
cyp11a	Fw	ACCGCGTGATTCTCAACAAG	ENSONIG00000015
	Rv	ACAAAATCCTGGCCCACTTC	391
hsd3β1	Fw	AAGGAACGCAGCTGCTTTTG	ENSONIG00000015
	Rv	TCACTTCAATGGTGCTGGTG	700
cyp17	Fw	TGAATTACCACCGCATGTGC	ENSONIG00000009
	Rv	ACTTCTTTGGCGTGCACATG	168
cyp19a1a	Fw	TTGCACAAAACCACGGTGAG	ENSONIG00000000
	Rv	ACGTGCGGGTTTTGTTTGAG	155
18s	Fw	GGACACGGAAAGGATTGACAG	JF698683
	Rv	GTTCGTTATCGGAATTAACCAGAC	
βactin	Fw	TGGTGGGTATGGGTCAGAAAG	XM_003455949
	Rv	CTGTTGGCTTTGGGGTTCA	

2.6 Statistical analysis

Data were checked for normality and homogeneity of variances by the Shapiro Wilk and Levene tests, respectively. Percentage data were transformed to homogenize variance by square-root arcsine. Data were submitted to one-way analysis of variance (ANOVA). Significant differences among means were determined using Tukey's HSD test. A probability level of 0.05 was used to reject the null hypothesis. Statistical analyses were done using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY, USA). Data are present as mean ± SEM.

3 RESULTS

3.5 Reproductive performance

Reproductive performance data of female Nile tilapia are present in Table 4. Increase in the number of eggs spawned per fish was observed in female Nile tilapia fed the 3.9 and 0.7 LA/LNA diets ($p<0.05$). No difference was observed on total spawning per tank or absolute and relative fecundity, and egg weight ($p>0.05$). Egg percentage hatchability and larval survival submitted to fasting were also not affected by the different treatments ($p>0.05$).

Table 4 - Reproductive performance female Nile tilapia fed experimental diets for 105 days.

	Diets				
Reproductive performance	**20.1**	**4.5**	**3.9**	**0.7**	SEM
Absolute fecundity[1]	682.4	666.4	707.9	702.4	19.7
Relative fecundity	4.8	4.7	4.5	4.7	0.1
Egg weight (g)	6.4	5.9	5.7	6.0	0.1
Total spawning per fish	4.06	4.03	4.33	4.22	0.3
Total spawning per tank	24.3	24.1	26.3	25.3	0.5
Total eggs spawned per fish	2589.6a	2683.7a	3051.1b	2987.9b	267.1
Hatchability (%)	74.1	74.3	79.4	72.9	3.2
Larval capacity survival (days)	20	20	21	20	0.3

Values are means of three tanks. SEM is pooled of standard error of mean. Mean in the same row with different letters are significantly different ($p < 0.05$).

3.6 Growth performance

At the end of experiment, final body weight of female Nile tilapia was not affected by dietary treatments ($p>0.05$) (Table 5). Fish fed the 0.7 LA/LNA diet presented higher specific growth rate ($p<0.05$). The GSI, HSI and VSI of female tilapia after 105 days fed experimental diets are presented in Table 5. No differences were found on body indexes, except for HSI that was significantly higher in fish fed the 4.5 LA/LNA diet than those fed the 0.7 LA/LNA diet.

Table 5 - Growth performance and body indices of Nile tilapia fed with different LA/ LNA diets for 105 days.

	Diets				
Growth performance	**20.1**	**4.5**	**3.9**	**0.7**	SEM
Initial body weight (g)	121.2	128.1	124.3	124.5	4.5
Final body weight (g)	179.1	169.1	187.9	191.4	10.1
Weight gain (g)	58.0	36.5	63.7	63.0	8.2
Specific growth rate[1]	0.4ab	0.3a	0.4ab	0.4b	0.01
VSI[2]	9.9	9.6	9.0	9.6	0.3
GSI[3]	4.4	3.9	4.4	4.3	0.2
HSI[4]	1.5ab	1.7b	1.6ab	1.4a	0.1

Values are means of three tanks. SEM is pooled of standard error of mean. Mean in the same row with different letters are significantly different ($p < 0.05$) by Tukey's test. [1]Specific growth rate: [(ln final weight - ln initial weight)/ time]*100; [2]VSI: viscerosomatic index; [3]HIS: hepatosomatic index; [4]GSI: gonadosomatic index.

3.7 Proximate and fatty acid composition

The observed mortality throughout the test was low and it was not affected by the experimental diets. Throughout the test, two motilities were verified, one fish in the 20.1 and 3.9 treatments each, that jumped out of the thanks. At the end of the experiment diets did not affect the whole-body content of moisture, protein, lipid or ashes (Table 6).

Table 6 - Whole-body (% dry weight) composition of female Nile tilapia fed the experimental diets for 105 days.

	Diets				
	20.1	**4.5**	**3.9**	**0.7**	SEM
Moisture	96.8	96.5	95.9	96.3	0.1
Protein	55.3	59.1	59.5	53.7	0.4
Lipid	15.9	16.1	16.0	15.8	0.3
Ash	19.0	18.8	18.3	18.5	0.2

Values are means of three tanks. SEM is pooled of standard error of mean. Mean in the same row with different superscript letters are significantly different (P<0.05).

