Original Article

HEMATOLOGICAL PARAMETERS FOR MALE NILE TILAPIA FED DIFFERENT OIL SOURCES

PARÂMETROS HEMATOLÓGICOS DE MACHOS DE TILÁPIA DO NILO ALIMENTADAS COM DIFERENTES FONTES DE ÓLEO

Rodrigo Diana NAVARRO[1]; Luis David Solis MURGAS[2]; Diego Vicente COSTA[3]; Rodrigo Fortes da SILVA[4]; Fernanda Keley Silva Pereira NAVARRO[5]

1. Laboratory of Aquaculture, University of Brasília, Campus Darcy Ribeiro, Brasília, DF, Brazil, navarrounb@gmail.com; 2. Federal University of Lavras - Department of Veterinary Medicine - Lavras - Minas Gerais – Brazil; 3. Federal University of Minas Gerais, Institute of Agricultural Sciences. University, Avenue Montes Claros, MG – Brazil; 4. Center of Agricultural Sciences, Environmental and Biological, Faculty of Fish Engineering, Federal University of Bahia, Campus Cruz das Almas, Bahia, Brazil Brazil; 5. Department of Academic Areas, Campus Aguas Lindas, Federal Institute of Education, Science and Technology of Goias - IFG, Aguas Lindas de Goias - Goias - Brazil.

ABSTRACT: Tilapia is one of the most bred species in Brazil because it is resistant to viral, bacterial and parasitic diseases when compared to other cultivated fish. Knowledge about the blood components and their function is important for normal and pathological balance. Different oil sources used to feed breeding males of Nile tilapia (*Oreochromis niloticus*) were evaluated on the hematological parameters. In the experiment, we used 80 male tilapias, 20 males for each treatment. The experiment was a completely randomized design with 4 treatments and four repetitions, which used a 32% CP diet and isoproteic isoenergetic 3,300 kcal DE kg-1 each implemented with a different oil source T1: linseed oil (OL), T2: soy oil (SO), T3: fish oil (OF) and T4: corn oil (CO).The feeding management consisted on two daily feeding at 8 am and 4 pm, in the amount of 3% body weight. The diet intake was 232.2 g/day. No significant difference was detected for the percentage of red cells, mean corpuscular volume, hematocrit, and mean leukocyte percentage. However, for the mean corpuscular hemoglobin concentration (CHCM) and hemoglobin, fish fed soybean oil presented significantly higher values than other treatments. The soybean oil, characterized by iron with a high bioavailability, led to higher levels of hemoglobin and CHCM in Nile tilapia, which possibly can reflect a greater oxygenation in fish. However, the fish oil originated greater values of both components of the blood (hematocrit, red cells) and immune (thrombocytes, monocytes, neutrophils and lymphocytes) system.

KEYWORDS: Fish nutrition. Mean corpuscular volume. Hemoglobin. Hematocrit.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is considered the most important fish of the XXI century, cultivated in over 100 countries, including Brazil, especially due its hardiness and rapid growth, with a high-quality meat (CASTRO et al., 2011).

The tilapia has good resistance against diseases, but the intensive culture and rapid growth may influence the immune status and increase the susceptibility to diseases. Several fish species have evidenced that the source of lipids in the diet may suppress or enhance some immune parameters. Nevertheless, only few studies are available for the immune system of tilapia (FERREIRA et al., 2011).

Researches have shown that differences in the formation and function of blood cells of animals may be due to the feeding management, primarily due to the inclusion of oil in the diet, and the knowledge of the hematological response to different diets is useful for new formulations (YILDIRIM-AKSOY, LIM, DAVIS; 2007; LIM, YILDIRIM-AKSOY, KLESIUS, 2011; COSTA et al., 2014).

In recent years, studies on some compounds usually used for fish feeding have indicated their benefits to immune responses. These substances, called immunostimulants, are promising for health management since they are used in chemotherapy and can be used to prevent diseases (SANTOS E OBA, 2009;HAN et al., 2012).

The essential polyunsaturated fatty acids are included in this category because they act on the immune system and on the anti-inflammatory response, competing with the mediators of the inflammation besides those related to the production performance (FALCON, BARROS, PEZZATO, 2008).

