Egypt. J. Aquat. Biol. & Fish., Vol. 4, No.2 : 1-25 (2000) *ISSN1110-6131*

EFFECT OF DIETARY LIPID LEVELS AND SOURCES ON IMMUNE RESPONSE OF NILE TILAPIA *OREOCHROMIS NILOTICUS* BROODSTOCK IN WINTER SEASON

Talaat M. Shehata and Ashraf M. Goda
National Institute of Oceanography and Fisheries

(Received : January 15, 2000)
Key words : Lipid level, Lipid source, Immune response, Tilapia *O. niloticus*, broodstock.

ABSTRACT

The present study was carried out for 120 days at Fish Research Station, belonging to the National institute of Oceanography and Fisheries at El-Kanater El Khairia. The aim of work was to detect the effect of lipid sources (Poultry oil, Corn oil and Linseed oil) and lipid levels (3 and 6 %) on immune response of Nile tilapia broodfish during the winter season.

Total serum protein (TSP), total serum albumin (TSA) and Total serum globulin (TSG) parameters were used as immunoglobulin indicators. The (TSP), (TSA) and (TSG) were not significantly ($P > 0.05$) affected by lipid source or lipid level at water temperature over 16.85 °C. But significant differences were found at water temperature below 11.66 °C. These data showed poultry oil with 3% lipid seemed to be adequate for optimal functioning of immunoglobulin.

The humoral response as measured by hydrogen peroxidase titer and hemolysin titer were not significantly ($P > 0.05$) affected by both lipid source or lipid level at water temperature over 16.85 °C while significant differences ($P \leq 0.05$) were found at water temperature below 11.66 °C. The data revealed that both corn oil and linseed oil with 6 % lipid seemed to be adequate for optimal functioning of humoral immune response.

INTRODUCTION

Fish, like other vertebrates, respond to infectious agents in both specific and non-specific manners. Also, fish appear to have most of the same cellular and humorol immune components as warm-blooded vertebrates. Several studies on fish have shown a correlation between immune response and disease resistance and fish nutrition (Ghittino, 1989; Landolt, 1989, Fracalossi and Lovell, 1994). Several studies have concentrated on vitamins and minerals though, lipid plays a role in fish immunity (Fracalossi and Lovell, 1994). In homeotherm nutrition research, dietary lipids have been studied as modulators of immunity and nonspecific disease resistance. The fatty acid composition of the cell membrane is especially important in disease resistance because many of the mechanisms and reactions are membrane-associated. Johnston (1988) reported that, the fluidity of the membrane depends on the unsaturation of the phospholipid fatty acids. Lipids supply essential fatty acids for production of eicosanoids, which modulate various steps of humoral and sell-mediated immunity (Kinsella and Lokesh, 1990). In mammals, fatty acid composition of cell membrane is primarily altered by diet. Fish, however, must also be able to alter the composition in response to temperature changes (Abruzzini *et al*, 1982). Low temperatures are immunsuppressive for fish (Ellis, 1982). Bly and Clem (1991) reported supperssion in fish T- and B-cell function after water temperature was lowered from 23 °C to 11°C. Bly and Clem (1991) and Bly *et al* (1990) suggested that the positive effect of n-3, n-6 on T-cell responses was due to an increase in cell membrane fluidity. So, both diet and temperature acclimation are very important in piscine cell-membrane composition.

Thus the present study was undertaken to detect the effect of lipid source (Poultry oil, Corn oil and Linseed oil) and lipid level (3 and 6%) in diet of tilapia (*O. niloticus*) brood fish on their immune response during winter season.

MATERIALS AND METHODS

1-Experimental Fish :

Brood fish of the Nile tilapia (*O. niloticus*) were collected from the common population of this species in fish research station belonging to the National Institute of Oceanography and fisheries at El Kanater El Khairia. The experimental fish were healthy, free of any parasites and had an average weight of 53.8 ± 1.9 g at collection time.

The brood fish were collected and transferred with special care to be weighed, then randomly distributed into the experimental groups in concrete ponds of (15 fish each). The actual experimental period lasted from December to the end of Febuary.

2-Experimental Technique:

Six concrete ponds (40 m^3 each) were used to represent six nutritional treatments. The treatments were divided into six groups received three diets that differed in oil source (poultry oil, corn oil and linseed oil) and two lipid levels (3 and 6 %) in factorial manner. The water temperature was measured every day and its average levels were illustrated in Table (1).