The fatty acid profiles of gonad, liver, and muscle differed in response to the experimental diets. Fatty acid composition of the gonad was significantly different (p<0.05) among treatments (Table 7). Lipid profile in the gonad generally reflected the fatty acid composition of the respective treatment. Highest levels of total PUFA n-6 were deposited in the gonads of fish fed the 20.1 LA/LNA diet compared to fish fed with the other diets, whereas more PUFA n-3 was observed in the gonads of fish fed the 0.7 LA/LNA diet. Following this trend, 18:2n-6 concentration was lower gonad of fish fed 0.7 LA/LNA diet, that also had lowest levels of ARA (p<0.05), due the lowest proportion of LA/ LNA. The highest levels of ARA were observed in 20.1 LA/LNA diet (p<0.05) and highest concentration of EPA and DHA on total fatty acid (TFA) were observed in fish fed 0.7 LA/LNA diet.

In liver, despite the abundance of LA in the 20.1 LA/LNA diet, its concentration was not different among dietary treatments. Similarly, ARA percentage in TFA also were not affected by diets in the liver (p>0.05). Content of n-3 fatty acids (LNA, EPA and DHA) was highest in fish fed 0.7 LA/ LNA diet. Fish fed 4.5 LA/ LNA diet had lowest SAFA content in TFA in the liver (Table 8).

Fish group fed 0.7 LA/ LNA diet showed lowest LA content of the TFA lipids on muscle (Table 9), but it was not different among other diets. Any significant difference was observed in ARA concentration in this tissue. LNA concentration on muscle lipids was highest in fish fed the 0.7 LA/ LNA diet. EPA concentration was significantly higher in fish fed the 0.7 LA/ LNA diet, compared to fish fed 20.1 LA/ LNA diet. Higher deposition of DHA on muscle was observed in fish fed 0.7 LA/ LNA diet, although it was not different of the group fed 3.9 LA/ LNA diet.

Table 7 - Gonad fatty acid (% total fatty acids) composition of Nile tilapia fed with different n-6/ n-3 fatty acid ratio diets.

	Dietary LA/LNA ratio				
Fatty acid	**20.1**	**4.5**	**3.9**	**0.7**	**SEM**
C14:0	3.9b	3.6a	3.4c	3.0c	0.4
C16:0	26.6b	25.9b	23.7a	22.7a	0.4
C18:0	8.0	7.6	8.3	8.2	0.2
C18:1n-9	27.2ab	31.0b	27.2ab	22.7a	0.6
C18:2n-6 LA	12.4b	12.7b	11.8b	7.6a	0.4
C18:3n-3 LNA	0.4a	1.8b	1.5b	5.0c	0.4
C20:3n-6	1.9	1.8	1.8	1.8	0,1
C20:4n-6 ARA	5.0c	4.2b	4.1b	2.7a	0.2
C20:5n-3 EPA	0.1a	0.2b	0.2b	0.5c	0.04
C22:6n-3 DHA	4.1a	6.0b	6.2b	8.6c	0.4
Σ SAFA	40.5b	36.2a	41.9b	41.8b	0.6
Σ MUFA	33.6b	34.5b	30.5a	30.1a	0.5
Σ PUFA	24.9a	28.4b	26.8a	27.4b	0.4
Σ CL-PUFA	10.7a	12.0ab	12.1ab	12.9b	0.3
Σ n-6	20.4c	19.9b	18.6b	12.2a	0.7
Σ n-3	4.6a	8.3b	8.1b	14.9c	0.8
n-6/n-3	4.6c	2.4b	2.3b	0.8a	0.3

Values are means of six fish. SEM is pooled of standard error of mean. Mean in the same row with different superscript letters are significantly different ($P < 0.05$).

Table 8 - Liver fatty acid (% total fatty acids) composition of Nile tilapia female fed with different n-6/ n-3 fatty acid ratio diets.

	Dietary LA/LNA ratio				
Fatty acid	**20.1**	**4.5**	**3.9**	**0.7**	**SEM**
C14:0	3.2	3.2	3.2	2.9	0.3
C16:0	25.1b	25.0b	23.0ab	21.1a	0.4
C18:0	11.2	9.7	10.9	12.1	0.5
C18:1n-9	27.2ab	31.0b	27.2ab	22.7a	0.9
C18:2n-6 LA	9.3	9.6	9.8	7.7	0.4
C18:3n-3 LNA	0.3a	0.8b	0.8b	2.8c	0.2
C20:3n-6	1.6	1.5	1.5	1.5	0.1
C20:4n-6 ARA	7.6	5.5	6.3	5.1	0.4
C20:5n-3 EPA	0,1a	0.2b	0.2b	0.8c	0.1
C22:6n-3 DHA	4.7a	5.4a	6.1a	13.4b	0.8
Σ SAFA	40.6ab	38.0a	41.8b	39.1ab	0.4
Σ MUFA	33.9ab	37.4b	33.3ab	28.5a	1.0
Σ PUFA	24.6ab	23.9a	25.7ab	30.7b	1.8
Σ CL-PUFA	13.9a	12.3a	13.8a	21.2b	1.0
Σ n-6	19.6b	17.5ab	18.5ab	15.0a	0.6
Σ n-3	5.0a	6.4a	7.2a	15.5b	1.0
n-6/n-3	4.0c	2.7b	2.5b	1.1a	0.3

Values are means of six fish. SEM is pooled of standard error of mean. Mean in the same row with different superscript letters are significantly different ($P<0.05$).

