FONTES DE ÓLEOS DA DIETA NA COMPOSIÇÃO DO MÚSCULO, LIPOPROTEÍNAS PLASMÁTICAS, IMUNIDADE INATA E RESISTÊNCIA DE TILÁPIAS DO NILO (*Oreochromis niloticus* L. 1757)

MILENA WOLFF FERREIRA

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do do curso de Doutorado em Zootecnia, área de concentração em Produção animal, para obtenção do título de "Doutor".

Orientadora

Profª. Drª. Priscila Vieira Rosa Logato

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APROVADA em 12 de março de 2008

Prof. Dr. Henrique César Pereira Figueiredo	**DMV/UFLA**
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LAVRAS
MINAS GERAIS – BRASIL

DEDICATÓRIA

À minha família, pelo apoio incondicional, respeito, confiança e por todas as lições de vida e amor que me ajudam a crescer.

Dedico

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SUMÁRIO

Páginas

RESUMO

FERREIRA, Milena Wolff. **Fontes de óleo da dieta na composição do músculo, lipoproteínas plasmáticas, imunidade inata e resistência de tilápias do nilo (*Oreochromis niloticus* L. 1757)** 2008. 57p. Tese (Doutorado em Zootecnia) – Universidade Federal de Lavras, Lavras.

Objetivou-se com esse estudo determinar a influência de alguns ácidos graxos da dieta na composição química, perfil de ácidos graxos e teor de colesterol total do músculo; na quantidade de colesterol sérico, triglicérides e lipoproteínas plasmáticas; em algumas funções imunes inata, na hematopoiese e na resistência de tilápias do Nilo (*Oreochromis niloticus* L.) a infecção por *Streptococcus agalactiae*. Cento e sessenta machos sexados de tilápia, com peso inicial médio de 72 g, foram distribuídos em 20 tanques circulares, com capacidade total de 100 L, em uma densidade de 8 peixes por tanque. Os peixes foram aclimatados as condições de laboratório e alimentados por 160 dias. Os tratamentos foram compostos por cinco dietas semi-purificadas, isoprotéicas e isoenergéticas, contendo aproximadamente 32 % de proteína bruta e 3200 Kcal/kg de energia digestível. A composição das dietas foram iguais exceto para as fontes de lipídeos, que foram adicionados 5% de óleo de soja (OS), óleo de milho (OM), óleo de linhaça (OL), óleo de peixe (OP) e óleo de oliva (OO). Após o período de alimentação, oito peixes de cada tratamento foram anestesiados com benzocaína, e amostras de sangue foram coletadas através de punção cardíaca, para as análises de colesterol sérico, triglicérides, lipoproteínas plasmáticas, porcentagem de hematócritos, fragilidade osmótica das hemácias, proteína total do soro, concentração de ferro e capacidade ligante de ferro do soro, capacidade bactericida, atividade do complemento e atividade de lisozima. Em seguida, os peixes foram sacrificados para as análises de composição química, perfil de ácidos graxos e colesterol total do músculo. O restante dos peixes permaneceram recebendo as dietas experimentais para posterior infecção experimental. Para a infecção experimental, oito peixes por tanque, de todos os tratamentos, foram anestesiados e desafiados por injeção intraperitonial com 0,1 mL *Streptococcus agalactiae* (10^4 UFC/peixe). A mortalidade dos peixes foi verificada durante 15 dias. Quanto às análises de composição muscular, as fontes de óleos da dieta não afetaram as porcentagens de umidade, cinzas e colesterol total do músculo ($P>0,05$). Os peixes alimentados com OS, OM e OO apresentaram maior quantidade de extrato etéreo quando comparados aos alimentados com OP e OL ($P<0,05$). A maior porcentagem de proteína foi

encontrada nos peixes alimentados com OP (P<0,05). O perfil de ácidos graxos do músculo foi influenciado pelas fontes de óleos da dieta (P<0,05), os grupos alimentados com OS e OM apresentaram maior concentração de C18:2 ω6, enquanto os peixes alimentados com OL apresentaram maior concentração de C18:3 ω3, e os peixes alimentados com OP apresentaram maior concentração de C22:6 ω3. Quanto aos parâmetros sanguíneos, os peixes alimentados com OP apresentaram maior concentração de colesterol total sérico, HDL e LDL (P<0,05), e os peixes alimentados com OS, OM e OL apresentaram maior concentração de VLDL e triglicérides (P<0,05). Com relação aos parâmetros hematológicos e imunológicos, a maior porcentagem de hematócritos e de proteína total do soro foi observada no grupo alimentado com OS (P<0,05). Os grupos alimentados com OP e OL apresentaram maior resistência das hemácias. Os peixes alimentados com OS, OM e OP apresentaram maior concentração de ferro e, conseqüentemente menor capacidade de ligação de ferro. A atividade do complemento não foi influenciada pelas fontes de lipídeos da dieta (P>0,05). Apenas os peixes alimentados com OP apresentaram menor capacidade bactericida e, a menor atividade de lisozima foi observada nos peixes alimentados com OO (P<0,05). A maior sobrevivência, após a infecção experimental, foi observada nos peixes alimentados com OS, seguida pelos peixes alimentados com OL e OP, e no grupo alimentado com OO ocorreu 100 % de mortalidade. Conclui-se com esse trabalho que, as dietas contendo óleo de soja, milho e oliva aumentaram a deposição lipídica no músculo; as dietas contendo óleo de peixe e óleo de oliva melhoraram os padrões de lipoproteínas plasmáticas; a dieta contendo óleo de soja melhorou as funções imunes e a resistência de tilápias do Nilo contra infecção por *Streptococcus agalactiae*; e as dietas contendo óleo de linhaça, óleo de peixe e óleo de oliva foram associadas a imunossupressão.

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ABSTRACT

FERREIRA, Milena Wolff. **Dietary oil sources in muscle composition, plasmatic lipoprotein, innate immunity and resistance of Nile tilapia (*Oreochromis niloticus* L. 1757)** 2008. 57p. Thesis (Doutor in Animal Science) – Universidade Federal de Lavras, Lavras.

The objetive of this study to determine the influence of some dietary fatty acids sources on the chemistry composition, fatty acids profile and total cholesterol in muscle; in cholesterol quantity, triglycerides and plasmatic lipoprotein; in some innate immunity functions; in hematopoiesis; and, in resistence of the Nile tilapia (*Oreochromis niloticus* L.) to *Streptococcus agalactiae* challenge. Hundred sixty sexed male Nile tilapia, with an initial average weight of 72 g, were randomly stocked in 20 circular tanks and maintained with a density of eight fish/tank. Fish were acclimated to laboratory conditions and fed with the different experimental diets for 160 days. The the treatments were composed for five semi-purified diets, isocaloric and isonitrogenous, contain approximately 32 % of crude protein and 3200 Kcal kg^{-1} of digestible energy. The composition of diets was the same except for the oil sources, what were added 5% the of soybean oil (SO), corn oil (CO), linseed oil (LO), fish oil (FO) and olive oil (OO). At the end of the feeding period, eight fish were randomly chosen from each tank, were anesthetized with benzocaine, and blood samples were collected by cardiac punction, to the cholesterol, triglycerides, plasmatic lipoprotein, hematocrit, osmotic fragility of the red blood cells, serum total protein, serum iro concentration and total iron biding capacity, serum bactericidal capacity, spontaneous haemolytic complement and serum lysozyme activity analysis. Subsequently, the fish were slaughtered to the chemistry composition, fatty acids profiles and muscle total choresterol analysis. The fish remainder continued receive experimental diet to challenge later. At experimental challenge, eight fish for tank, of all treatments were anesthetized and challenge by intraperitoneal injection with 0.1 mL of *Streptococcus agalactiae* inoculum (10^4 CFU $fish^{-1}$). Fish mortality was recorded three times a day during 15 days. With relationship to the analyses of muscular composition no difference was found in the moisture, ash and total cholesterol muscle ($P>0.05$).The fish fed SO, CO and OO diets showed high percentage of ether extract compared to fish fed LO and FO diets ($P<0.05$). The high percentage of protein was found in fish fed FO diet ($P<0.05$). The fatty acids profiles was

