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# Effect of different sources of lipids on the performance and immune response in broiler chicken

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## Abstract

A study was conducted to investigate the effect of different sources of lipid on performance indexes and immune responses in broiler chicken. Four hundred and twenty four one-day-old broiler chicks (Ross 308), randomly divided into five treatments as a completely randomized design, consisting four replicates of 21 birds per each. Chicks were assigned to receive one source of lipid either tallow or corn oil or sunflower oil or flaxseed oil or olive oil in their diet. Antibody titre against IBD (Gumboro) and IBV (Bronchite) and Heterophile to Lymphocyte (H/L) ratio as indexes of immune responses, at days 28 and 42 of age and feed conception, weight gain and feed efficiency at whole of period, as indexes of performances, were measured. The diet contain corn oil, showed the best performance indexes with significant different than other treatments ($P<0.05$). In comparison of diet consists of corn oil, flaxseed oil containing high ratio of n-3 PUFA, had a higher H:L ratio ($P<0.05$). Result indicated that antibody production in chicks fed flaxseed oil against IBV at day 28 and against IBD at day 42 of age were less than the other chicks. Although the chicks fed Sunflower oil showed lower antibody production against IBD at day 28 of age. In this study, it was concluded that the use of flaxseed oil as only source of lipid in diet can result in decreased antibody production because of lower efficiency of energy usage. Also H:L ratio was affected by lipid source and corn oil among used lipids had the lowest H:L ratio and higher performance indexes, that is useful for wellbeing and health of broiler chicks.

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## Introduction

Lipid is an important component of the rations, as the sources of energy and essential fatty acids, which poultry need for basic functions, including growth and maintenance of healthy tissues (Yaqoob, 2004). In recent years, researchers have studied the impact of type and quantity of oil to increase the efficiency of feed utilization, carcass quality and meat quality of chickens. In this regard, some researches are performed by Nobakht *et al.* (2011) and found that 4% of pure soybean, rapeseed and sunflower oil, and their mixtures in the diets for broiler chickens have significant impact on production performance. DeWitt *et al.* (2009) showed that the introduction of 6% sunflower and fish oil leads to improved feed conversion of broiler chickens, which is consistent with previous research of El Yamany *et al.* (2008). The research of Stanaćev *et al.* (2012), which aimed to investigate the effect of different vegetable oils in the diet of broiler chickens, concluded that the use of 4 and 8% flaxseed oil does not exhibit significant differences in production parameters and carcass quality. It has shown that poly unsaturated fatty acids (PUFAs) are related to metabolic function, regulation of the immune system, lipid levels in plasma (Stulnig, 2003). The inflammatory process can decrease performance (body weight gain, feed consumption, and feed conversion efficiency) of broiler chicks (Klasing *et al.,* 1987; Klasing and Barnes, 1988; Benson *et al.,* 1993). Immune cells enriched in n-3 PUFA in membrane release lower amounts of inflammatory mediators, (e.g., eicosanoids) and enhance antibody production (James *et al.,* 2000). Omega-6 and omega-3 must be consumed in a balanced proportion. Some researcher (Parmentier *et al.,* 1997) found that n-6 PUFA resulted in an increase, antibody response against antigens or immunoglobulin production but other researchers (Sijben *et al.* 2000, Van heugten *et al.* 1996) reported that high levels of oils containing n-6 PUFA resulted in a decrease antibody response. Heterophils are first phagocytic leukocyte, and proliferate in blood in response to inflammation and stress (Thrall, 2004). Leukocyte number is particularly useful in the statistic physiology because they are varied by inflammation and stress. Inflammation and stress increase the numbers of heterophils and decrease the lymphocyte numbers. Heterophils to lymphocyte (H:L) ratio is used to determine inflammation and stress condition (Davis and Maerz, 2008). In other hand H:L ratio is affected by lipid sources. Lymphocyte proliferation was significantly decreased after feeding broiler chickens diets rich in Fish oil (Al-Khalifa *et al.* 2012). The H:L ratio appears to be a more reliable indicator of inflammation and stress (Gross and Siegel , 1983). In the literature, reports on the effects of different lipid sources (Peck, 1994; De Pablo *et al.*, 2000) on functionality of immune system are numerous; however, no study exists showing the effect of different lipid source on antibody response against IBD and IBV. Therefore this study was conducted to examine the effect of the different dietary source fat on performance, H:L ratio and serum antibody titre in chicken.