Table 9 - Muscle fatty acid (% total fatty acids) composition of Nile tilapia fed with different n-6/ n-3 fatty acid ratio diets.

	Dietary LA/LNA ratio				
Fatty acid	**20.1**	**4.5**	**3.9**	**0.7**	**SEM**
C14:0	1.8a	1.3a	5.1b	2.9ab	0.4
C16:0	24.6	24.2	22.9	23.2	0.3
C18:0	9.6ab	9.9b	7.4a	8.6ab	0.3
C18:1n-9	21.4	21.6	23.3	22.1	0.6
C18:2n-6 LA	14.3b	14.2b	13.9b	11.3a	0.3
C18:3n-3 LNA	0.3a	1.0b	1.6b	3.9c	0.3
C20:3n-6	2.5	2.2	1.8	1.8	0.1
C20:4n-6 ARA	10.7	9.8	6.3	6.2	0.8
C20:5n-3 EPA	0.04a	0.3ab	0.3ab	0.8b	0.1
C22:6n-3 DHA	3.6a	5.2ab	6.7bc	8.6c	0.1
Σ SAFA	38.5	37.2	39.5	37.6	0.4
Σ MUFA	24.0	24.7	28.2	26.3	0.9
Σ PUFA	32.3	33.5	32.5	35.1	2.1
Σ CL-PUFA	19.1	17.0	14.9	18.3	0.6
Σ n-6	28.5c	27.4bc	23.0ab	21.0a	0.9
Σ n-3	3.9a	6.3ab	8.8b	13.8c	0.9
n-6/n-3	7.5c	5.8b	2.7b	1.5a	0.8

Values are means of six fish. SEM is pooled of standard error of mean. Mean in the same row with different superscript letters are significantly different ($P < 0.05$).

3.8 Plasma and Gene Expression

No significant differences were found on plasma triglycerides and estradiol. Total plasma cholesterol was higher in fish fed 4.5 and 3.9 LA/LNA diets than those fed the 20.1 LA/LNA diet (Table 10).

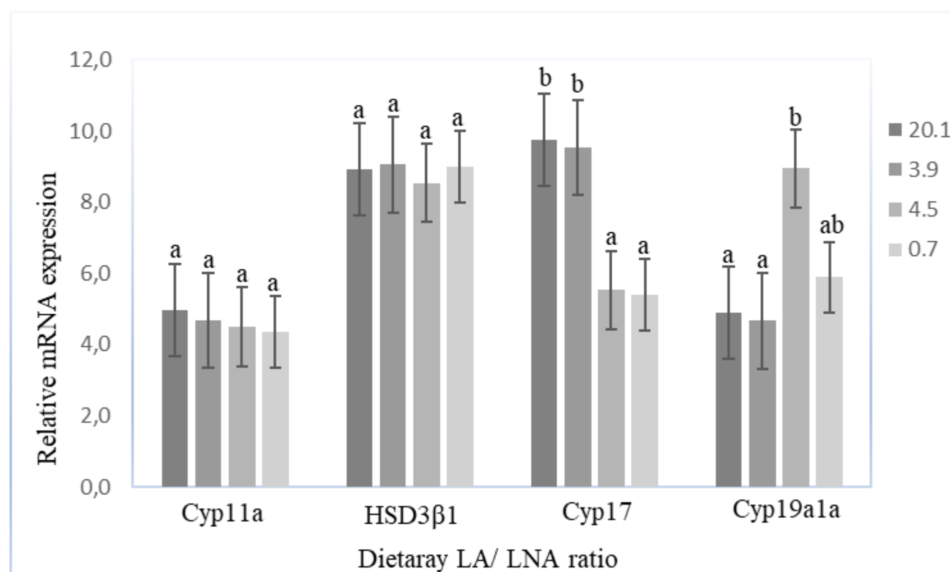
Table 10 - Plasma Nile tilapia parameters fed different LA/LNA diets for 105 days.

	Diets				
	20.1	**4.5**	**3.9**	**0.7**	SEM
Cholesterol (mg dL-1)	168.0a	208.9b	220.0b	183.0ab	5.6
Triglycerides (mg dL-1)	243.5	256.5	202.6	212.5	9.9
Estradiol (pg mL-1)	2596.0	2822.9	2518.5	2748.0	186.8

Values are means of three tanks. SEM is pooled of standard error of mean. Mean in the same row with different letters are significantly different ($p < 0.05$).

Data on expression levels of key steroidogenesis-related genes (*hsd3β1*, *cyp11a*, *cyp17* and *cyp19a1a*) in the gonad of female Nile tilapia fed the experimental diets are present in Figure 3. No difference on expression levels of *cyp11a* and *hsd3β1* were observed. *cyp19a1a* (aromatase) expression was higher in fish fed diet 3.9 LA/LNA than fish fed the 0.7 LA/LNA diet, while fish fed diets 20.1 and 4.5 LA/LNA showed highest expression of the *cyp17* in the gonad.

Figure 3 - Gene expression of key protein related to steroidogenesis in the gonads of female Nile tilapia fed different LA/LNA diets.



4 DISCUSSION

Several studies have been highlighted the importance of the dietary n-6 and n-3 ratio in the reproductive performance and egg quality in different fish species (Furuita et al., 2007; Mazorra et al., 2003; Wilson, 2009). In the present work, fish fed 3.9 and 0.7 LA/ LNA diets had higher number of eggs spawned per fish. However, no effects were observed on absolute and relative fecundity. Similar absolute fecundity was reported by Ng and Wang (2011). Hajizadeh et al. (2008) showed that fecundity in Nile tilapia fed a mixture of palm and fish oils was better than groups fed with these oils alone.