influenced by dietary lipid sources (P<0.05). The group fed SO and CO diets showed high level of C18:2 ω-6, while the fish LO diet showed high level of C18:3 ω-3, and the fish FO diet showed high level of C22:6 ω-3. With relationship to the sanguine parameters The fish fed FO diet showed high total cholesterol, HDL and LDL concentrations, and the fish fed SO, CO and LO diets showed high VLDL and tryglicerides concentrations (P<0.05). Regarding to hematological and immunological parameters, the percentage higher of hematocrit and serum total protein was in group fed SO diet (P<0.05). The higher resistance of erytrocytes was found in cell of fish fed LO and FO diets (P<0.05). The fish fed SO, CO and FO diets showed higher serum iron concentration and consequently low iron binding capacity (P<0.05). The activity of spontaneous haemolytic complement no influencied by dietary lipid sources (P>0.05). Only fish fed FO diet showed lower bactericidal activity and, the lower lysozyme activity was observed in the fish fed OO diet (P<0.05). The higth survival, after the experimental challenge, was observed in the fish fed SO diet, continued to fish fed LO and FO diets, and in the grup fed OO diet occured 100 % mortality. Concluded with this work what, the diets contained soybean, corn and olive oil higher the muscle lipid deposition; the diets contained fish and olive oil improve the lipoprotein standart; a dieta conteined soyabean oil improve in the immune functions and resistence of Nile tilapia against *Streptococcus agalactiae* challenge; and teh diets contained linseed, fish and olive oil were associated to immunosupression.

Advisor: Prof[a]. Priscila Vieira Rosa Logato

INTRODUÇÃO

A tilápia do Nilo (*Oreochromis niloticus* L.), nativa de diversos países africanos e introduzida no Brasil em 1971, é uma espécie bastante cultivada em regiões tropicais, e apresenta uma participação crescente na aqüicultura global. Segundo dados da FAO (2006), a produção brasileira de tilápia foi de 69 mil toneladas em 2004, ocupando a sexta posição mundial. A expansão no cultivo desta espécie deve-se ao seu bom índice zootécnico e grande aceitação pelo mercado.

Os lipídeos são importantes componentes da dieta, como principal fonte de energia e de ácidos graxos essenciais. A composição de ácidos graxos da dieta afeta o metabolismo dos peixes, influenciando o crescimento, a composição corporal, o perfil de ácidos graxos e o transporte de lipídeos através das lipoproteínas plasmáticas.

A quantidade de energia das dietas de peixes com a elevação da porcentagem de lipídeos induz a uma melhora na conversão alimentar e um aumento no ganho de peso. Entretanto, isso determina um aumento no conteúdo de lipídeos nos tecidos e mudanças nas concentrações de lipoproteínas plasmáticas, triglicérides e colesterol sérico. Porém, foi demonstrado em algumas espécies de peixes que, as fontes de óleos utilizadas nas formulações das dietas influenciam esses parâmetros.

Atualmente o mercado consumidor procura alimentos com menor teor de gordura e melhor perfil de ácidos graxos. E isso pode ser manipulado pelas fontes de óleos utilizadas nas formulações da dieta produzindo pescados com menor deposição lipídica e perfil de ácidos graxos desejável.

Em geral, a tilápia é bastante resistente a doenças, porém, o cultivo intensivo e o crescimento acelerado podem influenciar o status imunológico e

aumentar a susceptibilidade a doenças. Em várias espécies de peixes foi evidenciado que as fontes de lipídeos da dieta podem suprimir ou estimular alguns parâmetros imunológicos.

Contudo, estudos sobre a inclusão de diferentes fontes de óleos e a deposição lipídica, transporte de lipídeos através das lipoproteínas e os relacionados ao sistema imune em tilápias ainda são bastante escassos.

Assim, objetivou-se com esse trabalho, avaliar a influencia de algumas fontes de óleo na composição química, perfil de ácidos graxos e teor de colesterol total do músculo; na quantidade de colesterol sérico, triglicérides e lipoproteínas plasmáticas, em algumas funções imunes inata, na hematopoiese e na resistência de Tilápias do Nilo (*Oreochromis niloticus* L.) a infecção por *Streptococcus agalactiae*. Os resultados são apresentados em dois artigos científicos a seguir.

Influence of dietary oil sources in muscle composition and plasma lipoprotein concentrations in Nile tilapia (*Oreochromis niloticus* L.)

(Preparado de acordo com as normas da revista "Aquaculture Nutrition")

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Abstract

This experiment was conducted to determine the effect of dietary oil sources on muscle proximate composition and fatty acid composition, and cholesterol, triglycerides and plasma lipoprotein concentrations in Nile tilapia (*Oreochromis niloticus*). Males, distributed in sixteen fish/treatment, received isocaloric (3200 Kcal kg^{-1} digestible energy) and isonitrogenous (32 % crude protein) semi-purified diet, containing different oil sources (soybean –SO, corn – CO, linseed – LO, fish –FO, olive – OO). To the end of 160 days of feeding, the blood was collected separated serum for the analyses. Subsequently, the fish were slaughtered to the muscle separation. With relationship to the analyses of muscular composition no difference was found in the moisture, ash and total cholesterol muscle ($P>0.05$).The fish fed SO, CO and OO diets showed high percentage of ether extract compared to fish fed LO and FO diets ($P<0.05$). The high percentage of protein was found in fish fed FO diet ($P<0.05$). The fatty acids profiles was influenced by dietary lipid sources ($P<0.05$). The group fed SO and CO diets showed high level of C18:2 ω-6, while the fish LO diet showed high level of C18:3 ω-3, and the fish FO

Advisor: Prof[a]. Priscila Vieira Rosa Logato

diet showed high level of C22:6 ω-3. With relationship to the sanguine

Introduction

The consuming market seeks victuals with smaller fat tenor and better profile of fatty acids. This can be manipulated by the sources of oils used in the formulations of the diet producing fish with smaller deposition lipídica and profile of desirable fatty acids.

Lipids are important components of fish diets, both as energy and as essential fatty acids sources, which fish cannot synthesize but need for basic functions, including growth and maintenance of healthy tissues

Relates of coronary atherosclerosis and vascular injury in salmonids is decribed since 1961 (Farrell, 2002). In mammals, atherosclerosis is characterized by accumulation of lipid, mostly cholesterol and its ester, extracellular matrix and inflammatory cells, resulting in dramatic reduction in lumen diameter (Kris-Etheton et al., 2002). However, this relations not was established in coronary lesions in fish (Seierstad et al., 2005). Nutritional factors, especially ω-3 polyunsaturated fatty acids (PUFA), have attracted particular interest in the research of vascular lesion development and these relationships with lipids transport (Torstensen et al., 2004).

The hypotriglyceridemic effects of ω-3 PUFA from fish oil are well established in human studies. The ω-3 PUFA from fish oil decreased serum triclyceride concentration, with accompanying increases in LDL and HDL (Kris-Etheton et al., 2002). Thus, there are researches relating the effect of dietary lipid sources and plasma lipoproteins in some fish species, such as european sea bass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) (Santulli et al., 1996; Torstensen et al., 2001; Torstensen et al., 2004; Richard et al.,

2006ab; Jordal et al., 2007). However, there is limited information with respect to tilapia species.