## Materials and methods

*Birds and experimental diets*

Four hundred and twenty four one-day-old broiler chicks (Ross 308) were divided randomly into five dietary treatments, four replicates with 20 chicks per each. Birds were housed in pens (1 × 1 m). Throughout the study, feed and water were provided for ad libitum consumption. Lighting schedule were 23L/1D while the temperature was gradually reduced 3 °C from initially 32 °C in each week. Experimental diets were formulated to be isoenergetic and isonitrogenous as follow: 1) diet containing Tallow as energy source; 2) diet containing corn oil; 3) diet containing sunflower oil; 4) diet containing flaxseed oil and 5) diet containing olive oil as shown in Tables 1-6. Fatty acid profile of each source of lipid (Table 7) was measured as described by Crespo and Esteve-Garcia (2001). Starter (1-10 days), grower (11-28 days) and finisher (29-42 days) diets were formulated based on corn-soybean meal presented in Table 1. As seen in Table 1, the only difference among diets was the source of lipid.

*Blood sampling and Blood Smear Preparation*

Immediately after collection, 900 µL of blood were

transferred to micro-tube containing 100 µL sodium citrate solutions (3.85 mg/100 µL) and immediately mixed. The reminder of collected blood was poured in sterile glass tube, kept at room temperature for two hours, then overnight at 4 °C in refrigerator and centrifuged at 1500 × g for 15 min. Serum was obtained and stored at -20°C until analyses of corticosterone concentration. Vaccination, serology and hematology Immunization program included vaccination against Bronchite disease (IBV) (day 1, eye drop; days 19 drinking) and infectious bursal disease (IBD) (D78, days 12 and 24, eye drop). Blood samples (3 ml) of 10 birds per treatment (each bird being considered one replication) were collected from the wing veins, using sterile syringes, on days 28 and 42 of age. These tubes transferred to Mabna Veterinary Laboratory (Karaj, Iran) for counting heterophil and lymphocyte according to the method of Ye *et al.* (2006).

*Performance record*

Body weight, feed consumption and feed conversion of all birds in each pen was recorded and calculated in each period.

*Statistical Analysis*

The chicken (10 determinations per treatment) was the experimental unit for white blood cells and antibody titres. All values were analyzed by one-way ANOVA using the GLM procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC). When the F-test for treatments was significant at P ≤0.05 in the ANOVA table, means were compared for significant differences using the Tukey test of SAS.

**Results and discussion**

*Performance*

Comparison of performance parameters are shown in Table 8. There was a significant difference in feed consumption in starter period (P<0.05). The chickens fed Tallow in their diet showed highest consumption in starter period and those fed corn oil had lowest feed consumption (P<0.05), although there was no significant difference in chickens fed corn oil, sunflower oil and olive oil (P>0.05). Reid *et al.* (1975) showed that feed consumption increase in presence of saturated source of lipid because of existing palmitic and stearic acid in the diet. These fatty acids produce less energy, lead to increase in feed consumption (Reid *et al.* 1975). In other hand poly unsaturated fatty acid (PUFA) decrease plasma triglyceride and cholesterol in comparison of saturated fatty acid (SFA) (Newman *et al.* 2002). It can result in decrease in bile secretion and consequently decrease lipid digestion and increase feed consumption. In growth period the chickens fed flaxseed oil and olive oil in their diets showed lower feed consumption. Lower feed consumption in chicken fed flaxseed and olive oil, is related to higher digestion and produce higher metabolizable energy. Also there are some antinutrient including phytic acid (decrease Ca absorption), cyanoglicosidse (linamarin) and B6 vitamin antagonist (Klosterman *et al.* 1967; Oomah *et al.* 1992; Summers *et al.*1988; Talebali *et al.* 2005). In other hand in mammals several mechanisms seem to be involved in lower energetic efficiency of PUFA that can occur in birds. Preferential oxidation of PUFA in peroxisomes prior to their oxidation in mitochondria leads to higher energy losses (Clarke, 2000). Furthermore, PUFA are involved in the induction of uncoupling proteins (UCP) in the mitochondria. UCP-3 is restricted to skeletal muscle in rats and is increased twofold by fish oil (Baillie *et al.,* 1999). These mechanisms would reduce the retention of dietary energy in animals fed PUFA with respect to those fed diets rich in saturated fatty acids (SFA).