3- Feeds and Feeding Practice:

Six diets were formulated and tabulated in Table (2). Fatty acid composition of poultry oil, corn oil and linseed oil is shown in Table (3). Also fatty acid composition of experimental diets is shown in Table (4). The diets were isocaloric of 3000 Kcal metbolizable energy / Kg. according to Hepher *et al.* (1983) and isonitrogenous of 30 % protein according to Siddique *et al.* (1988). Fish were fed at a level of 1 % whole body weight per day. The weight of samples of brood fish were made every two weeks due to determine the performance parameter and amount of feed needed.

The diet mixture was processed into California Pellet Meal (CPM) machine. Pellets were processed through a mincer of 2 mm diam.

4- Blood Immune Assay:

a- Non-Specific Immune Factors:

The experimental fish were injected with 0.2 ml. of the suspension of sheep red blood cells (SRBC 10%) intramuscularly in the dorsal muscle. The (SRBC) was obtained from the central biotechnology laboratory for poultry, Faculty of Agriculture, Cairo University.

After 21 day from injection with SRBC, five fish were bled from the caudal vein, while the blood was allowed to clot at room temperature for 1 hr. Blood serum was obtained by centrifugation of the blood sample at 3000 rpm for 3 minutes and then was stored in deep freezer at –20 °C for further analysis of concentration of the total serum protein (TSP), total serum albumin (TSA) and total serum globulin (TSG) according to biured method (Reinnold, 1953).

b- Fish Red Blood Cell Peroxidase Assay:

A 500 ml sample of whole blood was transferred from the collection tube (5 fish) to polysteren conical centrifuge tubes. Phosphate bufferd saline (PBS, pH 7,2) was added (5 ml) to each tube. Samples were then centrifuged for a minute at 844 x gravity (xg) and the supernatant was removed and discarded. The process was repeated twice and the cells were resuspended in enough PBS to make a 2 % red blood cell suspension. The resuspended samples were immediately tested for resistance to oxidative hemolysis according to a modified method of Gyorgy *et al* (1952) and Wise *et al* (1993 a, b). The dynated micro titer system was used to make a two fold serial dilution of 0.296 % hydrogen peroxide (50 ml) in PBS in 96-well conical micro titer plates. The red blood cell suspension (50 ml) was then added to each well. A control well containing 25 ml deionized water, 25 ml PBS and 50 ml red blood cell

suspension was prepared for each sample. Plates were placed in a humidor and incubated overnight at room temperature. The oxidative titer was determined as the highest dilution (as log 2x) of hydrogen peroxide that caused pellet formation due to lysis of sheep red blood cell membrane.

c- Hemolysin Assay :

Hemolysin assay were determined according to Sakai (1981) and Blazer and Wolker (1984 a, b). Two-fold serial dilutions (double dilutions) were prepared in plastic plates (400 ml in each small cup) according to Herbert (1977). The sheep blood cell suspension (80 ml) was added to each plastic cup. Plates were incubated at room temperature for 30 minutes. The Hemolysin titer was determined as the last dilution (as log 2x +1) showing complete lysis according to Blazer and Wolker (1984 a).

5- Statistical Analysis:

Statistical analysis was made by factorial design (3 x 2) according to the procedure reported by Steel and Torrie (1980). Duncan's test was applied between treatments and control group when ever possible to test the mean differences (Duncan, 1955).

RESULTS AND DISCUSSION

Recently, the relationship between nutrition and immunology disease resistance in homeotherms has received considerable attention (Sheldon and Blazer, 1991; Bell *et al.*, 1991; Erdal *et al.*, 1991; Meydani *et al.*, 1991; Chang *et al.*, 1992; Fracalossi, 1993 and Fracalossi and lovell, 1994). It has been suggested that the minimal daily requirements which have been set for brood fish may not be adequate for optimal functioning of the immune system. The effect of lipid source and level on immune response have been described for tilapia brood fish in summer

season (Blazer and Wolker, 1984 a; Bell *et al.* 1990; Sheldon and Blazer, 1991). Although recommended supplement levels have been set, it is recognized that there can be no one requirement under winter season conditions. It is the hypothesis of this study that the brood fish may be marginally deficient in immune response without showing overt signs of such a deficiency. The brood fish appear healthy and continue to live in winter season, but their natural resistance and immune systems may be compromised. Bly and Clem(1991) reported suppression in fish T- and B-cell functions after water temperature was lowered from 23 °C to 11 °C. It is well established that major disease out-breaks most often occur after times of stress (handlying, transport, spawning) and also in the winter season when the water temperature begins to decline. If immunopotentiation could be initiated by feeding supplemental suitable kinds of oil in their diet, it would be efficacious during the winter season.

