www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Effects of different oil sources on serum lipid profile, immune response, and histology of liver, spleen and bursa of fabricius in broiler

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Abstract

An experiment was conducted to study the effects of different oil sources on performance, serum lipoproteins, antibody titre, histology and fat accumulation in broiler chicks. In a completely randomized design, 320 one-day-old broiler chickens (Ross 308) were randomly allocated to four dietary treatments *viz.,* 1) diet without oil as control, 2) diet with fish oil, 3) diet with soybean oil and 4) diet with olive oil at the rate of 2.15% in the starter and 3% in the grower and finisher diet. The results showed that the broilers fed diet containing soybean oil had better feed conversion ratio (FCR) in grower and finisher period ($P<0.05$). At day 42 of age, antibody titre against Newcastle in chicks fed a diet containing fish oil was higher than the other groups without affecting immunity against sheep red blood cells (SRBC). Effects of different sources of oil on histological studies of liver showed lower lipid deposition in birds fed fish oil in comparison with other treatments. The lymphocytes proliferation in Bursa of Fabricius was more with central nucleus than the other treatments. These results suggested that addition of fish oil to the poultry diet resulted in improved immune response while soybean oil enhanced the performance.

Keywords: Oil sources; antibody titre; lipid profile; broiler chickens

To cite this article: Sadeghi AA, A Safaei and M Chamani, 2014. Effects of different oil sources on serum lipid profile, immune response, and histology of liver, spleen and bursa of fabricius in broiler. Res. Opin. Anim. Vet. Sci., 4(12): 669-675.

Introduction

Among the nutrients of poultry diets, oil is a good source of energy that influences metabolism and immune development. Earlier studies indicated that unsaturated fatty acids could induce beneficial health effects. Some unsaturated fatty acids such as linoleic and linolenic acid are essential for birds and must be added into their diet (Schwalfenberg, 2006). Also, fish oil (rich in polyunsaturated fatty acids of n-3 series), soybean oil (rich in polyunsaturated fatty acids of n-6 series) and olive oil (rich in monounsaturated fatty acids of n-9 series) may affect immune response and blood lipid profile in animal (Parmentier et al., 1997; Koletzko and Goulet, 2010). In this regard, Saez et al. (2010) reported that consumption of diets containing polyunsaturated fatty acids resulted in decrease triglycerides (TG) and low-density lipoprotein (LDL), but increased the beneficial high-density lipoprotein (HDL)..Other researchers reported that diets containing fish and soybean oil had no effect on chicken serum TG and very low-density lipoprotein (VLDL) concentration (Alparslan and Ozdogan, 2006). Freidman (1997) reported that addition of fish oil led to improved antibody titre against Newcastle in turkeys, but another researcher reported that antibody production decreased in the chickens fed fish oil (Parmentier et al., 1997). In the literature, there are some controversial reports on different sources of oil on immune response and serum lipoproteins. Therefore, the objective of the current study was to test the effects of different sources of oils on performance, serum lipoprotein levels, antibody titre and histology of liver and some lymphoid organs in broiler chickens.

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Materials and Methods

Three hundred and twenty one-day-old healthy broiler chicks (Ross 308) were weighed individually and randomly allocated to four dietary treatments (4 replicates with 20 each). Environmental temperature was set at 32°C on day 1 and lowered gradually to 24°C for the rest of the experimental period. Relative humidity and ventilation were near standard conditions. Lighting schedule was 23 h light and 1 h dark. The birds had free access to feed and water throughout the trial. Birds were fed experimental diets from day 1 until day 42 of age in three periods: The starter (1-7 days), grower (8-28 days) and finisher (29-42 days). Diets were formulated based on the corn-soybean meal (Table 1). Treatments included: 1) diet without oil, 2) diet with fish oil, as source of ω-3, 3) diet with soybean oil, as source of ω-6) and 4) diet with olive oil as source of ω-9 (Table 1). Fatty acid composition was determined by gas chromatography in each treatment. The soybean oil, fish and olive oil used in this study contained 56.69%, 40.23% and 72.43% oleic acid. Average feed intake, body weight gain and feed conversion ratio (FCR) of all birds in each pen was recorded at the end of each feeding period. At days 42 of age, blood samples were drawn from vein of two birds in each pen. The blood samples were poured in tubes that had no anticoagulant and centrifuged at 1500 × g for 15 min. Serum was collected for assessment of antibody titre. The antibody titre against Newcastle disease was determined by haemagglutination inhibition test (Allan and Gough, 1974). At day 21 of age, sheep red blood cell (SRBC) suspension (5% in sterile phosphate buffered saline) was injected in breast muscle of birds. Seven days after each sensitization (day 28 of age) antibody titre against SRBC was measured and expressed as the log 2 of the reciprocal of the highest serum dilution giving complete agglutination (Vander and Leenstra, 1980). At the end of the experimental period, the concentration of TG,