The number of total spawning per tank and per fish showed no significant differences among treatments, although the observed values for these parameters were slightly higher in fish fed 3.9 and 0.7 LA/ LNA diets, which may have influenced the significant effect of the total eggs spawned in broodfish

fed with these diets. The spawning performance values observed in this study are generally within the range reported elsewhere in Nile tilapia breeding experiments (El-Sayed et al., 2005; Hajizadeh et al., 2008; Ng and Wang, 2011). Furuita et al. (2007) demonstrated in a freshwater fish (*Anguilla japonica*) that egg production was lower in fish fed pollack oil as lipid source compared to fish fed a mixture of corn and pollack oils or corn oil alone. El-Sayed et al. (2005) did not found any significant difference on spawning performance of Nile tilapia reared in freshwater fed diets supplemented with fish oil, vegetable oil or a mixture of them. In zebrafish, a mixture of squid and linseed oils promoted the highest egg production (Jaya-Ram et al., 2008). ARA is recognized as the main precursor of eicosanoids in fish cells. Series-2 of prostaglandin are formed from ARA via cyclooxygenase pathway and have been shown to be important in reproductive process (Bell and Sargent, 2003), concerning to the control of steroidogenesis, oocyte maturation and ovulation (Mercure and Van Der Kraak, 1996; Patiño et al., 2003; Sorbera et al., 2001). However, EPA competes for the same enzyme system to eicosanoids production, that have lower bioactive effect, and thus exerts a modulating influence in the synthesis and efficacy of eicosanoid produced from ARA (Bell et al., 1997). Therefore, the ratio of ARA/ EPA may be a critical nutritional factor for ovulating process.

In the present study, hatchability rate varied from 72.9 to 79.4% and the larvae survival capacity was 20-21 days. Despite the lack of significant differences, high hatchability ratios were achieved in all treatments, and they are higher compared to values reported by other laboratories with Nile tilapia breeding (El-Sayed et al., 2005; Lupatsch et al., 2010; Ng and Wang, 2011; Tsadik and Bart, 2007). Ng and Wang (2011) observed no significant difference on larvae tolerance from Nile tilapia fed palm, linseed or fish oil-based diets submitted to a salinity challenge. High hatchability rate observed in the present work, may have been due the strain used in the trial. Broodstock nutrition has

been reported to influence the quality and viability of larvae (Bhujel et al., 2001; Izquierdo, M. S., Fernandez-Palacios, H., & Tacon, 2001), because it provides exogenous nutrient required for gonadal development. Furthermore, egg yolk is the major source of nutrients for embryonic development in fish. We consider that the levels of dietary protein and energy adopted in the current work were sufficient to meet dietary nutrient requirements of female broodstock and provide good larvae performance.

Female Nile tilapia is characterized to be a mouth-brooder and during spawning fish do not eat while they are incubating their eggs in the mouth. Despite fish fed 0.7 LA/LNA diet had higher specific growth rate than other groups, the final weight gain was not different. Tilapia species can suppress the growth to maintain their reproductive capacity (Coward and Bromage, 1999). In Nile tilapia female fed linseed oil or cod liver oil, an inverse relationship between growth performance and reproduction was observed (Ng and Wang, 2011; Santiago and Reyes, 1993). Highest HSI was observed in the fish fed 4.5 LA/LNA diet. Female Nile tilapia fed palm oil-based diets had higher GSI compared to fish fed linseed or fish oil-based diets, but no difference were observed in the HSI (Ng and Wang, 2011). The increase of the HSI in the present work may be due to an increase of the hepatic metabolic activity. It is important to note that liver express estradiol receptors to vitellogenin synthesis, a phospholipoglycoprotein, which is deposited in the oocytes during gonadal development.

Studies confirm the ability of freshwater fish to elongate and desaturate the precursors LA and LNA in their respective LC-PUFA, namely ARA, EPA and DHA (AGABA et al., 2004). Evidences have been suggested that tilapia and zebrafish express a fatty acid desaturation and elongation pattern in hepatocytes (Jaya-Ram et al., 2008; Tocher et al., 2002). The lower concentration of 18:2n-6 and 18:3n-3 in tissues as compared to diets combined with higher concentration

of LC-PUFA, demonstrate biosynthesis activities to form LC-PUFA. Furthermore, activities of desaturation and elongation in freshwater fish are nutritionally regulated, being higher with elevated availability of precursor for biosynthesis pathway (Francis et al., 2007; Tocher et al., 2002) and during limited dietary intake of LC-PUFA (Bell et al., 2003), as it occurs when fish are fed with vegetable oil sources.

The fatty acid composition of fish gonads and eggs is recognized to be directly influenced by dietary fatty acid composition as well as in muscle (El-Sayed et al., 2005; Furuita et al., 2007; Lewis et al., 2010). In the present study, the fatty acid n-6/ n-3 ratio of fish tissues generally reflected the respective treatment. Fish group fed 20.1 LA/ LNA diet (26.7% 18:2n-6 TFA) presented the highest levels of ARA in the gonads compared to the other fish groups, but its concentration was not significantly different in the liver and muscle, suggesting that LA may have contributed to increase the concentration of ARA in TFA in the ovaries. Highest levels of EPA and DHA were observed in fish fed 0.7 LA/ LNA diet (18.4% 18:3n-3 TFA) and high concentrations of DHA contributed to elevate total LC-PUFA in the liver and ovaries, but not in the muscle. In some species reduced feed intake was observed during spawning, and muscle may be a relevant source of LC-PUFA in this period (Almansa et al., 2001; Pérez et al., 2007; Rodríguez et al., 2004).