Several studies on the effect of lipids and fatty acids added to the diet on the immune response and resistance of fish have been accomplished, but with contradictory results. Mourent, Good and Bell (2005) observed a decrease in the number of circulating leukocytes in *Dicentrarchus labrax* fed with vegetable oil (linseed, olive and sunflower). Bell et al. (1996) did not observe a significant effect

Received: 04/01/17
Accepted: 05/12/17

on the hematocrit percentage. Lee et al. (1985) demonstrated the negative effect of diet with highly unsaturated fatty acids (HUFA n-3) on the immune response, caused by a reduced production of leukotriene B, considered an important mediator of the inflammatory process. Li, Lim and Klesius (2013) working with tilapia hybrid noted that different linolenic acid levels ω-3 and ω-6 linoleic acid showed no significant differences in hematocrit values. Diverse fish species have evidenced that different sources of lipids in the diet may suppress or enhance some hematological parameters. This way, this study evaluated the effects of different dietary lipid sources in the diet on the hematocrit levels, hemoglobin, mean corpuscular volume (VCM), mean corpuscular hemoglobin concentration (CHCM), and differential leukocyte count in the blood of breeding female of Nile tilapia (*Oreochromis niloticus*).

MATERIAL AND METHODS

The experiment was conducted with adult males of tilapia (*O. niloticus*) with initial weight of 50 ± 12 g in the facilities of the Fish Farming Station of the Federal University of Lavras, Lavras, Minas Gerais State, during 60 days, from January to March 2009.

In the experiment, we used 80 male tilapias, 20 males for each treatment. The experiment was a completely randomized design with 4 treatments four repeat, which used a 32% CP diet and isoproteic isoenergetic 3,300 kcal DE kg-1 implemented four oil sources T1: linseed oil (OL), T2: Soy oil (SO), T3: fish oil (OF) and T4: corn oil (CO) (NAVARRO et al., 2006;PARRA et al., 2008) (Table 1).

Table1. Compositionspercentage, calculatedfromchemicalandexperimental diets

	Diets			
Ingredients (%)	Soy Bean	Linseed	Fish	Corn
Soy bean bran (%)	60.00	60.00	60.00	60.00
Wheat bran (%)	21.16	21.16	21.16	21.16
Corn bran (%)	9.80	9.80	9.80	9.80
Soy bean oil (%)	6.75	-	-	-
Linseed bran (%)	-	6.75	-	-
Fish oil (%)	-	-	6.75	-
Corn oil (%)	-	-	-	6.75
Bicalsic Fosfate	0.10	0.10	0.10	0.10
Mineral e vitamin premix (%)	0.60	0.60	0.60	0.60
Calcareous (%)	0.62	0.62	0.62	0.62
Antioxidant (BHT)	0.02	0.02	0.02	0.02
Metionine (%)	0.55	0.55	0.55	0.55
Salt (%)	0.40	0.40	0.40	0.40
Nutritional levels				
Crude protein(%)[1]	31.98	31.98	31.98	31.98
Crude energy (kcal/kg)[1]	3299.02	3299.02	3299.02	3299.02
Ethereal Extract (%)[1]	8.01	8.01	8.01	8.01
Crude fiber (%)[1]	5.71	5.71	5.71	5.71
Calcium (%)	0.48	0.48	0.48	0.48
Phosphorus (%)	0.59	0.59	0.59	0.59

1 According to laboratory analysis at DZO ; 2 Commercial mineral and vitamin premix (5 kg/ton), with guaranteed levels to the gram scale: Vit. A, 1.200.000 UI; Vit. D3, 200.000 UI; Vit k3, 2.400 mg; Vit B3. 4.800 mg; Vit B2 , 4.800 mg, Vit B6, 4.000 mg, Vit B12, 4.800 mg,. Pholic acid, 1.200 mg; pantotenato Ca 12.000mg; Vit. C, 48.000 mg; biotine, 48 mg; coline choret , 108.000 mg; niacine, 24.000 mg; and commercial mineral premix (1 kg/ton), with guaranteed levels Fe, 50.000 mg; Cu, 3.000 mg; 20.000 mg; Mn, 20.000 mg; Zn, 3.000 mg; I, 100 mg; Co, 10 mg; Se, 100 mg.