Table 1: Ingredients and chemical composition of experimental rations[1]

Ingredients (%)	Starter		Grower		Finisher	
Tretments	T_1	T_2, T_3, T_4	T_1	T_2, T_3, T_4	T_1	T_2, T_3, T_4
Corn	54.82	55.45	58.81	55.84	63.6	60.61
Soybean Meal (44% CP)	35.6	35.69	32.5	33.07	27.7	28.27
Starch	5	0	5	0	5	0
Oil	0	2.15	0	3	0	3
DCP	1.91	1.91	1.61	1.62	1.51	1.52
$CaCO_3$	1.18	1.18	0.96	0.95	0.93	0.92
DL- Methionine	0.29	0.29	0.19	0.2	0.16	0.16
L-Lysine HCl	0.21	0.20	0.04	0.03	0.02	0.01
L-Threonine	0.11	0.11	0.03	0.03	0.01	0.01
Salt	0.27	0.27	0.32	0.33	0.32	0.33
$NaHCO_3$	0.11	0.11	0.04	0.03	0.04	0.03
Zeolite	0	3.14	0	4.4	0.21	4.64
Vitamin Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Calculated chemical composition						
Metabolizable Energy (kcal/kg)	2840		2890		2940	
Crude Protein (%)	20.57		19.26		17.5	
Methionine (%)	0.59		0.48		0.43	
Met+Cys (%)	0.88		0.76		0.69	
Lysine (%)	1.22		1.06		0.89	
Threonine (%)	0.78		0.66		0.59	
Tryptophan (%)	0.22		0.21		0.19	
Arginine (%)	1.30		1.20		1.1	
Valine (%)	0.90		0.90		0.8	
Isoleusine (%)	0.90		0.88		0.8	
Calcium (%)	0.98		0.82		0.78	
Available Phosphorus (%)	0.46		0.41		0.39	
Sodium (%)	0.16		0.16		0.16	
Chloride (%)	0.23		0.23		0.23	
Potassium (%)	0.86		0.81		0.73	

1- T_1: dite without oil, T_2: dite with fish oil, T_3: dite with soybean oil and T_4: dite with olive oil; 2- Vitamin-mineral premix (each kg contained): calcium (Ca), 195 g; potassium (K), 70 g; sodium (Na),18 g;magnesium (Mg), 6 g; zinc (Zn), 1,200 mg; iron (Fe), 2,000 mg; copper (Cu), 400 mg; manganese (Mn), 1,200 mg;selenium (Se), 8 mg; cobalt (Co), 20 mg; iodine (I), 40 mg; vitamin A, 200,000 IU; vitamin D3, 80,000 IU; vitamin E,1,072 IU; vitamin K3, 34 mg; ascorbic acid, 1,300 mg; thiamine, 35 mg; riboflavin, 135 mg; niacin, 1,340 mg;vitamin B6, 100 mg; folic acid, 34 mg; vitamin B12, 670 µg; and biotin, 3,350 µg.

Table 2: Effect of different sources of oil on performance of broilers in starter, grower and finisher period

	Treatments	Starter	Grower	Finisher
Feed intake (g)	Control	289.33±21.37	980.04^{a}±7.12	3287.37±257.90
	Fish oil	290.03±9.33	1007.33^{a}±33.76	3305.66±262.23
	Soybean oil	270.66±3.12	920.02^{b}±28.45	3383.32±291.77
	Olive oil	279.02±7.94	981.00^{a}±11.05	3571.01±86.52
	SEM	3.459	9.735	60.7369
Weight gain (g)	Control	159.66±6.68	604.91^{c}±13.88	1558.05^{c}±39.54
	Fish oil	169.33±7.06	734.00^{a}±29/20	1081/04^{d}±65.64
	Soybean oil	160.66±7.14	722.66^{ab}±10.98	1910.66^{a}±77.36
	Olive oil	173.33±11.78	665.00^{bc}±77.17	1713.00^{b}±62.27
	SEM	2.403	16.2937	80.3587
Feed conversion ratio	Control	1.81^{a}±0.06	1.62^{a}±0.04	2.11^{b}±0.21
	Fish oil	1.71^{ab}±0.03	1.37^{bc}±0.07	3.06^{a}±0.19
	Soybean oil	1.69^{ab}±0.06	1.27^{c}±0.04	1.77^{c}±0.22
	Olive oil	1.62^{b}±0.15	1.49^{ab}±0.18	2.08^{b}±0.03
	SEM	0.0265	0.0405	0.1306