The total serum protein (TSP), total serum albumin (TSA) and total serum globulin (TSG) were not significantly ($p \geq 0.05$) affected by oil source, or oil level at water temperature over 16.85 °C (Tables 5, 8 and 11). But, significant results ($p < 0.05$) were found at water temperature below 11.66 °C.

Concerning oil source, the data in Tables (6, 9 and 12) showed that there were no significant differences ($p \geq 0.05$) in (TSP) (TSA) and (TSG) at water temperature over 16.85 °C. While, there were significant differences ($p \leq 0.05$) at water temperature below 11.66 °C. The highest values occurred when fish were fed diets supplemented with poultry oil or corn oil.

With respect of oil level, the data in Tables (7, 10 and 13), showed no significant differences ($p \geq 0.05$) found in (TSP), (TSA) and (TSG) at water temperature over 16.85 °C. But, significant differences ($p \leq 0.05$) were found at water temperature below 11.66 °C. The highest values were recorded when fish fed diet was supplemented with 3% lipid. The present

data indicated that both poultry oil or corn oil with 3% lipid level seemed to be adequate for optimal functioning of immunoglobulin.

It is worthy to note that the values of (TSP), (TSA) and (TSG) at water temperature over 16.85 °C in the present study were higher than the recommended values at water temperature over 16 °C reported by Blazer and Wolker (1984a) where these values reflect the higher level of immunoglobulin. Helmy *et al.* (1974) reported that the increase in serum protein would result only when anabolic processes exceeded catabolic ones, and reserve proteins were produced in greater quantity to meet increased metabolic requirements of the fish. They added that, an increased catabolic rate would explain the decreases in serum protein level. Miller *et al.* (1949) studied physiological properties of blood protein and found that the half-life time of globulin molecule is about 3 days which is less than half that of albumin. This fact may explain the rapid metabolic rate in globulin, which is more sensitive, reflecting quickly the changes in the catabolic – anabolic relationship. Thus, the humoral response as measured by Hydrogen peroxidase titer and with Hemolysin titer are specific immune response.

The humoral response as measured by Hydrogen peroxidase titer (Tables 14, 15 and 16) and Hemolysin titer (Tables 17, 18 and 19) was not significantly affected ($p \geq 0.05$) by both oil source and level at water temperature over 16.85 °C. But significant differences ($p \leq 0.05$) were found between all treatments and the control group at water temperature below 11.66 °C. The highest values were reported when fish fed diet was supplemented with corn oil or linseed oil at 6 % oil.

The present data showed that both corn oil and linseed oil with 6 % fat level seemed to be adequate for optimal functioning of humoral respose as measured by Hydrogen peroxidase titer and Hemolysin titer. Therefore, the diet contained either n-3 or n-6 fatty acids may have been responsible for the greater resistance of brood fish to infection. The

reduced resistance to infection in tilapia brood fish fed diets contained poultry, corn oil or linseed oil (n-3, n-6) at high temperature may be similar to the response of warm blooded animals to dietary oils, which is proposed to be caused in part, by the competitive inhibition of a metabolism by n-3 or n-6 fatty acids. This competitive inhibition among fatty acids of the n-6 , n-3 families for desaturases is responsible for the synthesis of polyunsaturated fatty acids (Hwang, 1989). For instance, through the lipoxygenase pathway, AA will be converted to leukotriene (B4) (Meydani, 1990). Leukotriene (B4) is an eicosanoid that has immuno stimulatory effects, promoting the proliferation of lymphocytes, stimulating the production of inter leukins 1 and 2, and acting as leukocyte chemotactic and chemokinetic agent (Goldman *et al.* 1983; Kragballe *et al.* 1987). Leitch *et al.* (1984) and Lee *et al.* (1985) demonstrated that the negative effect of dietary n-3 HUFA on the immune response was caused by a decrease in leukotriene (B4) production, and an increase in leukotriene (B5) by macrophages and neutrophils. Leukotriene (B4) is produced mainly from 20: 4n 6, whereas leukotriene (B5) is another eicosanoid that has immunosuppessive effect (Goldman *et al*, 1983) and is produced primarily from 20: 5n-3. Fracalossi and lovell (1994) demonstrated production of leukotrienes in the anterior kidney of fish fed diet containing different lipid sources, but leukotrienes (B4) and (B5) were not separately determined.