Fish were acclimated in the net cages for five days before receiving the experimental diet, totaling 60 days in the field phase. The feeding management included two daily feeding at 8 am and 4 pm, in the amount of 3% body weight. The diet intake was 232.2 g/day.

Water temperature and dissolved oxygen was measured daily before the feeding, using a digital oximeter (YSI, USA).

At the end of the field phase, 10 tilapias from each treatment were taken for sampling blood parameters. Fish were anesthetized with benzocaine, and the blood was sampled by cardiac puncture, using syringes containing EDTA (10%). This blood was used in the preparation of blood smears stained by Rosenfeld method (1947) to evaluate the effect of dietary lipids on the leukocyte count and differentiation. At first, the blade was analyzed under optical microscope, with lower magnification and then with oil-immersion objective with the highest magnification (100x). The cell count was performed from the middle to the end of the smear edge, by counting 50 cells on the top, and 50 on the bottom of the smear. Among the leukocytes, it was distinguished the thrombocytes, lymphocytes, neutrophils, monocytes, basophils and eosinophils.

The microhematocrit technique was employed to determine the hematocrit (Mourente et al., 2005). The blood collected was placed into capillary tubes and taken to the centrifuge for 15 min at 12,000 rpm. The reading was made with a standard scale and the values expressed in %. Among the red blood cells, the hemoglobin was quantified according to Collier (1944); the mean corpuscular volume, mean corpuscular hemoglobin concentration according to Wintrobe (1934), and total count of red cells in Neubauer chamber.

Data normality was tested according to Kolmogorov-Smirnov (Massey Junior 1951). Statistical analyzes were performed with SAS software (2007), and the mean values compared by (SNK) Student-Newman-Keuls test at 5% level of significance.

RESULTS AND DISCUSSION

The mean values for temperature (27.23 ± 0.63°C), pH (7.05 ± 0.58), dissolved oxygen (5.80 ± 0.85 mg L^{-1}) and the values Secchi depth (100 ± 10 cm) remained within the optimal range for the species growth, according to (Navarro et al., 2012). At the end of the 60 experimental days, the males reached a mean final weight of 142.25 ± 15 g. The results of the hematological analysis are listed in Tables 2 and 3.

Table2. Mean levels for hematocrit and red blood cells from male Nile tilapia fed different lipid sources

Tratament	Hematocrit (%)	Eritrocits (10^6/mm^3)
Soy bean	35.12 ± 10.77	9.55 ± 4.36
Corn	41.00 ± 10.35	11.37 ± 4.11
Linseed	42.00 ± 6.00	11.16 ± 3.52
Fish	44.12 ± 9.18	11.45 ± 4.04
Variance Coeficient	27.24	8.23

*Average values ± standard deviation; Averages on the same column with different subscriptions are significantly different according to SNK test (P>0,05).

No significant difference was observed for hematocrit and erythrocyte (Table 2). No significant difference was observed for corpuscular volume (MCV). For hemoglobin percentage values in treatments with soy, corn and linseed oils were compared to the treatment with fish oil. Soy, Linseed and corn oil provided higher values for Corpuscular Hemoglobin Media. (Table 03). There was no significant difference to average percentages of leukocytes (Table 04). Araujo et al. (2011), observed a reduction in the hemoglobin rate in tilapia fed with linseed oil after cold stimulation. Another author Salvador et al. (2013) observed an increase in hematocrit and hemoglobin rates in tilapia fed with fatty acid and vaccinated supplementation.

The erythropoiesis and leucopoiesis are influenced by biological factors such as reproduction, sex, gonad maturation, age, nutritional and health status, and stress. They are also influenced by environmental factors, such as temperature, pH, dissolved oxygen and seasonality (TAVARES-DIAS; MORAES, 2004).

Table 3. Mean levels of hemoglobin, MCV and MCHC in Nile tilapia fed different lipid sources

Tratament	Hemoglobine (%)	MCV (µ3)	MCHC (%)
Soy bean	18.65 ± 4.95 a	389.26 ± 88.59	59.04 ± 25.29 a
Corn	18.00 ± 5.36 a	383.22 ± 97.70	49.63 ± 23.88 ab
Linseed	16.38 ± 3.26 a	410.29 ± 90.57	39.88 ± 10.95 b
Fish	12.53± 3.25 b	423.80 ± 100.01	30.02 ± 12.78 b
V.C.	16.67	4.79	27.99

*Average values ± standard deviation; Averages on the same column with different subscriptions are significantly different according to SNK test (P>0.05).