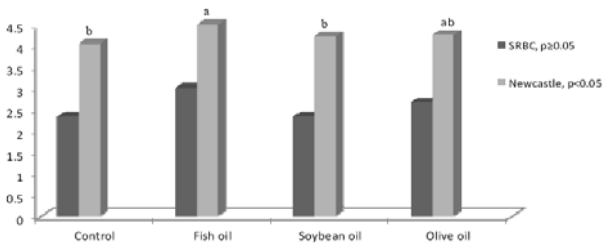

Fig 1: Effects of different sources of oil on antibody titre against Newcastle (log2) and SRBC (log2)

total cholesterol, LDL cholesterol and HDL cholesterol were determined using commercial enzymatic kits (Pars azmoon commercial, Iran). At the end of experiment, one bird from each replicate was randomly selected and killed by cervical dislocation and their liver, spleen and bursa of Fabricius were removed and fixed in 10% neutral buffered formalin. Samples were dehydrated, cleared and embedded in paraffin. Tissue samples were sectioned at 6 µm thickness, placed on glass slides, processed by hematoxylin-eosin and evaluated under light microscope (Billiar et al., 1988). At the end of the period, two birds per replicate were randomly selected and weighed. The birds were killed and abdominal fat and gizzard fat was removed and weighed individually. Their weight was expressed as a ratio of body weight.

Statistical analysis

All analyses were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute (SAS 2002). Means were compared with Duncan's multiple range test (Duncan, 1955). P<0.05 was considered as statistically significant.

Results

The effects of different sources of oil on feed intake, body weight gain and FCR of the birds in starter, grower and finisher are shown in Table 2. There were no significant effects on feed intake and body weight gain in starter (P≥0.05). The birds fed olive oil had the best FCR than the other groups during starter period. In grower period, there were significant effects on feed intake, body weight gain and FCR among treatments (P<0.05). The lowest feed intake was observed in soybean oil fed group with better FCR. Weight gain was significantly high (P<0.05) in group fed fish oil at this stage. There were no significant effects on feed intake in finisher period (P≥0.05). The broilers fed with soybean oil had better weight gain and FCR during finishing period.

Effects of different sources of oil on serum antibody titre against Newcastle and SRBC are presented in Fig. 1. Significantly high antibody titre against Newcastle was observed in the birds fed fish oil (P<0.05). Different sources of oil on antibody titre against SRBC, showed no significant effect among the treatments.

Effects of different sources of oil on histological studies of liver are presented in Fig. 2. In the birds fed control diet, their liver showed many lipid droplets with irregular nuclei arrangement. Lipid deposition in birds fed fish oil in comparison with other treatments was lower with no swelling and all the nuclei were regular and homogenous among other birds. In those fed with soybean oil and olive oil in comparison with control group, less lipid droplets deposition, sinusoid, dilation and swelling were observed. Effects of different sources of oil on histological studies of spleen are presented in Fig. 3. In birds fed oil free diet, spleen showed white and red pulps and lymphocytes proliferation was homogenous, but in comparison with other treatments, sinusoid were more separated and somewhat inflamed. In birds fed soybean oil, fish oil and olive oil, sinusoid was normal in shape and no inflammation was seen. Effects of different sources of oil on histological studies of Bursa of Fabricius are presented in Fig. 4. In bird fed

fish oil, nucleus of lymphocytes was centrally placed, pallor and more active than other treatments.

The effects of different sources of oil on deposition of fat in abdominal cavity and gizzard of the birds are shown in Fig. 5. There were significant effects on deposition of fat in abdominal cavity and gizzard ($P<0.05$). The highest fat deposition was observed in birds fed soybean oil. No significant effect of the oil source was observed on TG, CHO, HDL and LDL (Fig. 6).