The objective of this study was to determine the influence of dietary oil sources on proximate composition, fatty acid profiles and total cholesterol in muscle, and the cholesterol total, triglycerides and plasma lipoproteins concentrations of Nile tilapia (*Oreochromis niloticus* L.).

Material and methods

Experimental fish

Hundred sixty sexed male Nile tilapia, with an initial average weight of 72 $\pm$3.8 g, were randomly stocked in 20, 100-L circular tanks and maintained with a density of eight fish/tank in a water recirculation system. Fish were acclimated to laboratory conditions for 10 days prior to begin experiment and fed with the different experimental diets for 160 days. Water was continuously aerated with air stones, and the photoperiod was maintained on a 12:12 h light/dark schedule. Dissolved oxygen and temperature were measured daily using an oxygen meter (YSI, USA) and pH, ammonia, nitrate and nitrite were measured once a week using commercial kits (Alcon, Brazil) following the manufacturer's

instructions. During the trial, water temperature averaged 27.11 ±1.34 °C, dissolved oxygen averaged 4.28 ±0.95 mg L^{-1}, pH averaged 7.00 ±0.2, ammonia averaged 0.41 ±0.17 ppm, nitrate 12.40 ±2.9 ppm, and nitrite 0.73 ±0.08 ppm.

Experimental diets and feeding

Five semi-purified, isocaloric, and isonitrogenous diets were formulated to contain approximately 32 % of crude protein and 3200 Kcal kg^{-1} of digestible energy, based on values reported in NRC (1993) utilizing albumin, gelatin, dextrin, vitamins and minerals premixes, and oils. The composition of diets was the same except for the oil sources. The lipid sources, soybean oil (SO), corn oil (CO), linseed oil (LO), fish oil (FO) and olive oil (OO), were added at a level of 5 %. The experimental diet formulation and proximate analysis (AOAC, 1998) are shown in Table 1. Fatty acid composition of diets was measured by methodology of Folch et al. (1957) (Table 2). All diets were stored at -4°C until use. Fish were fed at 3 % of body weight by tank, four times a day.

Analytical methods

At the end of the feeding period, eight fish were randomly chosen from each tank and anesthetized with benzocaine at 50 mg L^{-1} and blood samples were collected by cardiac punction, after a 24 hours starvation period. It sample of blood centrifuged at 500 xg for 15 min, and serum was stored frozen at -80 °C for subsequent assays. Next, the fish were slaughtered and muscle separated and stored at -4°C for analyses posteriors.

Composition analyses of diets and muscle were made following standartd methods (AOAC, 1998): dry matter after desiccation in an oven (105°C for 24 h), ash (incineration at 550°C for 8 h), crude protein (Microkjedahl nitrogen, *N* X 6.25), ether extract (Soxhlet extraction) and energy (calorimeter bomb).

The lipids were extracted following the method of Folch et al. (1957). The samples were transmethylated according to methodology of Hartman & Lago (1973), that consists of the saponification and conversion of the fatty acids in methyl esters. The methyl esters of fatty acids was submitted the chromatograph gas, using equipment Varian 3800, with flame detector, injector in the way " splintless ", capillary

column of melted silica DB-WAX (30m x 0.25mm x 0.25 μm), coupled to a software (Borwin, JMBS, Developpements). The temperature program was 75 to 235°C at 10°C min^{-1}. the carrier gas was nitrogenous at 2 mL min^{-1}, at a constant flow. Each of the fatty acids was identified relative to known external standards (Supelco, 37 FAME Mix).

The total cholesterol in muscle was determined according to Bragagnolo & Rodriguez-Amaya (1995). The absorbances of the samples were measured spectrophotometrically at 490 nm, and converted to cholesterol concentration (g kg^{-1}) using a standard curve.

The analyses of total cholesterol, HDL, LDL, VLDL and triglycerides, through specific enzymatic-photometric method using commercial kits (Gold-Analisa, Brazil) following the manufacturer's instructions.

All analyses were done in duplicate.

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) using the SAS program (Statistic Analysis Systems, SAS

Institute, Inc., Cary, NC, 2001) and the means of the treatment compared by Scott-Knott test. It was adopted a significance level of 5%.

Results

No difference was found in the moisture, ash and muscle cholesterol total ($P>0.05$).

Protein and ether extract muscle were affected ($P<0.05$) for dietary lipids sources (Table 3).

The fish fed SO, CO and OO diets showed high percentage of ether extract compared to fish fed LO and FO diets. The high percentage of protein was found in fish fed FO diet ($P<0.05$).

Muscle fatty acid composition was influenced ($P<0.05$) by fatty acid composition of diets (Table 4). Fish fed SO and CO diets showed high level of C18:2 ω-6, and consequently high level of ω-6 total. The fish LO showed high level of C18:3 ω-3, and the fish FO diet showed high level of C22:6 ω-3, both showed high levels of ω-3 total.

The means of the serum cholesterol total, HDL, LDL, VLDL, triglycerides and muscle cholesterol total is found in Table 5. The fish fed FO diet showed high total cholesterol, HDL and LDL concentrations

comparing to fish fed SO, CO, LO and OO diets ($P<0.05$). The level of triglycerides was lower in fish fed FO and OO diets.

Discussion

Generally, no expected difference in muscle proximate composition of fish fed with isocaloric and isonitrogenous diets (Menoyo et al., 2003; Regost et al., 2003; Francis et al., 2005; Huang et al., 2007; Yildirim-Aksoy et al., 2007). However, it differences were observed in the experiment. Fish fed SO, CO (rich in ω-6 fatty acids) and OO diets (rich in ω-9 fatty acids) showed high ($P<0.05$) muscle lipid deposition, when compared with fish fed LO, rich in linolenic acid (C18:3 ω-3), and FO diets, rich in eicosapentaenoic acid (EPA, C20:5 ω-3) and docosaexaenoic acid (DHA, C22:6 ω-3), both LO and FO ω-3 PUFA, and only the FO group had increase of the protein. Similar results were reported for Ribeiro (2007), this author relates the largest lipid deposition to a larger activity of lipogenic enzymes, favored by the composition of fatty acids of the present lipids diet, what can have provided a process lipogenic more accentuated. The average of moisture and ash not showed difference ($P>0.05$) between treatment, and were similar that results

reported (Furuya et al., 2000; Aiura et al., 2003; Bahurmiz et al., 2007; Ribeiro, 2007).

It has been demonstrated that tissue fatty acid patterns of fish reflect those of dietary lipids (Stickney & McGeachin, 1983; Henderson & Tocher, 1987; Moreira et al., 2001; Maina et al., 2003; Francis et al., 2006; Yildirim-Aksoy et al., 2007). In a general way, the substitution of fish oil (rich in ω-3 PUFA) for soybean and corn oil (rich in ω-6 PUFA) in the diets it results in the decrease of the fatty acids of long chain of the series ω-3, EPA and DHA, and increase in the levels of fatty acids of 18 carbons, such as, oleic, linoleic and linolenic acids, in the tissue of some species as trout rainbow, Atlantic salmon, largemouth bass (*Micropterus salmoides*), and Nile tilapia (Boggio et al., 1985; Hardy et al., 1987; Greene & Selivonchick, 1990; Subhadra et al., 2005; Yildirim-Aksoy et al, 2007). Similar observations were made in the present study, particularly with reference to the type of ω-6 and ω-3 PUFAs. The fish fed SO and CO diets showed increase in linoleic acid (C18:2 ω-6) level ($P<0.05$), while the fish fed LO diet showed increase in linolenic acid (C18:3 ω-3) level ($P<0.05$), and the fish fed Fodiet showed increase in DHA (C22:6 ω-3) level ($P<0.05$).