In conclusion, it is apparent that diet contained n-3 and / or n-6 had significant effect on the immune response in tilapia brood fish at water temperature below 16.85 °C.

The average growth performance in terms of final body weight and specific growth rate (SGR) was important and reliable indicator of ideal oil source and level for tilapia brood fish during winter season. The data (Tables 20 and 21) revealed that the linseed oil with 6 % level is the best treatment. The corresponding values of fish in control group were

lower than the initial weight. The decrease of body weight (SGR) and gain values can be attributed to lack of supplementary feeding which negatively affected the anabolic/catabolic relationship. These findings accord well with those reported by Helmy *et al.* (1974) who noticed that the anabolic / catabolic relationship could be greatly affected by the dietary status of the reared fish.

REFERENCES

Abruzzini, A.F; Ingram, L.O. and Clem, L.W. (1982). Temperature-mediated process in teleost immunity; homeoviscous adaptation in teleost lymphocytes. Proc. Soc. Exp. Biol. & Med. *169*: 12-18.

Bell, J.G.; McVicar; A.H.; Park, M.T. and Sargent, J.R. (1991). High dietary linoleic acid affects the fatty acid composition of individual phospholipids from tissues of Atlantic salmon *Salmo salar*: association with stress susceptibility and cardiac lesion. J. Nutr., *121* : 1163 – 1172 .

Blazer, V.S. and Wolke, R.E. (1984a). The effects of alpha – tocopherol on the immune response and non-specific resistance factors of rainbow trout *Salmo gairdneri Richadson*. Aquacult. *37*: 1 – 9.

Blazer, V.S. and Wolke, R.E. (1984b). Effect of diet on the immune response of rainbow trout. Can.J. Fish. & Aquat. Sci., *41*: 1244 – 1247.

Bly, J.E.; Buttke, T.M. and Clem, L.W. (1990). Differential effects of temperature and exogenous fatty acids on mitogen – induced proliferation in channel catfish T and B Lymphocytes Comp. Biochem. Physiol., *95* A: 417-424.

Bly, J.E and Clem, L.W. (1991). Temperature mediated processes in telost immunity: in vitro immunosuppression induced by in vivo low temperature in channel catfish. Vet .Immunol. Immunopathol., *28* : 365.

Chang, H.R.; Dulloo, I.R.; Vladoianu, I.R.; Piguet, P.F.; Arsenijevic, D.; Girardier, L. and Pechere, J.C. (1992). Fish oil decreases natural resistance of mice to infection with *Salmonella typhimurium.* Metabol., *41* : 1 – 2 .

Duncan, D.B. (1955). Multiple range and multiple F –tests. Biomet., *11*: 1

Ellis, A.E. (1982). Differences between the immune mechanisms of fish and higher vertebrates. In : R.J. Roberts (Editor), Microbiol Diseases of Fish. Academic Press. New York : 29pp.

Erdal, J.I.; Evensen, O.; Kaurstad, O.K.; Lillehaug, A.; Solbakken, R. and Thorud, K. (1991). Relationship between diet and immune response in Atlantic salmon *Salmo salar*, L. after feeding various levels of ascorbic acid and omega 3-fatty acids. Aquacult., *98* : 363-379.

Fracalossi, D.M (1993). Effects of dietary lipids and temperature on growth, liver fatty acid composition, and immune responses in channel catfish. Ph.D. dissertation, Auburn University, Auburn, *Al*, 109 pp.

Fracalossi, D.M. and Lovell, R.T. (1994). Dietary lipid sources influence responses of channel catfish *Ictalurus punctatus* to challenge with the pathogen *Edwardsiella ictaluri.* Aquacult., *119* : 287 – 298 .

Ghittino, P. (1989). Nutrition and fish disease. Page 681-713 in J.E. Halver, editor. Fish nutrition, 2nd edition. Academic Press, New York.

Goldman, D.W.; Pickett, W.C. and Goetzl, E.J. (1983). Human neutrophil chemotatic and degranulating activities of leukotriene B (LTB) derived from eicosapentaenoic acid. Biochem. Biophys. Res. Commun., *117*: 282-288.

Gyorgy, P; Cogan, G. and Rose, C.S. (1952). Availability of vitamin E in the newborn infant. Proc. Soc. Exp. Biol. & Med. *81*: 536-538.

Helmy, A. M.; Badawi, H. K. and EL-Bishry, A. (1974). Seasonal variations in the protein composition blood serum of (*Anguilla vulgaris*, and *Mugil cephalus*). Bull. Tnst. Ocean and Fish. ARE., *4*: 369-381.