The chickens fed corn oil and tallow had highest weight gain in starter period (P<0.05) and those fed tallow and flaxseed oil showed lowest weight gain in growth period (P<0.05). In finisher period, diet containing tallow, sunflower and flaxseed oil showed lower weight gain (P<0.05). As we seen in this study, Turner *et al.* (1999) showed that unsaturated fatty acid had more efficient digestion rather than saturated fatty acid in young pullets. According these results feed conversion rat had no significant difference in starter period (P>0.05). The chicken fed corn oil and olive oil in their diet had lowest feed conversion and those fed tallow and flaxseed oil in

their diet showed higher feed conversion in growth period (P<0.05).

In finisher period diets containing corn and olive oil had best feed conversion rate (P<0.05). Absorption rate of different source of oil is varied and this can lead to different energy production of them (Nistan *et al.* 1997). This variation is proceed from several factors like form of lipid, PUFA to UFA ratio in mixture, sequence of PUFA and UFA in triglycerides, number of double bond and length of fatty acids. Although there is an interaction between additional lipid and existing lipids in diet (Ried *et al.* 1975). The positive effects of feeding corn oil on bird performance can be attributed to better relation of its fatty acids, better interaction of corn oil and diet lipid, and higher availability of this fat source.

**Table 1.** Feed compositions (%) of the diets.

| | starter | grower | Finisher |
|---|---|---|---|
| Corn | 58.62 | 64.26 | 63.48 |
| Soybean meal | 35.49 | 31.31 | 29.66 |
| Fat source [1] | 2 | 1.28 | 4.02 |
| DCP | 1.04 | 1.29 | 1.48 |
| Oyster powder | 1.19 | 1.01 | 1.03 |
| Common salt | 0.34 | 0.23 | 0.18 |
| Vitamins and minerals * | 0.25 | 0.25 | 0.25 |
| Minerals | 0.25 | 0.25 | 0.25 |
| Metyonin | 0.3 | 0.03 | - |
| Lysine | 0.3 | - | - |

[1] In different treatment, the relative fat source was used included of tallow, corn oil, sunflower oil, flaxseed oil and olive oil.

*Vitamin and mineral provided per kilogram of diet: vitamin A, 365000 IU; vitamin D3, 800000 IU; vitamin E, 7250 IU; vitamin K3, 810 mg; vitamin B1, 715 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2650 mg, vitamin B3, 3950 mg; vitamin B5, 12100 mg; vitamin B6, 1250 mg; vitamin B12, 6 mg; Choline chloride, 195000 mg, Manganese; 39500 mg; Iron, 19700 mg; Zinc, 39000 mg; Copper, 3900mg; Iodine, 400 mg.

**Table 2.** Nutritional composition (%) of diet containing tallow.

| | Starter | Growth | Finisher |
|---|---|---|---|
| ME (kcal/kg) | 2950 | 2950.0 | 3100.0 |
| CP | 21.70 | 19.50 | 18.00 |
| Ca | 1.00 | 0.90 | 0.80 |
| Total P | 0.68 | 0.68 | 0.64 |
| Available P | 0.68 | 0.45 | 0.40 |
| CF | 2.53 | 2.54 | 2.54 |
| Met+Sys | 0.90 | 0.79 | 0.67 |
| Lys | 1.40 | 1.27 | 1.10 |
| Na | 0.20 | 0.15 | 0.12 |
| Cl | 0.29 | 0.20 | 0.15 |
| 3-n | 0.07 | 0.07 | 0.07 |
| 6-n | 0.67 | 0.73 | 0.78 |
| 9-n | 0.57 | 0.64 | 0.66 |

*Antibody production*

Antibody titre comparison among diets has been shown in Table 9. In day 28 of age, the chickens fed diet containing sunflower had highest and those fed flaxseed oil had lowest ($P<0.05$). In a study, Broutghton *et al.* (1991) reported that effects of n-3 PUFAs on immune mediators production, is depend on total UFA and n-6 to n-3 ratio in diet. Zaki and Hadi (1995) observed that flaxseed oil increase antibody production in comparison to tallow. Probably lower antibody production in chicken fed flaxseed is related to decreasing triglyceride and cholesterol (Newman *et al.* 2002) lead to lower production of bile and lower lipid digestion. In other hand, lower levels of n-3 fatty acids may have positive effects on antibody production. Torki *et al.* (2000) and Kidd *et al.* (2004) showed lower levels of fish oil can improve immunity and antibody production in broilers.