The transport of fatty acids and other lipid-soluble components to peripheral tissues is predominantly mediated by lipoproteins (Babin & Vernier, 1989). Both the amount and fatty acid composition of dietary lipids are reported to affect plasma lipoprotein composition and metabolism in different species (Torstensen et al., 2000; Torstensen et al., 2001; Torstensen et al., 2004; Richard et al., 2006ab; Jordal et al., 2007).

The fish fed FO diet presented greater concentration of serum total cholesterol (P<0,01) comparing to fish fed SO, CO, LO and OO diets. Similar results were found for Richard et al. (2006a) in seabass fed with anchovy oil diet. The increase in the content of cholesterol in fish fed FO diet, just the probable the presence of levels cholesterol in the diet, for being of animal origin only.

The HDL was the predominant lipoprotein class in plasma of Nile tilapia, as in all teleosts (Babin & Vernier, 1989). The level of HDL in the fish fed FO diet showed higher, when compared the fish fed SO, CO, LO and OO diets (P<0,05). This increase of HDL can be due to presence of EPA and DHA in the diet, for being the only diet that presents larger concentration.The higher level of HDL is important for lower of

cardiovascular disease, that which of cholesterol have reverse path, collectoring the tissue cholesterol and removing by liver.

In many species, plasma LDL concentration increase with the addition of the cholesterol to the diet (Torstensen et al., 2004; Richard et al., 2006ab). In the present study, the lower LDL when Nile tilapia were fed LO and OO diets could be related to the higher levels of oleic and linolenic acids, respectivement. Several studies have shown that the effect of dietary fatty acids on the amount of plasma LDL is mainly mediated by modifications of plasma LDL clearance which occur via LDL-R-mediated uptake. Thus hepatic LDL-R activity was found to be impaired by saturated fatty acids and increased by PUFA (Fernandez & West, 2005).

The fish fed FO and OO diets showed lower plasma VLDL and triglycerides, reflecting its major role in the transport of triglycerides from the liver to the tissues where they will be stored or oxidezed (Tocher, 2003). Probably for higher quantity of poliinsaturated fatty acid of long chain (EPA and DHA) that constituent the FO and for higher delivered of lipoprotein lipase by ω-3 fatty acids This result at clarence factor must rapid, lower the time that tryglicerides circulation in blood.

Although, VLDL has been lower in the fed fish feed FO diet when compared the other diets, LDL in this group was larger. In mammals, most LDL appears to be formed from VLDL, but there is evidence for some production directly by the liver (HARPER, 1998). However, it seems to be that in fish the hepatic production of LDL is larger.

Conclusion

The proximate composition and fatty acid profiles in the muscle, and cholesterol, triglycerides and plasma lipoproteins were affected by dietary lipid sources tested.

The total cholesterol muscle not was affected by dietary oil sources tested.

Fish oil dietary and olive oil dietary improved standart of the plasma lipoproteins and also lower the levels of plasma triglycerides of Nile tilapia (*Oreochromis niloticus*), been created under these conditions. The fish fed fish oil and olive oil dietary showed lower of risk factors for the cardiovascular disease development.

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Table 1. Formulation and proximate composition of the experimental diets.

Ingredients (%)	Diets				
	SO	**CO**	**LO**	**FO**	**OO**
Dextrin	49.00	49.00	49.00	49.00	49.00
Albumin	32.80	32.80	32.80	32.80	32.80
Gelatin	8.30	8.30	8.30	8.30	8.30
Cellulose	3.38	3.38	3.38	3.38	3.38
Soybean oil	5.00	-	-	-	-
Corn oil	-	5.00	-	-	-
Linseed oil	-	-	5.00	-	-
Fish oil	-	-	-	5.00	-
Olive oil	-	-	-	-	5.00
Bicalcium fosfate	1.00	1.00	1.00	1.00	1.00
Vitamin premix [(1)]	0.33	0.33	0.33	0.33	0.33
Mineral premix [(2)]	0.17	0.17	0.17	0.17	0.17
Antioxidant (BHT)	0.02	0.02	0.02	0.02	0.02
Proximate composition					
Crude protein (%)	32.16	32.85	33.05	32.18	32.20
Crude energy (kcal kg^{-1})	4059.46	4087.45	3998.78	4028.90	4046.13
Ether extract (%)	5.86	5.13	5.10	5.48	5.16
Moisture (%)	3.37	3.18	3.05	3.20	3.56

[(1)] Vitamin premix composition (quantity/kg): vitamin A 1500 U.I.; vitamin B2, 15 mg; vitamin B3, 1000 U.I.; vitamin B12, 1000 U.I.; vitamin E, 25 mg; vitamin PP, 120 mg; coline, 2000 mg; calcium pantothenate, 80 mg; folic acid, 2 mg; BHT, 170 mg.

[(2)] Mineral premix composition (quantity/kg): Mn, 80 mg; Fe, 24 mg; Zn, 50 mg; Cu, 8 mg; I, 3 mg; Se, 0.10 mg.

Table 2. Fatty acid composition of the experimental diets.

Fatty acids (%)	Diets				
	SO	**CO**	**LO**	**FO**	**OO**
C 16:0	16.07	21.06	12.73	20.38	18.65
C 16:1 ω-7	0.02	4.01	0.04	0.01	4.88
C 18:0	19.78	24.36	18.85	15.27	26.55
C 18:1 ω-9	9.90	7.20	9.29	6.06	18.35
C 18:2 ω-6	35.06	27.30	17.13	11.26	12.41
C 18:3 ω-3	6.57	0.53	31.34	8.70	0.73
C 20:1 ω-9	0.60	1.66	0.41	3.15	4.32
C 20:2 ω-6	0.15	1.51	0.06	0.80	1.29
C 20:3 ω-6	1.10	0.73	0.01	0.31	1.38
C 20:4 ω-6	6.23	0.74	0.04	0.85	3.40
C 20:5 ω-3	0.06	2.20	1.09	7.49	1.46
C 22:6 ω-3	0.19	1.86	2.92	10.98	1.59
Totals					
ω-3	6.82	5.59	35.35	27.17	3.78
ω-6	42.54	30.28	17.24	13.22	18.48
ω-9	10.50	8.86	9.70	9.21	21.67
Saturate	35.85	45.06	31.58	40.65	45.20
Monoinsaturate	10.52	12.87	9.74	9.22	24.55
Poliinsaturate	42.36	41.87	52.59	40.39	24.26
ω-3/ω-6	0.19	0.15	2.05	1.68	0.20

SO – soybean oil, CO – corn oil, LO – linseed oil, FO – fish oil, OO – olive oil.

Table 3. Muscle composition of Nile tilapia (*Oreochromis niloticus*) fed diets containing distinct lipid sources.

	Moisture (%)	Protein (%)	Ether extract (%)	Ash (%)
SO	76.39 ±095 ^{A}	53.46 ±0.48 ^{B}	14.60 ±0.48 ^{A}	4.77 ±0.40 ^{A}
CO	76.42 ±1.30 ^{A}	53.74 ±0.81 ^{B}	14.41 ±0.59 ^{A}	5.00 ±0.24 ^{A}
LO	76.77 ±1.36 ^{A}	53.67 ±0.76 ^{B}	7.37 ±0.28 ^{B}	4.80 ±

Table 4. Muscle fatty acids composition of Nile tilapia (*Oreochromis niloticus*) fed diets containing distinct lipid sources.