Table 4. Average Percentage of leukocytes of female Nile tilapia fed different sources of oil.

	Trombocits	Monocits	Neutrofiles	Linfocits
Soy bean	3.72 ± 2.44	2.20 ± 1.50	48.41 ± 15.80	45.62 ± 10. 58
Corn	3.54 ± 2.42	1.48 ± 0.89	46.92 ± 8.66	48.04 ± 9.45
Linseed	4.58 ± 1.74	1.92 ± 1.22	51.77 ± 11.67	41.72 ± 10.80
Fish	5.87 ± 3.97	2.98 ± 0.89	36.57 ± 12.74	54.57 ± 9.00
V.C.	24.10	29.40	14.27	11.35

NM – Not Meaningful (P>0.05). *Average values ± standard deviation.V.C. Variance coeficient; Different letters on the same line indicate significant difference according to the test SNK test (P<0.05).

No significant difference (P>0.05) was verified for the hematocrit percentage, as shown in Table 2. However, tilapia treated with fish oil presented higher hematocrit values, reflecting the percentage of red cells in the blood, in relation to its total composition. This oil stands out for the presence of a great omega-3 amount compared to vegetable oils (Navarro et al., 2012). Some studies have reported the influence of polyunsaturated fatty acids (PUFA) on the osmotic resistance of erythrocytes in tilapia, besides its role in the integrity of cell membranes (NG, LIM; BOEY 2001). This suggests that the higher concentration of omega-3 polyunsaturated fatty acid may have caused a greater resistance in the cell wall of erythrocytes and consequently a higher percentage of hematocrit.

Similar results were found by Klinger, Blazer and Echevarria (1996), which observed an increased percentage of hematocrit in animals fed fish oil. Nevertheless, Mourent et al. (2005) did not verify a significant difference in the hematocrit percentage when fed linseed, canola, fish or olive oil. Other study had registered a higher value of hematocrit in fish fed with soybean oil, compared to those fed linseed, fish, or olive oil (Ferreira et al., 2011). On the other hand, Bell et al. (1996) observed no significant effect of dietary lipid on the percentage of hematocrit in Atlantic salmon.

Hemoglobin, the major component of mature erythrocytes, is responsible for transporting oxygen to the several tissues (TAVARES-DIAS; MORAES, 2004). The treatment with soy oil presented the highest hemoglobin value in relation to the other vegetable oils and fish oil, but significant only for the latter. Among the vegetable products, the soybean oil has a high amount of iron (9 - 13 mg/100g), with a good bioavailability. This term related to iron reflects the fraction of dietary iron that can be absorbed by the gastrointestinal tract, and subsequently stored and incorporated to the heme group of hemoglobin (Bianchi, Silva and OLIVEIRA,1992). Thus, vegetable oils, especially the soy oil may allow a greater oxygen transport, important molecule for cellular respiration, to fish body tissues.

Moreover, this formation of heme group of hemoglobin by the high bioavailability of iron in the soybean oil may also justify the higher values of mean corpuscular hemoglobin concentration (CHCM), presented in tilapia exposed to this source of oil.

Given the above, although the fish oil had had the higher values of hematocrit, erythrocyte, and VCM, possibly due to the role of PUFA to ensure the integrity of cell membranes, this cannot reflect a greater oxygen transport. This greater oxygenation may probably be ensured by the soy oil, considering its high iron concentration with high bioavailability, leading to the synthesis of heme groups, which bind to oxygen molecules.

The higher hemoglobin levels in the treatment with soy oil are likely associated with its high iron content, essential for the production of this oxygen-carrying protein. Besides that, this relationship can also justify the higher values of mean corpuscular hemoglobin concentration (CHCM), defined as the concentration of hemoglobin in a red blood cell, in tilapia exposed to the diet with soybean oil.