**Table 3.** Nutritional composition (%) of diet containing corn oil.

| | Starter | Growth | Finisher |
|---|---|---|---|
| ME (kcal/kg) | 2950.00 | 2950.00 | 3100.00 |
| CP | 21.70 | 19.50 | 18.00 |
| Ca | 1.00 | 0.90 | 0.80 |
| Total P | 0.68 | 0.68 | 0.64 |
| Available P | 0.45 | 0.45 | 0.40 |
| CF | 2.53 | 2.54 | 2.54 |
| Met+Sys | 0.90 | 0.79 | 0.67 |
| Lys | 1.40 | 1.27 | 1.10 |
| Na | 0.20 | 0.15 | 0.12 |
| Cl | 0.29 | 0.20 | 0.15 |
| 3-n | 0.16 | 0.19 | 0.21 |
| 6-n | 2.25 | 2.40 | 2.51 |
| 9-n | 1.16 | 1.28 | 1.35 |

**Table 4.** Nutritional composition (%) of diet containing sunflower oil.

| | Starter | Growth | Finisher |
|---|---|---|---|
| ME (kcal/kg) | 2950 | 2950 | 3100 |
| CP | 21.70 | 19.50 | 18.00 |
| Ca | 1.00 | 0.90 | 0.80 |
| Total P | 0.68 | 0.68 | 0.64 |
| Available P | 0.45 | 0.45 | 0.40 |
| CF | 2.53 | 2.54 | 2.54 |
| Met+Sys | 0.90 | 0.79 | 0.67 |
| Lys | 1.40 | 1.27 | 1.10 |
| Na | 0.20 | 0.15 | 0.12 |
| Cl | 0.29 | 0.20 | 0.15 |
| 3-n | 0.02 | 0.02 | 0.02 |
| 6-n | 2.82 | 2.95 | 2.99 |
| 9-n | 1.16 | 1.28 | 1.35 |

Antibody titre against IBD (Gumboro) at day 28 of age was lowest for chickens fed sunflower oil. Sunflower oil had highest ratio of n-6 to n-3. This result was consistent whit other study (Sijben *et al.* 2000 , Friedman and Sklan , 1995) which high levels of oils containing n-6 poly unsaturated fatty acid (n-6 PUFA) decreased antibody response and in other hand was inconsistence with finding of some authors (Parmentier *et al.* 1997), in which n-6 PUFA increased antibody production. At day 42 of age there was no significant different between treatments in antibody production against IBV (Bronchite) but the difference in antibody production against IBD (Gumboro) was statically significant ($P<0.05$). The chicken fed flaxseed oil had lower antibody titre against IBD. Using Flaxseed oil can reduce efficiency of lipid absorption and decline cholesterol and bile and consequently decrease protein and antibody production. Moreover, high amount of n-3 fatty acid in addition of competition with n-6 fatty acids can inhibits cyclooxygenase. It can decrease the useful mediators involving antibody production ($PGE_3$, $LTB_5$). Also n-3 fatty acid increase peroxidation andit can be a reason of lower immune responses.

**Table 5.** Nutritional composition (%)of diet containing flaxseed oil.

| | Starter | Growth | Finisher |
|---|---|---|---|
| ME (kcal/kg) | 2950.00 | 2950.00 | 3100.00 |
| CP | 21.70 | 19.50 | 18.00 |
| Ca | 1.00 | 0.90 | 0.80 |
| Total P | 0.68 | 0.68 | 0.64 |
| Available P | 0.45 | 0.45 | 0.40 |
| CF | 2.53 | 2.54 | 2.54 |
| Met+Sys | 0.90 | 0.79 | 0.67 |
| Lys | 1.40 | 1.27 | 1.10 |
| Na | 0.20 | 0.15 | 0.12 |
| Cl | 0.29 | 0.20 | 0.15 |
| 3-n | 0.55 | 0.88 | 0.95 |
| 6-n | 1.77 | 1.86 | 1.94 |
| 9-n | 1.21 | 1.34 | 1.41 |