Fatty acids	**Diets**				
(%)	**SO**	**CO**	**LO**	**FO**	**OO**
C 16:0	21.27 ±0.82 ^{A}	21.67 ±0.77 ^{A}	23.52 ±1.41 ^{A}	22.34 ±1.32 ^{A}	22.21 ±0.72 ^{A}
C 16:1 ω-7	4.01 ±1.21 ^{A}	4.99 ±0.52 ^{A}	3.78 ±1.89 ^{A}	4.62 ±0.56 ^{A}	4.09 ±1.25 ^{A}
C 18:0	11.59 ±4.67 ^{B}	20.00 ±1.11 ^{A}	7.27 ±2.86 ^{B}	13.05 ±2.38 ^{B}	10.42 ±0.96 ^{B}
C 18:1 ω-9	15.27 ±1.68 ^{A}	11.84 ±1.43 ^{A}	14.58 ±1.91 ^{A}	12.83 ±4.58 ^{A}	15.96 ±1.90 ^{A}
C 18:2 ω-6	19.62 ±0.94 ^{A}	18.88 ±0.97 ^{A}	13.22 ±1.48 ^{B}	14.02 ±0.47 ^{B}	13.68 ±0.97 ^{B}
C 18:3 ω-3	1.19 ±0.48 ^{B}	1.22 ±0.67 ^{B}	7.73 ±0.39 ^{A}	1.43 ±0.49 ^{B}	1.18 ±0.46 ^{B}
C 20:1 ω-9	0.65 ±0.46 ^{B}	1.66 ±0.28 ^{A}	0.85 ±0.45 ^{B}	1.43 ±0.12 ^{A}	1.43 ±0.11 ^{A}
C 20:2 ω-6	1.53 ±0.07 ^{A}	1.62 ±0.08 ^{A}	1.34 ±0.27 ^{A}	1.47 ±0.08 ^{A}	1.55 ±0.27 ^{A}
C 20:3 ω-6	1.75 ±0.30 ^{A}	1.79 ±0.13 ^{A}	1.62 ±0.39 ^{A}	1.79 ±0.22 ^{A}	1.78 ±0.32 ^{A}
C 20:4 ω-6	6.55 ±1.57 ^{B}	6.22 ±1.26 ^{B}	6.32 ±3.79 ^{A}	5.80 ±0.70 ^{B}	8.12 ±2.34 ^{A}
C 20:5 ω-3	1.35 ±0.6 ^{A}	1.28 ±0.32 ^{A}	1.31 ±0.92 ^{A}	2.45 ±0.50 ^{A}	0.47 ±0.10 ^{A}
C 22:6 ω-3	5.52 ±1.46 ^{B}	4.86 ±1.46 ^{B}	5.00 ±1.11 ^{B}	9.30 ±0.69 ^{A}	5.87 ±0.99 ^{B}
Totals					
ω-3	8.05 ±2.53 ^{B}	7.36 ±2.45 ^{B}	14.04 ±2.42 ^{A}	14.18 ±1.67 ^{A}	7.52 ±1.54 ^{B}
ω-6	29.44 ±2.89 ^{A}	28.50 ±2.43 ^{A}	25.50 ±3.93 ^{B}	23.07 ±1.47 ^{B}	25.12 ±3.91 ^{B}
ω-9	15.92 ±2.14 ^{A}	13.50 ±1.71 ^{A}	15.43 ±2.36 ^{A}	14.26 ±4.70 ^{A}	17.39 ±2.01 ^{A}
Saturated	32.86 ±5.49 ^{B}	41.66 ±1.88 ^{A}	30.76 ±4.26 ^{B}	35.38 ±3.70 ^{B}	32.62 ±1.68 ^{B}
Mono	19.93 ±3.34 ^{A}	18.49 ±2.23 ^{A}	19.21 ±4.25 ^{A}	18.87 ±5.27 ^{A}	21.48 ±3.26 ^{A}
Poli	37.49 ±5.42 ^{A}	35.86 ±4.88 ^{A}	36.54 ±4.35 ^{A}	37.25 ±3.14 ^{A}	30.64 ±2.45 ^{B}
ω-3/ω-6	0.27 ±0.14 ^{B}	0.25 ±0.20 ^{B}	0.55 ±0.05 ^{A}	0.61 ±0.05 ^{A}	0.30 ±0.11 ^{B}

Means in the same line with different superscripts are significantly different at test Scott-Knott ($P<0.05$).
SO – soybean oil, CO – corn oil, LO – linseed oil, FO – fish oil, OO – olive oil.

Table 5. Means serum cholesterol (SC), HDL, LDL, VLDL, triglycerides and muscle cholesterol (MC) of Nile tilapia (*Oreochromis niloticus*) fed diets containing various sources of lipid.

	SC (mg dL^{-1})	**HDL (mg dL^{-1})**	**LDL (mg dL^{-1})**	**VLDL (mg dL^{-1})**	**triglycerides (mg dL^{-1})**	**MC (g kg^{-1})**
SO	108.30 ±11.35 ^{B}	80.194 ±8.66 ^{B}	15.94 ±0.85 ^{A}	12.16 ±4.94 ^{A}	60.84 ±4.26 ^{A}	29.57 ±1.16 ^{A}
CO	110.70 ±11.11 ^{B}	88.85 ±8.69^{B}	11.19 ±			

Influence of dietary lipid sources on the innate immunity and resistance of Nile tilapia (*Oreochromis niloticus* L.) to *Streptococcus agalactiae* challenge

(Preparado de acordo com as normas da revista "Fish Phisiology and Biochemistry")

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Abstract

One hundred sixty Nile tilapia were fed with five isocaloric and isonitrogenous semi-purified diets, but varied in the oil sources used (soybean, corn, linseed, fish and olive oils). Subsequently, the effect of dietary lipids on immune function and resistance to Streptococcus agalactiae challenge were evaluated. The hematocrit and serum protein were higher in fish fed soybean diet. The fish fed linseed and fish oil diets presented higher erythrocyte resistance. The highest serum iron concentration was in the fish fed soybean, corn and fish oil diets, while fish olive oil diet showed the highest the iron binding capacity. Only the fish fed fish oil diet showed low serum bactericidal capacity, and low lysozyme activity was observed in the fish fed olive oil diet. The highest survival, 15 days post challenge, was found in fish soybean oil diet. The results show that the lipid sources analyzed affects immunological parameters in Nile tilapia. The increased survival of fish fed soybean oil diet, rich in ω-6 fatty acids, suggests that this lipid source increases the resistance of Nile tilapia against bacterial infection.

Key Words: innate immunity, fish, lipid, diet, Nile tilapia, *Streptococcus agalactiae*

Introduction

Nile tilapia (*Oreochromis niloticus*, L.) is a major farmed fish in tropical regions, with an increasing participation in the global aquaculture market. In the environment Nile tilapia seems to be very resistant to infectious diseases, but under cultivation in high stock densities the susceptibility may be increased (Ndong et al. 2007).

The status of the innate immune system in fish is strongly influenced by environmental factors such as water temperature and quality, pollutants, hormones, and components of the diets (Lin & Shiau 2003; Dominguez et al. 2005). For various fish species there are evidences that distinct dietary lipids sources can suppress or stimulate some immunological parameters, as lysozyme activity, alternative complement pathway, serum bacterial killing activity, immunoglobulin levels, phagocytosis and resistance to pathogen challenge (Lin & Shiau 2003; Montero et al. 2003; Wu et al. 2003; Puangkaew et al. 2004; Mourente et al, 2005; Blafry et al. 2006; Lin et al. 2007; Montero et al. 2007). The effect of diets containing vegetable oils or fish oil in the fish immune system seems to be particular for each fish species, probably due to the different lipid requirements among them (Erdal et al. 1991;

Fracalossi & Lovell 1994; Kiron et al. 1995; Tort et al. 1996; Montero et al. 1998; Wu et al. 2003; Puangkaew et al. 2004; Blafry et al. 2006; Lin & Shiau 2007).

At the present, there are few available data on the effect of distinct lipid sources in the immune system of Nile tilapia. Yildirim-Askoy et al. (2007) studied the effect of many lipid sources (corn oil, menhaden fish oil, linseed oil, and beef tallow), alone or in combination, in the immunity and resistance of Nile tilapia to *Streptococcus iniae* challenge. It was found that hematological parameters were similar in fish feeding the different oils, expect to fish oil, that induced high red and white blood cell counts. Lysozyme and complement activities were significantly reduced in fish feeding beef tallow (a source of saturated fatty acids). However, the resistance to challenge with *Streptococcus iniae* was higher in fish fed with this lipid source.