Discussion

The main purpose of the present study was to examine the effects of different sources of oil on performance, antibody titre, serum lipoprotein levels, histology of some organs and fat accumulation in broiler chickens. The results showed that chicks fed diets containing soybean oil showed better FCR than the other groups in grower and finisher period. Our result is consistent with the finding of some other studies (Tabeidian et al., 2005; Dewitt et al., 2009). The improved FCR of broilers fed soybean oil were probably because of fatty acid composition of this oil; a long-chain n-6 fatty acid that enhances growth. Lack of meaningful differences in feed intake and body weight gain in starter period could be secondary to incomplete recycling of bile salts and also poorly development of digestive system of birds (Wiseman et al., 1991). In this study, we hypothesized that among the different sources of oil, a source of n-3 polyunsaturated fatty acids results in increase antibody titre against Newcastle and SRBC. The results showed that the birds fed diets containing fish oil had better antibody titre against Newcastle on day 42 of age. These results may be due to the beneficial effect of fish oil on high antibody titre (Parmentier et al., 1997). The results agreed with finding which reported that amega-3 polyunsaturated fatty acids supplementation enhanced antibody production (Billiar et al., 1988). Antibody titre against SRBC showed no significant effect among treatments at 42 day of age. These results were inconsistent with other reports (Torki et al, 2002; Kidd, 2004). The discrepancy may be due to the type and dose of used oil. It was concluded that the addition of fish oil in the diet may enhance antibody titre against Newcastle, because of metabolic function of eicosapentaenoic acid and docosahexaenoic acid that improve the immune response (Sugano et al., 2000). Immune system of the birds is mainly composed of lymphatic vessels and lymphoid tissues. Primary tissue such as the bursa of Fabricius is considered as primary organ of immune response in poultry while spleen is secondary immune organ.

At least four mechanisms of action have been proposed to explain the action of fatty acids on the modulation of animal's immune system: changes in the composition of membrane phospholipids, lipid peroxidation, alteration of gene expression, or productions of eicosanoids, cytokines and arachidonic acid (Peres et al., 2005). The n-6 polyunsaturated fatty

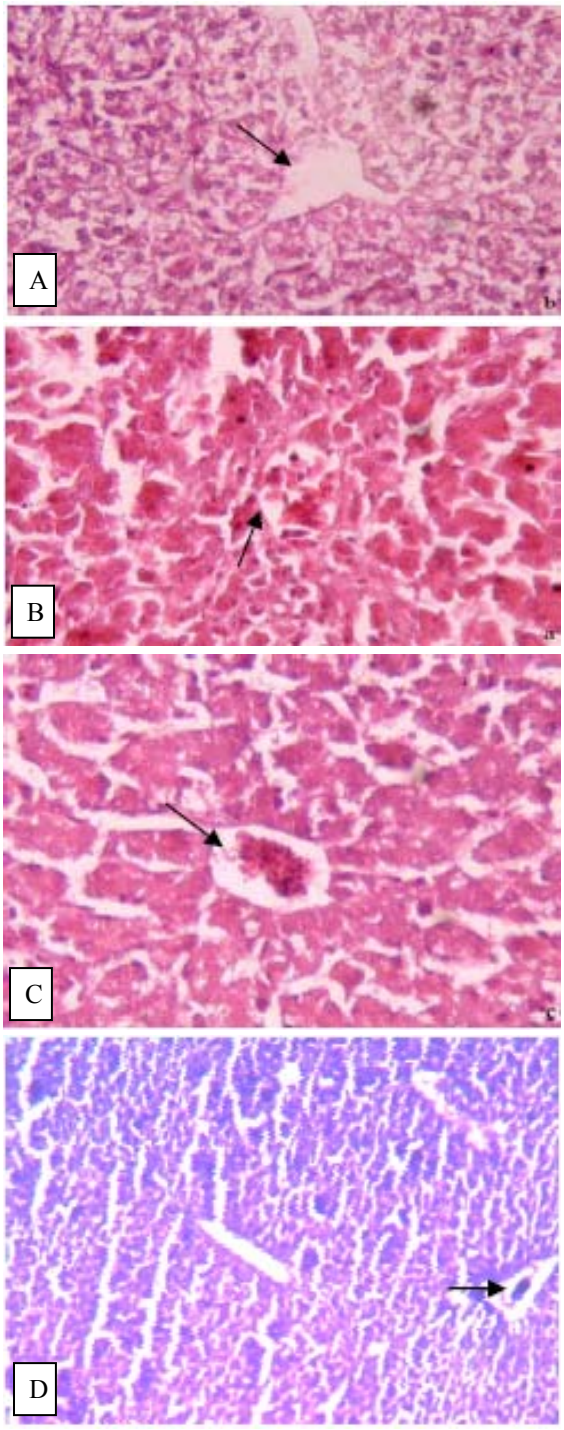

Fig 2: Effects of different sources of oil on histological studies of liver. Birds were fed with soybean oil (A), without oil (B), fish oil (C) and olive oil (D).