Hepher, B; Liao, I.C.; Cheng, S.H. and Hsich, C.S. (1983). Food utilization by red tilapia, effects of composition, feeding level and temperature on utilization efficiencies for maintenance and growth Aquacult., *32*: 255.

Herbert, W.J. (1977). Veterinary immunology Blakwel Scientific publication, Oxford, London, Dinburgh, Melbourne P. 367.

Hwang, D. (1989). Essential fatty acids and immune response, FASEBJ, *3: 2052-2061.*

Johnston, P.V. (1988). Lipid modulation of immune responses. 37-86 in R.K. Chandra, editor. Nutrition and immunology R.Liss, New York.

Kinsella, J.E. and Lokesh, B. (1990). Dietary lipids, eicosaniods and the immune system. Crit. Care /Med., *18*: 94 –113.

Kragballe, K.; Voorhees, J.J. and Goetzl, E.J. (1987). Inhibition by Leukotriene B of Leukotriene B4. Induced activation of human Keratinocytes and neutrophils. J. Invest. Dermatol., *88 : 555-558.*

Landolt, M.L. (1989). The relationship between diet and the immune response in fish. Aquacult., *79* : 193-206.

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. and Austen, K.F. (1985). Effects of dietary enrichment with eicosapentaeonic and docosahexaenoic acids on in vitro neutrophil and monocyte Leukotriene generation and neutrophil function. N. Engl.J. Med., *312*: 1217 – 1224.

Leitch, A.G.; Lee, T.H.; Ringel, E.W.; Prickett, J.D.; Robinson, D.R.; Pyne, S.G.; Corey, E.J.; Drazen, J.M.; Austen, K.F. and Lewis, R.A., (1984). Immunologically induced generation of tetraene and pentaene Leukotrienes in the peritoneal cavities of menhaden-fed rats. J. Immunol., *132* : 2559 – 2565 .

Meydani, S.N. (1990). Deitary modulation of cytokine production and biologic functions. Nutr. Rev., *48*: 361 – 369.

Meydani, S.N. Endres, S.; Woods, M.M.; Goldin, B.R.; Soo, C.; Morrill – Labrode, A.; Dinarello, C.A. and Gorbach, S.L., (1991). Oral (n-3) Fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. J. Nutr., *121* : 547 – 555 .

Miller, L.L.; Bale, W.F.; Yuile, C.L.; Masters, R.E.; Tishkoff, G.H. and Wipple, G.H. (1949). The Use of radioactive lynne in studies of protein metabolism synthesis and utilization of plasma proteins. J. Exp. Med., *90*:297.

Reinnold, J .G. (1953). Determination of total portein and albumin in the serum. Submitted by to standard method in chemical chemistry. M.Reiner (ed.) Vol.1, Academic press N. Y. 88pp.

Sakai, D. K. (1981). Heat inactivation of complements and immune hemolysin reactions in rainbow trout, masu trout ,coho salmon, goldfish and tilapia. Bull. Jpn. Soc. Sci. Fish., *47*:565.

Sheldon, W. M., Jr., and Blazer, V.S. (1991). Influence of dietary lipid and temperature on bactericidal activity of channel catfish macrophages. J. Aqua. Anim. Health, *3:* 87.

Siddiqui, A. O.; Howlader, S. M. and Adam, A. (1988). Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile tilapia, *Oreochomis niloticus*. Aquacult., *70*: 63.

Steel, R. G. D. and Torrie, J. H. (1980). Principles and procedures of statistics. McGrow- Hill Boof co., New York .

Wise, D. J.; Tomasso, J. R.; Gatlin, D. M.; Bai, S. C. and Blazer V.S. (1993a). Effects of dietary selenium and vitamint E on red blood cell peroxidation, glutathione peroxidase activity, and macrophage superoxide, anion production in channel catfish . J. Aqua. Anim. Health, *5*: 177.

Wise, D.; Tomasso, J.; Schwedler, T. E.; Blazer, V. S. and Gatlin, D. M. (1993b). Effect of vitamin E on the immune response of channel catfish to *Edwardsiella ictaluri*. J. Aqua. Anim. Health, *5*: 183.

Table 1. The average water temperature during different experimental period.