Also, essential fatty acids, such as ω-3 polyunsaturated fatty acid (PUFA), are important for maintaining the structure, fluidity and function of fish cell membranes (Tort et al. 1996; Montero et al. 1998).

The objective of this study was to determine the influence of some dietary lipid sources on the innate immune function, hematopoiesis and

resistance of Nile tilapia (*Oreochromis niloticus* L.) to *Streptococcus agalactiae* challenge.

Material and methods

Experimental fish

Sexed male Nile tilapia, with an initial average weight of 72 ±3.8 g, were randomly stocked in 20, 100-L circular tanks and maintained with a density of eight fish/tank in a water recirculation system. Fish were acclimated to laboratory conditions for 10 days prior to begin experiment and fed with different experimental diets for 160 days. Water was continuously aerated with air stones, and the photoperiod was maintained on a 12:12 h light/dark schedule. Dissolved oxygen and temperature were measured daily using an oxygen meter (YSI, USA) and pH, ammonia, nitrate and nitrite were measured once a week using commercial kits (Alcon, Brazil) following the manufacturer's instructions. During the trial, water temperature averaged 27.11 ±1.34 °C, dissolved oxygen averaged 4.28 ±0.95 mg L^{-1}, pH averaged 7.00 ±0.2, ammonia averaged 0.41 ±0.17 ppm, nitrate 12.40 ±2.9 ppm, and nitrite 0.73 ±0.08 ppm.

Experimental diets and feeding

Five semi-purified, isocaloric, and isonitrogenous diets were formulated to contain approximately 32 % of crude protein and 3200 Kcal kg^{-1} of digestible energy, based on values reported in NRC (1993) utilizing albumin, gelatin, dextrin, vitamins and minerals premixes, and oils. The composition of diets was the same except for the oil sources. The lipid sources, soybean oil (SO), corn oil (CO), linseed oil (LO), fish oil (FO) and olive oil (OO), were added at a level of 5 %. The experimental diet formulation and proximate analysis (AOAC, 1998) are shown in Table 1. Fatty acid composition of diets was measured by methodology of Folch et al. (1957) (Table 2). All diets were stored at -4°C until use. Fish were fed at 3 % of body weight by tank, four times a day.

Haematological and immunological assays

At the end of the feeding period, eight fish were randomly chosen from each tank, anesthetized with benzocaine at 50 mg L^{-1}, and blood samples were collected by cardiac punction, after a 24 hours starvation period. One aliquot was collected with sodium citrate 0.1 M to the

analysis of hematocrit and osmotic fragility and other one for serum separation. Haematocrit of each fish was determined using microhaematocrit method (Mourente et al. 2005). Red blood cells were collected by centrifugation (3000 x g, 10 min.) and washed three times in phosphate buffered saline (PBS). Serum samples were obtained from whole blood collected using non-heparinised syinges and allowed to clot for 1 h at room temperature for 5 h at 4 ^{o}C, followed by centrifugation (2000 x g 5 min at 4 ^{o}C). Aliquots of serum were stored at -20 ^{o}C until use.

The osmotic fragility of the red blood cells was determined according to Kiron et al. (2004), with some modifications. A 25 µL aliquot of red blood cells was added to a series of 2,5 mL saline solutions (0.20 - 0.50 mg mL^{-1} in 5 mM phosphate buffer, pH 7.4). 0.85 and 0 mg mL^{-1} NaCl solutions were also included to provide values for inherent and maximal hemolysis, respectively. After gentle mixing and 60 min incubation at room temperature, the suspensions were centrifuged at 500 x g for 10 min and the absorbance of the supernatants was measured spectrophotometrically at 540 nm. Results are given as the salinity

causing 50 % lysis of the red blood cells based on a curve of maximal hemolysis versus the salt concentrations.

Serum total protein concentration (mg mL^{-1}) was determined using commercial kits (Amresco, USA) following the manufacturer's instructions.

Serum iron concentration and total iron binding capacity (TIBC) were determined using commercial kits (Gold-Analisa, Brazil) following the manufacturer's instructions.

Serum bactericidal capacity was determined according to Sunyer & Tort (1995), with some modifications. Briefly, *Escherichia coli* (ATCC 11229) was grown for 20 h in 20 mL of tryptic soy broth (TSB, Difco, USA) at 25 °C on an orbital incubator at 200 rev min^{-1}. Bacterial density was adjusted to an absorbance of 0.2 at 570 nm. Eight hundred μL of bacterial suspension were added to 1600 μL of serum (diluted 8x in saline solution). A negative control (blank) was made with serum dilution, with no addition of the bacterial suspension. The mixture was incubated for 1 h at 25 °C on an orbital incubator at 200 rev min^{-1}. Results were given as percentages of absorbance at time 0.

Spontaneous haemolytic complement was determined according to Sunyer & Tort (1995), with some modifications. Briefly, a volume of 25 µL of rabbit red blood cells in Alsever's solution (Bier, 1985) were added to Nile tilapia serum that had serially diluted in cold phosphate buffer saline (PBS-EGTA) solution (0.85% PBS, 0.1% gelatin, 0.01M EGTA-Mg^{2+}). Tubes were incubated at room temperature for 30 min with occasional shaking. The reaction was stopped by 1 mL of cold PBS-EDTA solution (0.85 % PBS, 0.1 % gelatin, 0.02 M EDTA). Tubes were centrifuged at 200 x g for 10 min and supernatant absorbance were measured spectrophotometrically at 414 nm and converted to percent haemolysis based on distilled water controls. The 50 % lysis was calculated by linear regression of each serum sample and expressed as the log dilution.

Serum lysozyme activity was determined according to Kiron et al. (2004) based on the lysis of the lysozyme-sensitive Gram-positive bacterium *Micrococcus lysodeikticus* (ATCC 4698, Sigma, USA). Hen egg white lysozyme (Sigma, USA) was used as an external standard. Serum (50 µL) and 750 µL of *Micrococcus lysodeikticus* suspension (75 µg mL^{-1}) were added to each tube and incubated for 20 min at 35 °C. The

initial and final absorbances of the samples were measured spectrophotometrically at 450 nm, and converted to lysozyme concentration (μg mL^{-1}) using a standard curve.

All hematological and immunological tests were done in duplicate.

Bacterial challenge

Streptococcus agalactiae (strain SA 16-06, originally isolated from Nile tilapia in Brazil) was used to challenge Nile tilapia by intraperitoneal injection.

Frozen stock-culture of *Streptococcus agalactiae* was grown in triptic soy broth for 20 h at 28 °C. The cell density was adjusted to an optical density of 0.6 at 540 nm, using a spectrophotometer, to give an estimated *Streptococcus agalactiae* inoculum of 10^5 colony-forming units (CFU) mL^{-1}.

Eight fish/tank of all treatments were anesthetized with 50 mg L^{-1} of benzocaine and challenged by intraperitoneal injection with 0.1 mL of *Streptococcus agalactiae* inoculum (10^4 CFU $fish^{-1}$). After injection, fish were returned to their respective tanks. Fish mortality was recorded three

times a day, during 15 days. All dead fish were submitted to bacteriological analysis.

Statistical analysis

Hematological and immunological parameters, and percent of survival to *Streptococcus agalactiae* infection were analyzed by one-way analysis of variance (ANOVA) using the SAS program (Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, 2001) and the means of the treatment compared by Scott-Knott test. It was adopted a significance level of 5 %.