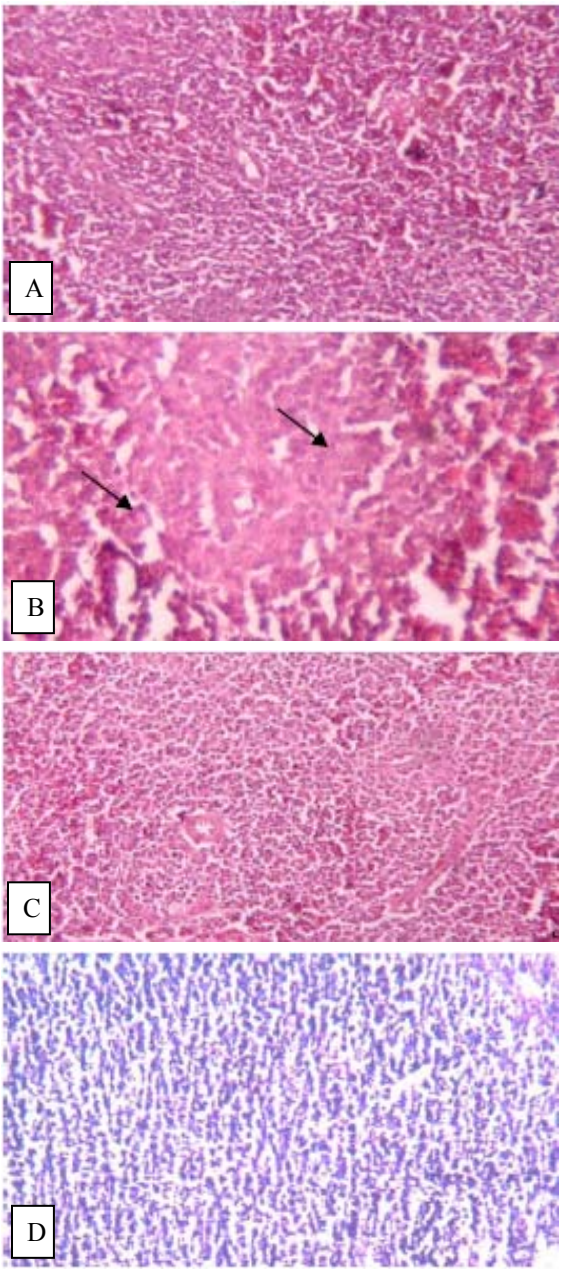

Fig 3: Effects of different sources of oil on histological studies of spleen. Birds were fed with soybean oil (A), without oil (B), fish oil (C) and olive oil (D).