In the present experiment, tilapia fed a diet with fish oil, which contains a higher concentration of omega-3, presented a greater amount, but not significant, of all classes of leukocytes, thrombocytes, monocytes and lymphocytes. The omega-3 fatty acids are limitedly synthetized by fish, and are important to maintain the integrity of cell membranes and to produce eicosanoids (NAVARRO et al., 2012).In the majority of osseous fish the blood composition varies according to feeding, gender, stage of gonadal development, stress and environmental pollution. (TAVARES DIAS; MORAES, 2004; SALVADOR et al., 2013). Subhadra, Lochmann and Rawles (2006) observed that *Micropterus salmoides* fed with diets with at least 7% n-3 fatty acids, presented a greater amount of lymphocytes than those fed diets with 5% or less of these acids. Mourente et al. (2005) replaced 60%

of fish oil by canola, linseed and/or olive oil in the diets to European sea bass (*Dicentrarchus labrax*), and verified a reduction in circulating leukocytes and respiratory activity. These results suggest that these polyunsaturated fatty acids play a key role in the maintenance of fish immune balance.

CONCLUSION

Different oil sources produce effects with varied potentials depending on the organic system. The soy oil, characterized by iron with a high bioavailability, led to higher levels of hemoglobin and CHCM in Nile tilapia, which possibly can reflect a greater oxygenation in fish. However, the fish oil created greater values of both blood (hematocrit, red cells) and immune (thrombocytes, monocytes, neutrophils and lymphocytes) system components. Thus, fish oil is recommended for tilapia breeding. This information can guide new experiments, improve fish health conditions in a culture system.

RESUMO: A tilápia é uma das espécies mais criadas no Brasil pois apresenta resistência a doenças virais, bacterianas e parasitárias, quando comparada a outros peixes cultivados. O conhecimento sobre os componentes do sangue e de suas funções é importante para o as condições de equilíbrio normais e patológicas. Foram avaliadas diferentes fontes de óleo na alimentação de machos reprodutoras de tilápia do Nilo (*Oreochromis niloticus*) sobre os parâmetros hematológicos. Para isso, foram utilizadas 80 machos reprodutores de tilápia do Nilo. Cada alimento foi suplementado com 6% de diferentes fontes lipídicas, sendo: óleo de soja; óleo de milho; óleo de linhaça; óleo de peixe. O manejo alimentar incluiu dois alimentação diária às 8 horas e 4 horas, no valor de 3% do peso corporal. A ingestão de dieta era 232,2 g / dia. Nenhuma diferença significativa foi detectada pela percentagem de glóbulos vermelhos, volume corpuscular médio, o hematócrito, eo percentual de leucócitos dizer. No entanto, para a concentração média corpuscular de hemoglobina (CHCM) e hemoglobina, peixes alimentados com óleo de soja apresentaram valores significativamente mais elevados do que outros tratamentos. O óleo de soja, caracterizada por ferro com uma elevada biodisponibilidade, levou a níveis mais elevados de hemoglobina e CHCM em tilápia do Nilo, que, possivelmente, podem refletir uma maior oxigenação nos peixes. No entanto, o óleo de peixe originado pelos maiores valores de ambos os componentes do sangue (o hematócrito, glóbulos vermelhos) e imunitários (trombócitos, monócitos, neutrófilos e linfócitos) do sistema.

PALAVRAS-CHAVE: Nutrição de peixes. Volume Corpuscular Médio. Hemoglobina. Hematócrito

REFERENCES

ARAUJO D. M.; PEZZATO, A. C.; BARROS, M. M.; PEZZATO, L. E.; NAKAGOME, F. K. Hematologia de tilapias do nilo alimentadas com dietas com óleos vegetais e estimuladas pelo frio. Pesquisa Agropecuária Brasileira., v. 46, n. 3, p. 294-302, 2011. https://doi.org/10.1590/S0100-204X2011000300010

BELL, J. G.; ASHTON, I.; SECOMBES, C. J.; WEITZEL, B. R.; DICK, J. R.;SARGENT, J. R. Dietary lipids affets phospholipid fatty acid compositions, eicosanoid production and immune function in Atlantic salmon (*Salmo salar*). **Prostaglandins, Leukotrienes Essential Fatty Acids**, Toronto, v. 54, n. 1, p. 173-182.Marc. 1996. http://dx.doi.org/10.1016/S0952-3278(96)90013-7

BIANCHI, M. L. P.; SILVA, H. C.; OLIVEIRA, J. E. D. Considerações sobre a biodisponibilidade do ferro dos alimentos. **Archivos Latino-americanos de Nutricion**. Chacao**,** v. 42, n. 2, p. 94-100. 1992.