Results

The values of hematocrit, erytrocytes osmotic fragility, serum total protein, serum iron concentration, and iron binding capacity were affected ($P<0.05$) by dietary lipid sources (Table 3). The percentage of hematocrit was higher ($P<0.05$) in fish fed SO diet. The higher resistance of erytrocytes ($P<0.05$) were found in fish fed LO and FO diets. The higher level of serum total protein was observed in fish fed SO diet and the lower in fish fed OO diet ($P<0.05$). The fish fed SO, CO and FO showed

higher ($P<0.05$) serum iron concentration and consequently low ($P<0.05$) iron binding capacity (TIBC).

The bactericidal activity (BA), lysozyme activity and survival were influenced ($P<0.05$) by lipid sources of diets, and no difference ($P>0.05$) was found in the activity of spontaneous haemolytic complement among them (Table 4). The bactericidal activity of the serum of fish fed SO, CO LO and OO diets were higher ($P<0.05$) than fish fed FO diet. Fish fed SO, CO, LO, and FO diets showed higher ($P<0.05$) lysozyme activity than fish fed OO diet.

The survival rates of Nile tilapia after the challenge with *Streptococcus agalactiae*, according to the lipid sources, are shown in the Table 4. A significant difference ($P<0.05$) was observed among treatments, with higher survival in the fish fed SO diet.

Discussion

The hematological and immunological parameters analyzed, and the resistance of Nile tilapia to *Streptococcus agalactiae* challenge were affected by different oil sources. However, no clear stimulation or suppression was observed for each lipid source tested**.**

The percentage of hematocrit was higher in fish fed SO diet (which contained high amounts of ω-6 fatty acids) than in fish that fed LO, FO and OO diets. The low amounts of ω-6 in LO, FO and OO suggests that these fatty acid was important in the erythropoiesis of Nile tilapia. Likewise, Klinger et al. (1996) reported that channel catfish (*Ictalurus punctatus* R.) fed soybean oil diet (predominantly ω-6 fatty acids) had significantly higher hematocrit in comparison with fish fed menhaden oil diet (rich ω-3 fatty acids). However, in gilthead seabream (*Sparus aurata* L.), largemouth bass (*Micropterus salmoides* L.) and Nile tilapia this relationship was not observed (Montero et al. 2003, Subhadra et al. 2006; Yildirim-Aksoy et al. 2007).

Erythrocytes from fish that fed FO and LO showed more resistance to the osmotic lysis. The higher concentration of ω-3 PUFA in fish oil and linseed oil diets seems to be necessary for strong cell membranes as indicated by the low fragility among erythrocytes in these groups. It is suggested that an increased incorporation of ω-3 PUFA in cell membranes may protect the cells from lysis (Kiron et al. 2004). Dietary fatty acids greatly influence fatty acid composition of cell membrane phospholipids, which in turn can have extensive effects on

disease resistance because many immune responses are based on leukocyte cell membrane interactions. Klinger et al. (1996) showed that erythrocytes from channel catfish fed with menhaden oil (rich in eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA) were most resistant to hemolysis, whereas the beef tallow (predominantly saturated fatty acids) diet group presented the most fragile cells. A similar effect of dietary ω-3 PUFA on erythrocyte membrane fragility to osmotic lysis was also reported in Atlantic salmon (*Salmo salar* L.) by Erdal et al. (1991).

Regarding immunological indicators, serum bactericidal capacity and lysozyme activity were influenced ($P<0.05$) by the diets. However, no difference ($P>0.05$) was found in the activity of spontaneous haemolytic complement. The decrease in bactericidal capacity of fish fed FO diet may be associated to the excessive levels of ω-3 PUFA since Nile tilapia is a warm water species and doesn't have high demand for ω-3 PUFA.

Lysozyme is known to act as a nonspecific immune mediator against bacterial infections and its concentration in fish blood can increase upon infection or invasion by foreign material (Puangkaew et al. 2004). The decrease of lysozyme activity in fish fed OO diet may be a

result of diet deficiency in both ω-3 and ω-6 fatty acids. This immune parameter was not different among fish fed the other dietary oil sources, which contained higher levels of ω-3 and ω-6. Yildirim-Aksoy et al. (2007) reported that Nile tilapia fed beef tallow diet (predominantly saturated fatty acids) had lower lysozyme activity in comparison to fish fed fish oil, corn oil and linseed oil diets. However, the direct association between dietary lipid and lysozyme production is still unknown.

The alternative complement pathway is considered as one of the main nonspecific immune responses and has been described in many fish species. Some authors have reported depletion in complement activity associated with a reduction in the ω-3 PUFA levels in the diet (Montero et al. 1998, Subhadra et al. 2006). However, in our study, spontaneous haemolytic complement (CH50) was not affected by dietary lipid sources. Similar results were reported by Montero et al. (2003) with gilthead seabream.

A higher survival post bacterial challenge was observed in fish fed SO diet. In this group the hematocrit and serum protein were significant higher and no immune suppression was observed, for any parameters

analyzed. The high resistance to *Streptococcus agalactiae* infection suggests good health status of this group of fish.

The survival of fish fed FO and LO diets were intermediate between fish fed SO and CO diets ($P<0.05$) and this could be indicative of an immunospressive effects of the excessive levels of ω-3 PUFA in FO and LO diets. Similar results have been reported for other fish species. Fracalossi and Lovell (1994) reported that menhaden oil (rich in EPA and DHA) or linseed oil (rich in linolenic acid) in diets increased the susceptibility of channel catfish to *Edwardsiella ictaluri*. Excessive levels of ω-3 PUFA have also been reported to increase the mortality of rainbow trout (*Oncorhynchus mykiss* W.) infected with *Aeromonas salmonicida* (Kiron et al. 1995). Erdal et al. (1991) reported the Atlantic salmon fed diets, higher in ω-3 PUFA, had decreased antibody titers after vaccination and reduced survival after challenge with *Vibrio salmonicida*.

Although both SO and CO diets are rich in ω-6 fatty acids, the ratio ω-3/ω-6 found in the SO (0.19) was higher than in CO (0.15) diet. The difference in ω-3/ω-6 ratios could be associated to the higher mortality observed in fish fed CO diet.

The highest mortality was observed in OO diet. It can be associated to the deficiency in eicosanoid procuction. The eicosnoids are derived from PUFAs that contains 20 carbons, mainly araquidonic acid and EPA, by the action of cyclooxygenase and lipoxigenase resulting in metabolites that include prostaglandins, leukotrienes and lipoxins that are know to influence a wide rage of immune functions (Tocher 2003). However, ω-9 fatty acids (predominant in OO diet) are not used in the eicosanoid synthesis.

The role of iron in the bacterial virulence is well established, since iron is an essential nutrient required for the bacterial growth inside the host. Welker et al. (2007) studied the effect of graded levels of bovine lactoferrin in the immune function and resistance of juvenile Nile tilapia to *Streptococcus iniae* infection. It was observed a decrease in iron concentration and an increase in TIBC in plasma of fish supplemented with lactoferrin. These authors observed that 800 mg kg^{-1} dietary lactofferrin provided 337.8 μg dL^{-1} TIBC, and this value of TIBC was sufficient to protect fish against *Streptococcus iniae* challenge. However, in our study the higher levels of TIBC (335.92 μg dL^{-1}) and low total iron observed in fish fed OO diet were not efficient to increase the survival

after *Streptococcus agalactiae* challenge. Recently, it was demonstrated that *Streptococcus agalactiae* has a siderophore-dependent acquisition system of iron (Clancy et al., 2006). It is a low-molecular mass organic chelators that liberates iron from transferrin and lactoferrin or solubilizes it from ferric oxyhydroxide, making it available for bacterial use. This mechanism might be involved in the establishment of infection in fish of the OO group, instead of the observed level of TIBC. The ability of *Streptococcus iniae* to express an iron acquisition system is unknown, and the possible benefits of high TIBC could be restricted to only some kinds of bacterial infections.