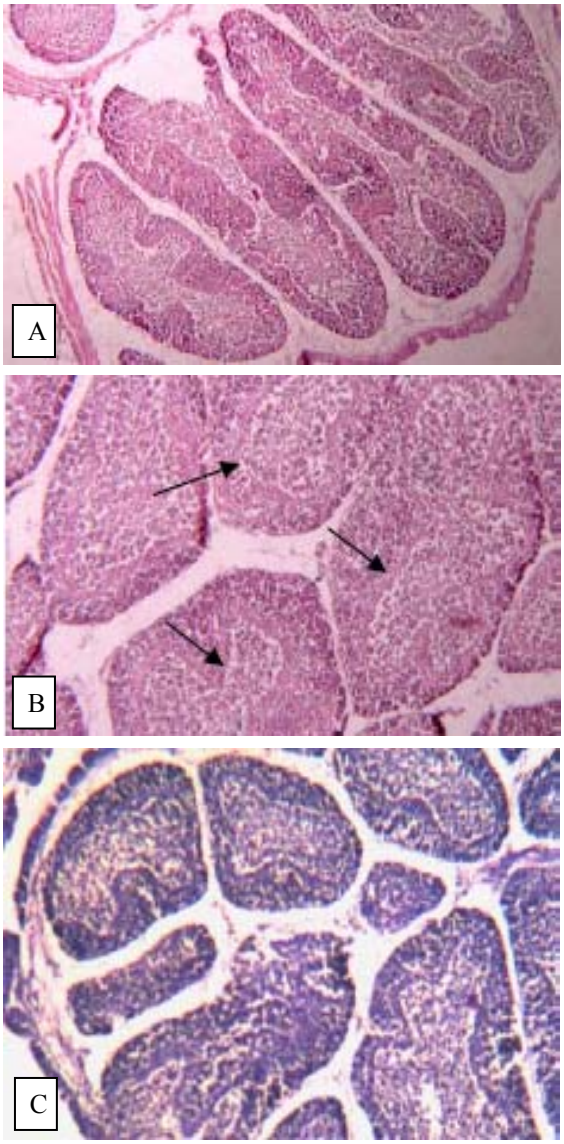

Fig 4: Effects of different sources of oil on histological studies of bursa of Fabriccuses. Birds were fed with soybean oil (A), without oil (B), fish oil (C) and olive oil (D).

acid in soybean oil is used in the biosynthesis of arachidonic acid and some prostaglandins. It was shown that addition of arachidonic acid can directly reduce lymphocyte proliferation and is toxic to these cells. As shown in Fig. 3B, lymphoid depletion occurred in spleen of chicks fed diet containing soybean oil. A study (Song and Horrobin, 2004) revealed that inclusion of soy oil in diet could induce significant increase in serum corticosterone concentration. Corticosterone has been found to be immune-suppressive, inhibiting the production and actions of antibodies, lymphocyte function and leukocyte population (Siegel, 1995).

In histological examination of bursa of Fabricius lymphocytes proliferation were more active and nucleus is central in position. It seems that adding fish oil to the diet has positive impacts on immune cell proliferation.

In addition, the results indicate that adding different sources of oil in birds diet result in decrease in blood lipid profile except HDL, but these effects were not statistically significant in comparison with birds fed with control. In histological study of the liver of birds, best results were observed in birds fed fish oil.

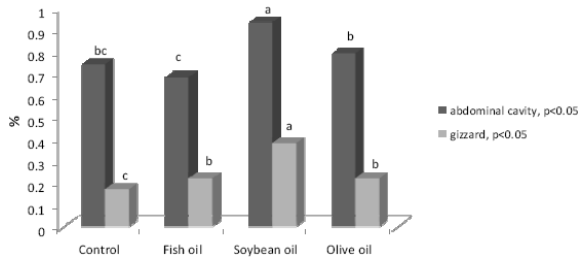

Fig 5: Effects of different sources of oil on deposition of fat (weight as a ratio of body weight.) in abdominal cavity and gizzard

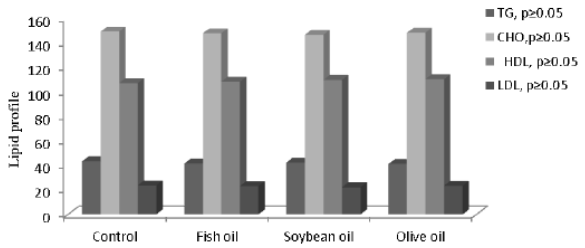

Fig 6: Effects of different sources of oil on Blood lipid profile (mg/dl).

Therefore, consumption of oil is highly recommended for optimal liver function. These results agreed with studies reporting that amega-3 polyunsaturated fatty acids supplementation have significant protective role on the liver (Koletzko and Goulet, 2010). These results suggest that diets containing unsaturated fatty acids have a positive impact on lipid metabolism in liver. Fat deposition was observed in the liver of chicks fed different sources of oil; however, comparatively more fat droplets were observed in the liver of chick fed oil free diet. The reason may be the conversion of starch into glucose in the small intestines and then into triglycerides in the liver. Soybean oil caused less accumulation of triglyceride in liver than the control, which were alternatively deposited in the abdomen and gizzard. Abdominal fat around the abdominal cavity and gizzard were not significantly altered in birds fed with or without oil. These results are inconsistent with result of Grunder and Chambers (1985) who reported that VLDL present in the blood is the core indicator of fat deposition due to its correlation with abdominal and fat content.

It was concluded that the addition of fish oil to the diet may lead to improved immune response against Newcastle while improved performance was observed in 3% soybean oil.

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