In the current work fish fed 4.5 and 3.9 LA/ LNA diets had highest levels of plasma cholesterol compared to fish fed 20.1 LA/ LNA diet, but no changes in triglycerides were observed. Cholesterol is the common substrate for the synthesis of all steroid hormones and its transfer from the cytoplasm to the inner of mitochondrial membrane is the rate limiting step of steroidogenesis (Manna et al., 2009; Stocco and Clarck, 1996). Although not determined, dietary cholesterol levels probably did not vary, since the only source of cholesterol was from fishmeal, which was added in constant amount in all diets. Therefore, the

observed differences in plasmatic cholesterol was due to the endogenous synthesis. Transcription of cholesterol and LC-PUFA biosynthesis genes in fish seems to be mediated by activation sterol regulatory element binding proteins (SREBPs) (Eberlé et al., 2004).

Previous studies have been shown that replacement of dietary fish oil by vegetable oil sources decreased the levels of plasma cholesterol in rainbow trout (*Oncorhynchus mykiss*) (Richard et al., 2006), but it had no effect in *Dicentrarchus labrax* juveniles (Castro et al., 2015).

Gonadal steroidogenesis regulation occurs through tropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (LUBZENS et al., 2010). This regulation is related to the expression of an array of genes that encodes steroidogenic enzymes, which ensure the ability of cells to produce steroids hormones from cholesterol and its derivatives (Guzmán et al., 2014; Ijiri et al., 2003; Kagawa, 2013; Lubzens et al., 2010). The first step for biosynthesis of steroid hormones is the conversion of cholesterol to pregnenolone through the side chain cleavage enzyme (P450scc) (Nagahama and Yamashita, 2008), encoded by *cyp11a*. Then, pregnenolone is converted to E2 by the action of many steroidogenic enzymes encoded by a number of genes, including *hsd3β1* (3β-hydroxysteroid dehydrogenase), *cyp17* (17α-hydroxylase and 17,20 lyase) and *cyp19a1a* (P450 aromatase) (Clelland and Peng, 2009). In this study, experimental diets differentially regulated the gene expression of the key proteins related to steroidogenesis. Expression levels of *cyp11a* and *hsd3β1* were not influenced by the diets. However, higher levels of *cyp17* were observed in fish fed 20.1 and 4.5 LA/LNA diets.

17α-hydroxylase and 17,20-lyase activities are required for testosterone synthesis, whereas only 17α-hydroxylase enzyme is required to produce 17α-hydroxyprogesterone, the precursor of the hormone which induce oocyte maturation in teleost (17α,20β-dihydroxy-4-pregnen-3-one, 17α,20β-DHP). A

shift on production of E2 to 17α,20β-DHP prior to oocyte maturation was reported in salmonids (Nagahama, 1997; Planas et al., 2000), Japanese eel (Kazeto et al., 2000) and rainbow trout (Sakai et al., 1992), suggesting the *cyp17* as a critical factor in the steroidogenic switch. Ings and Van der Kraak (2006) reported decrease of the *cyp17* levels with the progress of primary growth to maturating follicles in zebrafish ovary.

Highest expression of *cyp19a1a* occurred in fish fed 4.5 LA/LNA diet. Expression of *cyp19a1a* gene has been related in vitellogenic follicles in several species (García-López et al., 2011; Gen et al., 2001; Ijiri et al., 2003; Ings and Van Der Kraak, 2006; Kumar et al., 2000) and its expression coincided with a peak of plasma E2 in rainbow trout (Nakamura et al., 2016). In the present work, despite the significant differences observed on *cyp19a1a*, E2 levels were not influenced by the different n-6 and n-3 fatty acid ratios. However, the increasing levels of plasma cholesterol (substrate for steroidogenesis) coincides with a higher *cyp19a1a* expression. Therefore, the increased levels of plasma cholesterol may be due to the increasing expression of *cyp19a1a.*

Fatty acids effects on ovary steroidogenesis have been reported in fish species, but the results are variables. ARA supplementation significantly reduced E2 levels in immature and mature female tongue sole (*Cynoglossus semilaevis*) (Xu et al., 2017) and any effects were observed in female *Solea senegalensis* with increasing of ARA in the diets (Norambuena et al., 2013). These studies indicated that ARA metabolism is different in male and female, and it may be more important in the

5 CONCLUSION

In conclusion, the dietary LNA/ LNA ratio for tilapia should be around 4.5 to highest expression of ovarian aromatase and better spawning performance.

Acknowledgements

We thank the Fundação de Amparo à Pesquisa de Minas Gerais, FAPEMIG, for the research funding granted to Priscila Vieira Rosa (PPM 00227/12) and the Ph.D. scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CAPES), granted to Tamira M. Orlando, which made possible the accomplishment of this work.