CASTRO T. S. S.; SANTOS, L. D.; FURUYA,W. M.; SILVA, L. C. R.; MATSUSHITA, M. Dietary conjugated linoleic acid (CLA) for finishing Nile tilapia. **Aquaculture Nutrition**, Bergen v. 17, n. 2, p. 70-81. 2011 http://2011. 10.1111/j.1365-2095.2009.00735.x

COLLIER, H. B. The standardization of blood haemoglobin determinations. **Canadian Medical Association Journal,** Ottawa v. 50, n. 1,p. 550-552. Jun.1944.

COSTA, D. V.; FERREIRA, M. W.; NAVARRO, R. D**.** ; ROSA, P. V. E.; MURGAS, L. D. S. Parâmetros hematológicos de tilápias do Nilo (*Oreochromis niloticus*) alimentadas com diferentes fontes de óleo. **Revista Brasileira de Saúde e Produção Animal**, Salvador, v. 15, p. 757-767. Jul/Set. 2014.

HAN, Y. Z.; REN, T. J.; JIANG, Z. Q.;JIANG, B. Q.;GAO, J. KOSHIO, S.;KOMILUS, C. F. Effects of palm oil blended with oxidized fish oil on growth performances, hematology, and several immune parameters in juvenile Japanesesea bass, *Lateolabrax japonicas*. **Fish Physiology and Biochemistry**, Amsterdam v. 38, p. 1785-1794.Jul. 2012. http://10.1007/s.10695.012.9615.4

FALCON, D. R.; BARROS, M. M.; PEZZATO, L. E.; SOLARTE, W. V. N.; GUIMARÃES I.G. Leucograma da tilápia-do-Nilo arraçoada com dietas suplementadas com níveis de vitamina C e lipídeo submetidas a estresse por baixa temperatura. **Ciência Animal Brasileira**, Goiânia v. 9, n. 3, p. 543-551, Jul/Set. 2008.

FERREIRA, M. W.; ARAÚJO, F. G.; COSTA, D. V.; LOGATO, P. R. ; FIGUEIREDO, H. C. P. ; MURGAS, L. D. S. Influence of Dietary Oil Sources on Muscle Composition and Plasma Lipoprotein Concentrations in Nile Tilapia, *Oreochromis niloticus*. **Journal of the World Aquaculture Society**, Hoboken v. 42, p. 24-33. Feb. 2011. http://10.1111/j.1749-7345.2010.00440.x.

KLINGER, R. C.; BLAZER, V. S.; ECHEVARRIA, C. Effects of dietary lipid on the haematology of channel catfish, *Ictalurus punctatus*. **Aquaculture,** Amsterdam, v. 147, n. 1, p. 225-233.Dec. 1996. http://10.1016/S0044-8486(96)01410-X.

LI, E.; LIM, C.; KLESIUS, P. H.; WELKER, T. L. Growth, Body Fatty Acid Composition, Immune Response,and Resistance to Streptococcus iniae of Hybrid Tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, Fed Diets Containing Various Levels of Linoleic and Linolenic Acids. **Journal of the World Aquaculture Society**, Hoboken v. 44, n. 1, p. 42-55. Feb.2013. http://10.1111/jwas.12014.

LIM, C.; YILDIRIM-AKSOY, M.; KLESIUS, P. Lipid and fatty acid requirements of tilapia. **North American Journal of Aquaculture**, Carbondale, v. 73, p. 188–193, May. 2011. https://doi.org/10.1080/15222055.2011.579032

LEE, T. H.; HOOVER, R. L.;WILLIAMS, J. D.; SPERLING, R. I.; RAVALESE, J.; SPUR, B. W.; ROBINSON, D. R.;COREY, E. J.; LEWIS, R. A.; AUSTEN, K. F. Effects of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyteleukotriene generation and neutrophil function. **New England Journal of Medicine**, Waltham v. 312, p. 1217-1224.May. 1985.