**Table 6.** Nutritional composition (%) of diet containing olive oil.

| | Starter | Growth | Finisher |
|---|---|---|---|
| ME (kcal/kg) | 2950.00 | 2950.00 | 3100.00 |
| CP | 21.70 | 19.50 | 18.00 |
| Ca | 1.00 | 0.90 | 0.80 |
| Total P | 0.68 | 0.68 | 0.64 |
| Available P | 0.45 | 0.45 | 0.40 |
| CF | 2.53 | 2.54 | 2.54 |
| Met+Sys | 0.90 | 0.79 | 0.67 |
| Lys | 1.40 | 1.27 | 1.10 |
| Na | 0.20 | 0.15 | 0.12 |
| Cl | 0.29 | 0.20 | 0.15 |
| 3-n | 0.04 | 0.05 | 0.05 |
| 6-n | 1.12 | 1.20 | 1.29 |
| 9-n | 2.39 | 2.75 | 2.82 |

At least four modes of action have been proposed to explain the potential action of fatty acids on the modulation of immune system in both animals and humans. Accordingly, immune system modulation by dietary lipids may be attributed to changes in the composition of membrane phospholipids, lipid peroxidation, alteration of gene expression, or productions of eicosanoids, cytokines and arachidonic acid (Peck, 1994; Friedman and Sklan, 1995; Depablo, 2000).

**Table 7.** Fatty acid composition (%) of lipids.

| Fatty acids | Sources of lipid | | | | |
|---|---|---|---|---|---|
| | Tallow | corn oil | Sunflower oil | Flaxseed oil | Olive oil |
| C14:0 | 3.25 | 0.25 | 0.15 | 0.12 | 0 |
| C15:0 | 0.48 | 0 | 0 | 0.09 | 0 |
| C16:0 | 27.5 | 10.55 | 6.79 | 5.35 | 12.4 |
| C16:1 | 2.64 | 0.15 | 0.14 | 0.06 | 0.89 |
| C18:0 | 21.5 | 1.82 | 4.52 | 3.85 | 2.8 |
| Tranc C18:1 n-9 | 4.25 | 0 | 0 | 0 | 0 |
| C18:1 n-9 | 33.2 | 27.3 | 26 | 17.1 | 69.2 |
| C18:1 n-7 | 1.78 | 0.64 | 0.58 | 0.75 | 2.49 |
| C18:2 n-6 | 4.21 | 58.15 | 62.3 | 14.9 | 9.69 |
| C18:3 n-6 | 0.03 | 0 | 0 | 0 | 0 |
| C20:0 | 0.21 | 0.45 | 0.33 | 0.2 | 0.48 |
| C18:3 n-3 | 0.47 | 1.14 | 0.1 | 55.5 | 0.81 |
| C20:1 n-9 | 0.32 | 0.25 | 0.19 | 0.18 | 0.35 |
| SFA | 52.94 | 13.07 | 11.79 | 9.61 | 15.68 |
| UFA | 44.26 | 87.48 | 89.17 | 88.43 | 82.54 |
| MUFA | 42.19 | 28.34 | 26.91 | 18.09 | 72.93 |
| PUFA | 4.71 | 59.29 | 62.40 | 70.40 | 10.50 |
| n-9 | 37.77 | 27.55 | 26.19 | 17.28 | 69.55 |
| n-6 | 4.24 | 58.15 | 62.30 | 14.90 | 9.69 |
| n-3 | 0.50 | 1.14 | 0.10 | 55.50 | 0.81 |
| n-6:n-3 | 8.48 | 51.01 | 623.00 | 0.27 | 11.96 |
| SFA:UFA | 1.20 | 0.15 | 0.13 | 0.11 | 0.19 |

*SFA **saturated fatty acids; UFA *unsaturated fatty acids; MUFA *monounsaturated fatty acids; PUFA **polyunsaturated fatty acids.