REFERENCES

Almansa, E., Martín, M. V., Cejas, J.R., Badía, P., Jerez, S., Lorenzo, A., 2001. Lipid and fatty acid composition of female gilthead seabream during their reproductive cycle: Effects of a diet lacking n-3 HUFA. J. Fish Biol. 59, 267–286. https://doi.org/10.1006/jfbi.2001.1650

Araújo, F.G., Costa, D. V., Machado, M.R.F., Paulino, R.R., Okamura, D., Rosa, P. V., 2017. Dietary oils influence ovary and carcass composition and embryonic development of zebrafish. Aquac. Nutr. 23, 651–661. https://doi.org/10.1111/anu.12432

Bell, J.G., Farndale, B.M., Bruce, M.P., Navas, J.M., Carillo, M., 1997. Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*). Aquaculture 149, 107–119. https://doi.org/10.1016/S0044-8486(96)01436-6

Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218, 491–499. https://doi.org/10.1016/S0044-8486(02)00370-8

Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R., Crampton, V.O., 2003. Altered fatty acid compositions in atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. J. Nutr. 133, 2793–2801.

Bhujel, R.C., Turner, W.A., Yakupitiyage, A., Little, D.C., 2001. Impacts of environmental manipulation on the reproductive performance of Nile tilapia (*Oreochromis niloticus*). J. Aquac. Trop. 16, 197–209.

Bruce, M., Oyen, F., Bell, G., Asturiano, J.F., Farndale, B., Carrillo, M., Zanuy, S., Ramos, J., Bromage, N., 1999. Development of broodstock diets for the European Sea Bass (*Dicentrarchus labrax*) with special emphasis on the importance of n−3 and n−6 highly unsaturated fatty acid to reproductive performance. Aquaculture 177, 85–97. https://doi.org/https://doi.org/10.1016/S0044-8486(99)00071-X

Castro, C., Corraze, G., Panserat, S., Oliva-Teles, A., 2015. Effects of fish oil replacement by a vegetable oil blend on digestibility, postprandial serum metabolite profile, lipid and glucose metabolism of European sea bass (*Dicentrarchus labrax*) juveniles. Aquac. Nutr. 21, 592–603. https://doi.org/10.1111/anu.12184

Clelland, E., Peng, C., 2009. Endocrine/paracrine control of zebrafish ovarian development. Mol. Cell. Endocrinol. 312, 42–52. https://doi.org/10.1016/j.mce.2009.04.009

Coward, K., Bromage, N.R., 1999. Spawning frequency, fecundity, egg size and ovarian histology in groups of Tilapia zillii mantained upon two distinct food ration sizes from first-feedind to sexual maturity. Aquat. living Resouces 12, 11–22.

Dias, M.A.D., de Freitas, R.T.F., Arranz, S.E., Villanova, G. V., Hilsdorf, A.W.S., 2016. Evaluation of the genetic diversity of microsatellite markers among four strains of *Oreochromis niloticus*. Anim. Genet. 47, 345–353. https://doi.org/10.1111/age.12423

Eberlé, D., Hegarty, B., Bossard, P., Ferré, P., Foufelle, F., 2004. SREBP transcription factors: Master regulators of lipid homeostasis. Biochimie 86, 839–848. https://doi.org/10.1016/j.biochi.2004.09.018

El-Sayed, A.F.M., Mansour, C.R., Ezzat, A.A., 2005. Effects of dietary lipid source on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. Aquaculture 248, 187–196. https://doi.org/10.1016/j.aquaculture.2005.04.024

Francis, D.S., Turchini, G.M., Jones, P.L., De Silva, S.S., 2007. Dietary lipid source modulates in vivo fatty acid metabolism in the freshwater fish, murray cod (M*accullochella peelii peelii*). J. Agric. Food Chem. 55, 1582–1591. https://doi.org/10.1021/jf062153x

Furuita, H., Hori, K., Suzuki, Sugita, T., Yamamoto, T., 2007. Effect of n-3 and n-6 fatty acids in broodstock diet on reproduction and fatty acid composition of broodstock and eggs in the Japanese eel *Anguilla japonica*. Aquaculture 267, 55–61. https://doi.org/10.1016/j.aquaculture.2007.01.039

García-López, Á., Sánchez-Amaya, M.I., Prat, F., 2011. Targeted gene expression profiling in European sea bass (*Dicentrarchus labrax*, L.) follicles from primary growth to late vitellogenesis. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 160, 374–380. https://doi.org/10.1016/j.cbpa.2011.07.006

Gen, K., Okuzawa, K., Kumakura, N., Yamaguchi, S., Kagawa, H., 2001. Correlation between messenger RNA expression of cytochrome P450 aromatase and its enzyme activity during oocyte development in the red seabream (*Pagrus major*). Biol. Reprod. 65, 1186–94.

Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquac. 1, 71–124. https://doi.org/10.1111/j.1753-5131.2009.01006.x

Guzmán, J.M., Luckenbach, J.A., Yamamoto, Y., Swanson, P., 2014. Expression profiles of fsh-regulated ovarian genes during oogenesis in coho salmon. PLoS One 9, 1–22. https://doi.org/10.1371/journal.pone.0114176

Hajizadeh, A. liI, Jaunecey, K., Rana, K., 2008. Effects of dietary dipid source on egg and larval quality of Nile tilapia, *Oreochromis niloticus* (L.), in: Elghobashy, H., Fitzsimmons, K., Diab, A.S. (Eds.), The Eight International Symposium on Tilapia in Aquaculture. American Tilapia Association, pp. 965–977.