Conclusion

The immune status and disease resistance were affected by dietary lipid sources tested.

The diet contained soybean oil, predominantly ω-6 fatty acids, improved immune functions and resistance of Nile tilapia against *Streptococcus agalactiae* challenge.

Diets with linseed oil, fish oil (rich in ω-3 fatty acids) and olive oil (rich in ω-9 fatty acids) were associated to immunosupression in Nile tilapia.

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Table 1. Formulation and proximate composition of the experimental diets.

Ingredients (%)	Diets				
	SO	**CO**	**LO**	**FO**	**OO**
Dextrin	49.00	49.00	49.00	49.00	49.00
Albumin	32.80	32.80	32.80	32.80	32.80
Gelatin	8.30	8.30	8.30	8.30	8.30
Cellulose	3.38	3.38	3.38	3.38	3.38
Soybean oil	5.00	-	-	-	-
Corn oil	-	5.00	-	-	-
Linseed oil	-	-	5.00	-	-
Fish oil	-	-	-	5.00	-
Olive oil	-	-	-	-	5.00
Bicalcium fosfate	1.00	1.00	1.00	1.00	1.00
Vitamin premix [(1)]	0,33	0,33	0,33	0,33	0,33
Mineral premix [(2)]	0,17	0,17	0,17	0,17	0,17
Antioxidant (BHT)	0,02	0,02	0,02	0,02	0,02
Proximate composition					
Crude protein (%)	32.16	32.85	33.05	32.18	32.20
Crude energy (kcal/kg)	4059.46	4087.45	3998.78	4028.90	4046.13
Ether extract (%)	5.86	5.13	5.10	5.48	5.16
Moisture (%)	3.37	3.18	3.05	3.20	3.56

[(1)] Vitamin premix composition (quantity/kg): vitamin A 1500 U.I.; vitamin B2, 15 mg; vitamin B3, 1000 U.I.; vitamin B12, 1000 U.I.; vitamin E, 25 mg; vitamin PP, 120 mg; coline, 2000 mg; calcium pantothenate, 80 mg; folic acid, 2 mg; BHT, 170 mg.
[(2)] Mineral premix composition (quantity/kg): Mn, 80 mg; Fe, 24 mg; Zn, 50 mg; Cu, 8 mg; I, 3 mg; Se, 0.10 mg.

Table 2. Fatty acid composition of the experimental diets.

Fatty acids (%)	Diets				
	SO	**CO**	**LO**	**FO**	**OO**
C 16:0	16.07	21.06	12.73	20.38	18.65
C 16:1 ω-7	0.02	4.01	0.04	0.01	4.88
C 18:0	19.78	24.36	18.85	15.27	26.55
C 18:1 ω-9	9.90	7.20	9.29	6.06	18.35
C 18:2 ω-6	35.06	27.30	17.13	11.26	12.41
C 18:3 ω-3	6.57	0.53	31.34	8.70	0.73
C 20:1 ω-9	0.60	1.66	0.41	3.15	4.32
C 20:2 ω-6	0.15	1.51	0.06	0.80	1.29
C 20:3 ω-6	1.10	0.73	0.01	0.31	1.38
C 20:4 ω-6	6.23	0.74	0.04	0.85	3.40
C 20:5 ω-3	0.06	2.20	1.09	7.49	1.46
C 22:6 ω-3	0.19	1.86	2.92	10.98	1.59
Totals					
ω-3	6.82	5.59	35.35	27.17	3.78
ω-6	42.54	30.28	17.24	13.22	18.48
ω-9	10.50	8.86	9.70	9.21	21.67
Saturate	35.85	45.06	31.58	40.65	45.20
Monoinsaturate	10.52	12.87	9.74	9.22	24.55
Poliinsaturate	42.36	41.87	52.59	40.39	24.26
ω-3/ω-6	0.19	0.15	2.05	1.68	0.20

SO – soybean oil, CO – corn oil, LO – linseed oil, FO – fish oil, OO – olive oil.

Table 3 – Means hematocrit, erythrocyte osmotic fragility, serum total protein, serum iron and iron binding capacity (TIBC) in Nile tilapia (*Oreochromus niloticus* L.) fed diets containing distinct lipid sources.

	Hematocrit	Fragility (% NaCl)	Serum protein mg mL^{-1}	Iron (µg dL^{-1})	TIBC (µg dL^{-1})
SO	39.33 ±1.03^{A}	0.3913 ±0.041^{A}	20.23 ±0.80^{A}	119.47 ±11.57^{A}	210.33 ±13.44^{C}
CO	28.66 ±4.05^{B}	0.3908 ±0.035^{A}	18.97 ±0.83^{B}	120.50 ±4.98^{A}	204.89 ±10.98^{C}
LO	23.33 ±3.73^{C}	0.3522 ±0.016^{B}	18.75 ±0.30^{B}	65.55 ±19.31^{B}	250.48 ±22.50^{B}
FO	22.83 ±3.17^{C}	0.3388 ±0.030^{B}	18.83 ±0.24^{B}	128.36 ±6.30^{A}	216.58 ±10.40^{C}
OO	18.66 ±2.40^{C}	0.3852 ±0.023^{A}	17.21 ±1.07^{C}	20.73 ±2.47^{C}	335.92 ±19.70^{A}

Means in the same column with different superscripts are significantly different at test Scott-Knott ($P<0.05$).

SO – soybean oil, CO – corn oil, LO – linseed oil, FO – fish oil, OO – olive oil.

Table 4. Means serum bactericidal activity (BA), spontaneous haemolytic complement (CH50), lysozyme activity and survival after challenge in Nile tilapia (*Oreochromis niloticus* L.) fed diets containing distinct lipid sources.

	BA (%)	Lysozyme (μg mL^{-1})	CH50 (un. mL^{-1})	Survival (%)
SO	28.41 ±6.45^{A}	12.54 ±1.91^{A}	134.47 ±13.79^{A}	56.25 ±7.21^{A}
CO	24.84 ±2.83^{A}	12.25 ±2.31^{A}	136.86 ±5.97^{A}	12.50 ±10.20^{C}
LO	27.58 ±2.59^{A}	13.45 ±1.49^{A}	134.80 ±6.38^{A}	28.12 ±6.25^{B}
FO	14.98 ±3.22^{B}	12.19 ±1.25^{A}	135.92 ±7.20^{A}	21.87 ±6.25^{B}
OO	28.32 ±2.82^{A}	9.23 ±0.98^{B}	134.00 ±6.16^{A}	0.0 ±0.0^{D}

Means in the same column with different superscripts are significantly different at test Scott-Knott ($P<0.05$).

SO – soybean oil, CO – corn oil, LO – linseed oil, FO – fish oil, OO – olive oil.

CONCLUSÕES GERAIS

As diferentes fontes de óleo utilizadas nas dietas influenciaram os parâmetros avaliados.

Uma menor deposição lipídica foi observada nos peixes alimentados com dietas formuladas com óleo de linhaça e peixe, ambos ricos em ácidos graxos da série ω-3.

O perfil de ácidos graxos do músculo refletiu o perfil de ácidos graxos das dietas fornecidas.

As dietas contendo óleo de peixe e óleo de oliva melhoraram os padrões de lipoproteínas plasmáticas.

A dieta contendo óleo de soja melhorou as funções imunes e a resistência a infecção por *Streptococcus agalacteai*.

As dietas contendo óleo de linhaça e óleo de peixe aumentaram a resistência osmótica das hemácias, mas não foram associadas a melhora nos parâmetros de resposta imune inata avaliados.

As dietas contendo óleo de linhaça, óleo de peixe e óleo de oliva foram associadas à imunossupressão.

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