MASSEY JUNIOR, F. J. The Kolmogorov-Smirnov test for goodness of fit. J. Am. Stat. Assoc. 46:68-78. 1951. http://dx.doi.org/10.1080/01621459.1951.10500769 https://doi.org/10.1080/01621459.1951.10500769

MOURENT, G.; GOOD, J. E.; BELL, J. G. Partial substitution of fish oil with rapessed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax*): effets immune function and effectiveness of a fish oil finishing diet. **Aquaculture Nutrition**, Bergen v. 11, n. 1, p. 25-40. 2005. http://10.1111/j.1365-2095.2004.00320.x.

NAVARRO, R. D.; MATTA, S. L. P.; LANNA, E. A. T.; DONZELE, J. L.; RODRIGUES, S. S.; SILVA, R. F.;CALADO, L. L.; RIBEIRO FILHO, O. P.Níveis de energia digestível na dieta de piauçu no desenvolvimento testicular em estágio pós-larval. **Zootecnia Tropical**, Caracas v. 24, n. 2, p. 53-163. Jun. 2006.

NAVARRO, R. D.; NAVARRO, F. K. S. P.; RIBEIRO FILHO, O.P.;FERREIRA, W. M.; PEREIRA, M. M.; SEIXAS FILHO, J. T. Quality of polyunsaturated fatty acids in Nile tilapias (*Oreochromis niloticus*) fed with vitamin E supplementation. **Food Chemistry**, Brussels v. 134, n. 6, p. 215-218.Sept. 2012. http://10.1016/j.foodchem.2012.02.097

PARRA, J. E. G.; RADÜNZ, N. J.; VEIVERBERG, C. A.; LAZZARI, R.; BERGAMIN, G. T.; PEDRON, F. A.; ROSSATO, S., SUTILI, F. J. Alimentação de fêmeas de jundiá com fontes lipídicas e sua relação com o desenvolvimento embrionário e larval. **Ciência Rural**, Santa Maria v. 38, n. 7, p. 2011-2017. Out. 2008.

ROSENFELD, G. Corante pancrômico para hematologia e citologia clinica. Nova combinnação dos componentes do May - Grunwald e do Giensa num só corante de emprego rápido. **Memórias do Instituto Butantan**, Rio de Janeiro v. 20, n. 1, p. 329-334.1947.

SANTOS, L. R. B.; OBA, E. T. Dieta: ferramenta importante para o manejo dos peixes no cultivo. In: TAVARES DIAS, M. Manejo e sanidade de peixe em cultivo**.** Embrapa Amapá, Macapá, 2009. pg 89 a 105.

ROGÉRIO, S.; CLAUDIANO, G. S.; LOUREIRO, B. A.; MARCUSSO, P. F.; ETO, S. F., PILARSKI, F.; TOAZZA, C. S.; MORAES, J. R. E.; MORAES, F. R. Desempenho e hematologia de tilapias do nilo alimentadas com *Saccharomyces cerevisiae* e vacinadas contra *Streptococcus agalactiae.* **Pesquisa Agropecuária brasileira**, v. 48, n. 8, p. 892-898, 2013. https://doi.org/10.1590/S0100-204X2013000800012

SAS. Statistical Analysis System Institute - SAS/STAT Procedure guide for personal computers. SAS Inst. 1999. p. 5: 334.

SUBHADRA, B.; LOCHMANN, R.; RAWLES, S.; CHEN, R. Effect of dietary lipid source on the growth tissue composition and hematological parameters of largemouth bass (*Micropterus salmoides*). **Aquaculture,Amsterdam** v. 255, n. 1-4, p. 210-222. May. 2006. http://10.1016/j.aquaculture.2005.11.043.

TAVARES-DIAS, M. E.; MORAES, F. R. Hematologia de peixes teleósteos**.** Ribeirão Preto: Villimpress Complexo Gráfico, 2004. 144p.

YILDIRIM-AKSOY, M.; LIM, C. D.; DAVIS, A.; KLESIUS, R. S. P. H. Influence of Dietary Lipid Sources on the Growth Performance, Immune Response and Resistance of Nile Tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* Challenge. **Journal of Applied Aquaculture**, Brunswick, v. 19, n. 2, p. 31-42. 2007. http://10.1300/J028v19n02_02.

WINTROBE, M. M. Variations on the size and haemoglobin content of erythrocytes in the blood of various vertebrates. **Folia Haematologica,** Leipzig v. 51, n. 1, p. 32-49. 1934.