Ijiri, S., Kazeto, Y., Lokman, P.M., Adachi, S., Yamauchi, K., 2003. Characterization of a cDNA encoding P-450 aromatase (CYP19) from Japanese eel ovary and its expression in ovarian follicles during induced ovarian development. Gen. Comp. Endocrinol. 130, 193–203. https://doi.org/10.1016/S0016-6480(02)00589-0

Ings, J.S., Van Der Kraak, G., 2006. Characterization of the mRNA expression of StAR and steroidogenic enzymes in zebrafish ovarian follicles. Mol. Reprod. Dev. 73, 943–954. https://doi.org/10.1002/mrd

Izquierdo, M. S., Fernandez-Palacios, H., & Tacon, A.G.J., 2001. Effect of broodtsock nutrition on reproductive performance of fish. Aquaculture 197, 25–42.

Jaya-Ram, A., Kuah, M.K., Lim, P.S., Kolkovski, S., Shu-Chien, A.C., 2008. Influence of dietary HUFA levels on reproductive performance, tissue fatty acid profile and desaturase and elongase mRNAs expression in female zebrafish *Danio rerio*. Aquaculture 277, 275–281. https://doi.org/10.1016/j.aquaculture.2008.02.027

Kagawa, H., 2013. Oogenesis in teleost fish. Aqua-BioScience Monogr. 6, 99–127. https://doi.org/10.5047/absm.2013.00604.0099

Kazeto, Y., Ijiri, S., Todo, T., Adachi, S., Yamauchi, K., 2000. Molecular cloning and characterization of Japanese eel ovarian P450c17 (CYP17) cDNA. Gen. Comp. Endocrinol. 118, 123–133. https://doi.org/10.1006/gcen.1999.7449

Kumar, R.S., Ijiri, S., Trant, J.M., 2000. Changes in the expression of genes encoding steroidogenic enzymes in the channel catfish (*Ictalurus punctatus*) ovary throughout a reproductive cycle. Biol. Reprod. 63, 1676–1682. https://doi.org/10.1095/biolreprod63.6.1676

Lavens, P., Lebegue, E., Jaunet, H., Brunel, A., Dhert, P., Sorgeloos, P., 1999. Effect of dietary essential fatty acids and vitamins on egg quality in turbot broodstocks. Aquac. Int. 7, 225–240. https://doi.org/10.1023/A:1009225028889

Lewis, H.A., Trushenski, J.T., Lane, R.L., Kohler, C.C., 2010. Effect of dietary marine lipids on female white bass ova compositions and progeny survival. Fish Physiol. Biochem. 36, 979–992. https://doi.org/10.1007/s10695-009-9376-9

Liang, M.Q., Lu, Q.K., Qian, C., Zheng, K.K., Wang, X.X., 2014. Effects of dietary n-3 to n-6 fatty acid ratios on spawning performance and larval quality in tongue sole *Cynoglossus semilaevis*. Aquac. Nutr. 20, 79–89. https://doi.org/10.1111/anu.12054

Little, D.C., Hulata, G., 2000. Strategies for tilapia seed production. Tilapias Biol. Exploit. 267–326. https://doi.org/10.1007/978-94-011-4008-9_8

Lubzens, E., Young, G., Bobe, J., Cerdà, J., 2010. Oogenesis in teleosts: How fish eggs are formed. Gen. Comp. Endocrinol. 165, 367–389. https://doi.org/10.1016/j.ygcen.2009.05.022

Lupatsch, I., Deshev, R., Magen, I., 2010. Energy and protein demands for optimal egg production including maintenance requirements of female tilapia *Oreochromis niloticus*. Aquac. Res. 41, 763–769. https://doi.org/10.1111/j.1365-2109.2009.02229.x

Manna, P.R., Dyson, M.T., Stocco, D.M., 2009. Regulation of the steroidogenic acute regulatory protein gene expression: Present and future perspectives. Mol. Hum. Reprod. 15, 321–333. https://doi.org/10.1093/molehr/gap025

Mazorra, C., Bruce, M., Bell, J.G., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M., Bromage, N., 2003. Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 227, 21–33. https://doi.org/10.1016/S0044-8486(03)00493-9

Mercure, F., Van Der Kraak, G., 1996. Mechanisms of action of free arachidonic acid on ovarian steroid production in the goldfish. Gen. Comp. Endocrinol. 102, 130–140. https://doi.org/10.1006/gcen.1996.0054

Nagahama, Y., 1997. 17α,20β-Dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: Mechanisms of synthesis and action. Steroids 62, 190–196. https://doi.org/10.1016/S0039-128X(96)00180-8

Nagahama, Y., Yamashita, M., 2008. Regulation of Oocyte Maturation in Fish. Dev. Growth Differ. 50, 195–219. https://doi.org/10.1016/S0070-2153(08)60565-7

Nakamura, I., Kusakabe, M., Swanson, P., Young, G., 2016. Regulation of sex steroid production and mRNAs encoding gonadotropin receptors and steroidogenic proteins by gonadotropins, cyclic AMP and insulin-like growth factor-I in ovarian follicles of rainbow trout (*Oncorhynchus mykiss*) at two stages of vitellogen. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 201, 132–140. https://doi.org/10.1016/j.cbpa.2016.06.035