Periods	Temperature °C
From 1 -10-1998 to 14 - 10 -1998	20.5
From 15 -10-1998 to 31 - 10 -1998	19.0
From 1 -11-1998 to 14 - 11 -1998	20.5
From 15 -11-1998 to 30 - 11-1998	17.1
From 1 -12 –1998 to 14 – 12-1998	14.3
From 15- 12-1998 to 31 - 12 -1998	14.8
From 1 - 1 –1998 to 14 - 1 - 1999	12.4
From 15 - 1- 1999 to 31 - 1 - 1999	13.3
From 1 - 2 –1999 to 14 - 2 - 1999	12.0
From 15 - 2 –1999 to 29 - 2 -1999	13.8
From 1 - 3 -1999 to 14 - 3 - 1999	12.9
From 15 - 3 –1999 to 30 - 3 - 1999	13.2
From 1 - 4 – 1999 to 14 - 4 -1999	16.0
From 15 - 4 –1999 to 30 - 4 – 1999	21.2

Table 2. Chemical composition of the experimental diets.

Ingredients	Experimental diets					
	Poultry oil		Corn oil		Linseed oil	
	3%	6%	3%	6%	3%	6%
Wheat bran	50	46	50	46	50	46
Soybean meal	25	25	25	25	25	25
Fish meal	19	20	18	20	20	20
Poultry oil	3	3	-	-	-	-
Corn oil	-	-	3	6	-	-
Linseed oil	-	-	-	-	3	6
Vitamin and mineral	3	-	3	3	3	3
Nutrient Composition						
Crude Protein, %	30.11	30.68	30.11	30.68	30.11	30.68
Ether extract, %	7.10	10.04	7.10	10.04	7.10	10.04
Total carbohydrate, %	51.63	48.48	51.63	48.48	51.63	48.48
Ash, %	11.16	10.80	11.16	10.80	11.16	10.80
Protein/Energy ratio	95.30	93.44	95.30	93.44	95.30	93.44
Gross energy (kcal/kg)[2]	4513.5	4690.7	4513.5	4690.7	4990.7	4513.5
Metabolizable energy (kcal/kg)[3]	3159.5	3283.5	3159.5	3253.5	3159.5	3283.5

1-Vitamin and mineral mixture each 1kg of mixture contains :

4.8 m.I.U Vit. A; 0.8 m.I.U D3; 4.0 g Vit. E ; 0.8 g. Vit. K; 4.0 g. Vit . B12 ; 4.0 g . Vit. B2; o.6 g. Vit. B6; 4.0 g.

Vit . Pantothenic acid ; 8.0 g . Vit . Nicotinic acid ; 400 mg ; Vit . folic acid ; 2o mg. Vit . Biotin ; 2oo g . Chorine chloride; 4 g .

Copper ; 0.4 g . Iodine ; 12 g . Iron ; 22 g . Manganese; 22 g ; 0.04 g . Selenium .

2-GE was calculated according to Hepher *et al* (1983) .

3-ME was calculated from gross energy as 75 as reported by Hepher *et al* (1983) .

Table 3. Fatty acid composition of Poultry oil , Corn oil and Linseed oil used in experimental diets .

Lipid source	14:0	16:0	16:1*n*9	18:0	18:1*n*9	18:2*n*6	18:3*n*3	20:1*n*9	Σ n 3	Σ n 6	Σ n 9	Σ n 3 : n 6
Poultry oil	0.9	21.6	3.7	6.0	37.3	19.5	1.0	0.1	1.0	19.6	41.1	0.05
Corn oil	-	10.9	-	1.8	24.2	58.0	0.7	-	0.7	58.0	24.2	0.01
Linseed oil	-	5.3	-	4.1	20.2	12.7	53.3	-	53.3	12.7	20.2	4.2

Cited from NRC , 1993 for fish

Table 4. Fatty acid composition of experimental diets.

Lipd sources	Experimental treatments					
	Poultry oil		Corn oil		Linseed oil	
	3%	6%	3%	6%	3%	6%
Types of fatty acids:						
Saturates:						
0:14	-	-	-	-	0.05	0.03
0:16	0.32	016	0.65	0.33	1.30	0.65
0:18	0.25	012	0.11	0.05	0.36	0.18
Monounsaturated:						
1:16*n*9	-	-	-	-	0.22	0.11
1:18*n*9	1.21	0.61	1.45	0.73	2.24	1.12
1:20*n*9	-	-	-	-	0.01	0.01
Diounsarurated:						
2:18n6	0.76	0.38	3.48	1.70	1.17	0.59
Triounsaturated:						
3:18n3	3.20	1.60	0.04	0.02	0.06	0.03
Profile variables:						
Total fatty acids	5.74	2.87	5.74	2.87	5.44	2.72
Total polyunsaturated	5.18	2.59	4.98	2.49	3.72	1.86
Total unsaturated / Total saturates	9.25	9.25	6.55	6.55	2.16	2.16
Total n9	1.21	0.61	1.45	0.733	2.46	1.23
Total n6	0.76	0.38	3.48	1.74	1.18	0.59
Total n3	3.20	1.60	0.04	0.02	0.06	0.03
Total n3 / n6	0.24	0.24	0.01	0.01	0.05	0.05
Total saturates	0.56	0.28	0.76	0.33	1.72	0.86
Total monotones	1.21	0.61	1.45	0.73	2.46	1.23
Total oiened	0.76	0.38	3.48	1.74	1.18	0.59
Total triened	3.20	1.60	0.04	0.02	0.06	0.03