Ng, W., Wang, Y., 2011. Inclusion of crude palm oil in the broodstock diets of female Nile tilapia, *Oreochromis niloticus*, resulted in enhanced reproductive performance compared to brood fi sh fed diets with added fi sh oil or linseed oil. Aquaculture 314, 122–131. https://doi.org/10.1016/j.aquaculture.2011.01.034

Norambuena, F., Estévez, A., Mañanós, E., Bell, J.G., Carazo, I., Duncan, N., 2013. Effects of graded levels of arachidonic acid on the reproductive physiology of Senegalese sole (*Solea senegalensis*): Fatty acid composition, prostaglandins and steroid levels in the blood of broodstock bred in captivity. Gen. Comp. Endocrinol. 191, 92–101. https://doi.org/10.1016/j.ygcen.2013.06.006

Oliveira, M.M., Ribeiro, T., Orlando, T.M., de Oliveira, D.G.S., Drumond, M.M., de Freitas, R.T.F., Rosa, P.V., 2014. Effects crude protein levels on female Nile tilapia (*Oreochromis niloticus*) reproductive performance parameters. Anim. Reprod. Sci. 150, 62–69. https://doi.org/10.1016/j.anireprosci.2014.08.006

Patiño, R., Yoshizaki, G., Bolamba, D., Thomas, P., 2003. Role of arachidonic acid and protein kinase C during maturation-inducing hormone-dependent meiotic resumption and ovulation in ovarian follicles of atlantic Croaker. Biol. Reprod. 68, 516–523. https://doi.org/10.1095/biolreprod.102.009662

Pérez, M.J., Rodríguez, C., Cejas, J.R., Martín, M. V, Jerez, S., Lorenzo, A., 2007. Lipid and fatty acid content in wild white seabream (*Diplodus sargus*) broodstock at different stages of the reproductive cycle. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 146, 187–196. https://doi.org/https://doi.org/10.1016/j.cbpb.2006.10.097

Planas, J. V, Athos, J., Goetz, F.W., Swanson, P., 2000. Regulation of ovarian steroidogenesis in vitro by follicle-stimulating hormone and luteinizing hormone during sexual maturation in salmonid fish. Biol. Reprod. 62, 1262–1269. https://doi.org/10.1095/biolreprod62.5.1262

Richard, N., Kaushik, S., Larroquet, L., Panserat, S., Corraze, G., 2006. Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). Br. J. Nutr. 96, 299. https://doi.org/10.1079/BJN20061821

Rodríguez, C., Acosta, C., Badía, P., Cejas, J.R., Santamaría, F.J., Lorenzo, A., 2004. Assessment of lipid and essential fatty acids requirements of black seabream (*Spondyliosoma cantharus*) by comparison of lipid composition in muscle and liver of wild and captive adult fish. Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 139, 619–629. https://doi.org/10.1016/j.cbpc.2004.07.013

Sakai, N., Tanaka, M., Adachi, S., Miller, W.L., Nagahama, Y., 1992. Rainbow trout cytochrome P -450 c17 (17α-hydroxylase/17,20-lyase) cDNA cloning, enzymatic properties and temporal pattern of ovarian P -450 c17 mRNA expression during oogenesis. FEBS Lett. 301, 60–64. https://doi.org/10.1016/0014-5793(92)80210-8

Santiago, C.B., Reyes, O.S., 1993. Effects of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia *Oreochromis niloticus* (Linnaeus) broodstock. J. Appl. Ichthyol. 9, 33–40.

Sorbera, L. a, Asturiano, J.F., Carrillo, M., Zanuy, S., 2001. Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrarchus labrax*). Biol. Reprod. 64, 382–389. https://doi.org/10.1043/0006-3363(2001)064(0382:eopfaa)2.0.co;2

Stocco, D.M., Clarck, B.J., 1996. Role of the steroidogenic acute regulatory protein (StAR) in steroidogenesis. Biochem. Pharmacol. 51, 197–205. https://doi.org/10.1007/s12020-015-0715-6

Tocher, D.R., 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aquaculture 449, 94–107. https://doi.org/10.1016/j.aquaculture.2015.01.010

Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquac. Res. 41, 717–732. https://doi.org/10.1111/j.1365-2109.2008.02150.x

Tocher, D.R., Agaba, M., Hastings, N., Bell, J.G., Dick, J.R., Teale, A.J., 2002. Nutritional regulation of hepatocyte fatty acid desaturation and polyunsaturated fatty acid composition in zebrafish (*Danio rerio*) and tilapia (*Oreochromis niloticus*). Fish Physiol. Biochem. 24, 309–320.

Tsadik, G.G., Bart, A.N., 2007. Effects of feeding, stocking density and water-flow rate on fecundity, spawning frequency and egg quality of Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture 272, 380–388. https://doi.org/10.1016/j.aquaculture.2007.08.040

Wilson, R., 2009. Dietary effects of n-3 highly unsaturated fatty acid levels on egg and larval quality, and the fatty acid composition of the eggs of Chilean flounder *Paralichthys adspersus* broodstock. Aquac. Res. 40, 1400–1409. https://doi.org/10.1111/j.1365-2109.2009.02238.x

Xu, H., Cao, L., Zhang, Y., Johnson, R.B., Wei, Y., Zheng, K., Liang, M., 2017. Dietary arachidonic acid differentially regulates the gonadal steroidogenesis in the marine teleost, tongue sole (*Cynoglossus semilaevis*), depending on fish gender and maturation stage. Aquaculture 468, 378–385. https://doi.org/10.1016/j.aquaculture.2016.11.002