Table 5. Total serum protein of fish fed diets different in oil source and oil level during the winter season.

Oil source / Oil level	Control	Poultry oil 3%	Poultry oil 6%	Corn oil 3%	Corn oil 6%	Linseed oil 3%	Linseed oil 6%	SE ±
Water temperature (°C) More than 16.85	2.71	2.71	2.71	2.71	2.71	2.71	2.71	-
Less than 11.66	5.33 c	7.11 a	7.32 a	6.71 ab	7.07 a	7.56 a	5.52 b	0.72

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 6. Total serum protein of fish fed diets different in oil source irrespective of oil level .

Water temperature (°C)	Control	Poultry oil	Corn oil	Linseed oil	SE ±
More than 16.85	2.71	2.71	2.71	2.71	-
Less than 11.66	5.33 c	7.22 a	6.89 a	6.54 b	0.34

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 7. Total serum protein of fish fed diets different in oil level irrespective of oil source.

Water Temperature (°C)	Control	3 %	6 %	SE ±
More than 16.85	2.71	2.71	2.71	-
Less than 11.66	5.33 c	7.13 a	6.64 b	0.35

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b.. etc. means in same row with different superscripts ($P \leq 0.05$).

Table 8. Total serum albumin of fish fed diets different in oil source and oil level during the winter season.

Oil source / Oil level	Control	Poltry oil 3%	Poltry oil 6%	Corn oil 3%	Corn oil 6%	Linseed oil 3%	Linseed oil 6%	SE ±
Water temerature(°C) More than 16.85	1.7 d	1.7 ac	1.7 b	1.7 ab	1.7 a	1.7 a	1.7 b	-
Less than 11.66	2.67	3.35	3.43	2.70	3.45	3.79	2.83	0.41

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 9. Total serum albumin of fish fed diets different in oil source irrespective of oil level.

Water Temperature (°C)	Control	Poultry oil	Corn oil	Linseed oil	SE ±
More than 16.85	1.7	1.7	1.7	1.7	-
Less than 11.66	2.67 c	3.39 a	3.8 b	3.3 a	0.16

SE, Standard error, calculated from residual mean square in the analysis of variance.

A,b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 10. Total serum albumin of fish fed diets different in oil level irrespective of oil source.

Water Temperature (°C)	Control	3%	6%	SE ±
More than 16.85	1.7	1.7	1.7	-
Less than 11.66	2.67 c	3.28 a	3.24 b	0.2

SE, Standard error, calculated from residual mean square in the analysis of variance.

A,b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 11. Total serum globulin of fish fed diets different in oil source and oil level during the winter season.

Oil source	Control	Poltry oil		Corn oil		Linseed oil		SE ±
Oil level (%)		3%	6%	3%	6%	3%	6%	
Water temperature (°C) More than 16.85	1.64	1.64	1.64	1.64	1.64	1.64	1.64	-
Less than 11.66	d 2.66	ac 3.67	bc 3.43	a 4.1	ac 3.62	ac 3.77	b 2.69	0.46

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same raw with different superscripts ($P \leq 0.05$).

Table 12. Total serum globulin of fish fed diets different in oil source irrespective of oil level.

Water Temperature (°C)	Control	Poultry Oil	Corn oil	Linseed oil	SE ±
More than 16.85	1.64	1.64	1.64	1.64	
Less than 11.66	c 2.66	a 3.60	a 3.82	b 3.20	0.31

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 13. Total serum globulin of fish fed diets differed in oil level irrespective of oil source.

Water Temperature (°C)	Control	3%	6%	SE ±
More than 16.85	1.64 c	1.64 a	1.64 b	-
Less than 11.66	2.66	3.85	3.25	0.42

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 14. Hydrogen peroxidase titer of fish fed diets different in oil source and level during the winter season.

Oil source	Control	Poultry Oil		Corn Oil		Linseed Oil		SE ±
Oil level		3%	6%	3%	6%	3%	6%	
Water temerature(°C) More than 16.85	2.75	2.75	2.75	2.75	2.75	2.75	2.75	-
Less than 11.66	4.29 d	5.57 bc	6.24 b	6.92 a	6.47 ab	5.66 bc	707 a	0.62

SE, Standard error, calculated from residual mean square in the analysis of variance.

A,b ..... etc. means in same raw with different superscripts are different ($P \leq 0.05$).

Table 15. Hydrogen peroxidase titer of fish fed diets differed in oil source irrespective of oil level .

Water Temperature (°C)	Control oil	Poultry oil	Corn oil	Linseed oil	SE ±
More than 16.85	2.75	2.75	2.75	2.75	-
	c	b	a	a	
Less than 11.66	4.29	5.91	6.70	6.35	0.40

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 16. Hydrogen peroxidase titer of fish fed diets different in oil level irrespective of oil source.

Water Temperature (°C)	Control	3 %	6 %	SE ±
More than 16.85	2.75	2.75	2.75	-
		c b	a	
Less than 11.66	2.73	6.05	6.56	0.36

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same raw with different superscripts ($P \leq 0.05$).

Table 17. Hemolysin titer of fish fed diets different in oil source and oil level during the winter season.

Oil source	Control	Poultry oil		Corn oil		Linseed oil		SE ±
Oil level		3%	6%	3%	6%	3%	6%	
Water temperature (°C) More than 16.85	2.50	2.50	2.50	2.50	2.50	2.50	2.50	-
	c	b	ab	b	a	b	a	
Less than 11.66	2.73	5.66	6.76	5.97	7.38	5.93	7.80	0.87

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same raw with different superscripts ($P \leq 0.05$).

Table 18. Hemolysin titer of fish fed diets differed in oil source irrespective of oil level.

Water Temperature (°C)	Oil source				SE ±
	Control	Poultry oil	Corn oil	Linseed oil	
More than 16.85	2.50	2.50	2.50	2.50	-
	c	b	a	a	
Less than 11.66	2.73	6.21	6.68	6.87	0.34

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 19. Hemolysin titer of fish fed diets different in oil level irrespective of oil source.

Water Temperature (°C)	Oil level			SE ±
	Control	3 %	6 %	
More than 16.85	2.50	2.50	2.50	-
	c	b	a	
Less than 11.66	2.71	5.85	7.31	1.03

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

Table 20. Average performance of tilapia brood fish during the winter season (120 days).

Oil source	Control	Poultry oil		Corn oil		Linseed oil		SE ±
Oil level		3%	6%	3%	6%	3%	6%	
Intial body weight (g)	51.13	56.5	55.38	53.63	54.83	51.75	53.75	1.65
		c	b	a	ab	b	a	a
Final body weight (g)	47.00	59.25	75.5	62.00	57.00	71.00	69.00	7.29
		c	b	a	ab	b	a	a
Specific growth rate (%/day)8	- 0.07	0.04	0.26	0.12	0.03	0.26	0.21	0.11
		c	b	a	ab	b	a	a
Gain in weight per fish	- 4.13	2.75	20.12	8.372.17		19.25	15.25	8.02

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b ..... etc. means in same row with different superscripts ($P \leq 0.05$).

* Specific growth rate (%/day) = 100 (ln final weight – ln initial weight) / days.

Table 21. The response of tilapia brood performance for dietary lipid sources and levels.

Oil source	Sources of oil					Levels of oil		SE
Oil level (%)	Control	Poultry oil	Corn oil	Linseed oil	SE ±	3%	6%	±
Intial body weight (g)	51.13	55.94	54.23	52.75	1.03	53.96	54.65	0.49
		d	b	c	a		b	a
Final body weight (g)	47.00	67.94	59.5	70.00	5.16	64.08	67.17	2.18
		d	b	c	a		b	a
Sepcific growth rate (%/day)*	- 0.07	0.15	0.08	0.24	0.04	0.14	0.17	0.02
		d	b	c	a		B	a
Grain in weight per fish	- 4.13	11.44	5.27	17.25	3.04	10.12	12.51	1.69

SE, Standard error, calculated from residual mean square in the analysis of variance.

A, b etc. means in same row with different superscripts ($P \leq 0.05$).

* Specific growth rate (%/day) = 100 (ln final weight – ln initial weight) / days.