**Table 8.** Performance of chickens fed different treatments.

| | Starter | Grower | Finisher |
|---|---|---|---|
| Feed consumed (g) | | | |
| T1 | $24.4^{a}$ | $88.2^{a}$ | $160.3^{ab}$ |
| T2 | $23.7^{c}$ | $86.1^{ab}$ | $161.6^{ab}$ |
| T3 | $24.0^{abc}$ | $86.0^{ab}$ | $163.8^{a}$ |
| T4 | $24.3^{ab}$ | $85.3^{b}$ | $159.7^{b}$ |
| T5 | $23.8^{bc}$ | $85.5^{b}$ | $160.4^{ab}$ |
| SE | 0.1 | 2.07 | 5.6 |
| Weight gain (g) | | | |
| T1 | $17.3^{ab}$ | $51.2^{bc}$ | $80.6^{c}$ |
| T2 | $16.8^{c}$ | $50.7^{c}$ | $82.2^{c}$ |
| T3 | $17.2^{bc}$ | $52.2^{ab}$ | $83.6^{bc}$ |
| T4 | $17.6^{a}$ | $52.4^{ab}$ | $87.7^{a}$ |
| T5 | $17.0^{bc}$ | $53.1^{a}$ | $85.8^{ab}$ |
| SE | 0.075 | 0.65 | 4.68 |
| Feed conversion | | | |
| T1 | $1.41^{a}$ | $1.72^{a}$ | $1.99^{a}$ |
| T2 | $1.41^{a}$ | $1.69^{ab}$ | $1.96^{ab}$ |
| T3 | $1.40^{a}$ | $1.64^{bc}$ | $1.95^{ab}$ |
| T4 | $1.37^{a}$ | $1.62^{c}$ | $1.82^{c}$ |
| T5 | $1.39^{a}$ | $1.61^{c}$ | $1.87^{bc}$ |
| SE | 0.001 | 0.001 | 0.0047 |

* T1: Tallow, T2: Corn oil, T3: Sunflower oil, T4: Flaxseed oil, T5: Olive oil.

*Heretophil to lynphocyte (H:L) ratio*

A shown in Table 10, at day 28 of age, sunflower oil had the highest level of heterophil and corn oil showed the lowest count of heterophil. There were no differences among treatments for these measured parameters in except of corn oil (P<0.05). Chicks fed corn oil and flaxseed oil showed the highest lymphocyte count and those fed diet containing sunflower oil had the lowest count of lymphocyte (P<0.05). Differences among treatment for H:L ratio was found (P<0.05). The chicken fed corn oil had the lowest H:L ratio and those fed sunflower oil had the highest ratio (P<0.05). It was reported that animals cannot convert n-6 to n-3 fatty acids and vice versa. High rang of n-3 fatty acids is considered to have beneficial effects on produce immune-mediated component (Calder, 2006) and n-6 PUFA and n-3 PUFA are detected to have competition (Hill *et al.*, 2007). At day 28 of age, H:L ratio for birds fed flaxseed oil, was less than birds fed tallow and sunflower.

**Table 9.** Antibody titres (log2) of chicks against IBV and IBD viruses.

| | Day | |
|---|---|---|
| | 28 | 42 |
| IBV | 749 $^{ab}$ | 586 |
| T1 | | |
| T2 | 670 $^{ab}$ | 529 |
| T3 | 856 $^{a}$ | 571 |
| T4 | 640 $^{b}$ | 551 |
| T5 | 721 $^{ab}$ | 557 |
| SEM | 190.6 | 199.6 |
| IBD | | |
| T1 | 2742 $^{ab}$ | 5371 $^{a}$ |
| T2 | 2762 $^{ab}$ | 4478 $^{ab}$ |
| T3 | 2552 $^{b}$ | 4689 $^{ab}$ |
| T4 | 2681 $^{ab}$ | 4133 $^{b}$ |
| T5 | 2904 $^{a}$ | 4818 $^{ab}$ |
| SEM | 249.2 | 1086 |

* T1: Tallow, T2: Corn oil, T3: Sunflower oil, T4: Flaxseed oil, T5: Olive oil.

**Table 10.** Heterophil to Lymphocyte ratio of chicks in different treatments.

| | Day | |
|---|---|---|
| | 21 | 42 |
| H/L | | |
| T1 | 0.35$^{ab}$ | 0.24$^{bc}$ |
| T2 | 0.23$^{d}$ | 0.20$^{c}$ |
| T3 | 0.39$^{a}$ | 0.37$^{a}$ |
| T4 | 0.28$^{c}$ | 0.27$^{b}$ |
| T5 | 0.31$^{bc}$ | 0.28$^{b}$ |
| SEM | 0.0011 | 0.001 |

* T1: Tallow, T2: Corn oil, T3: Sunflower oil, T4: Flaxseed oil, T5: Olive oil.

No significant difference was found between bird fed flaxseed oil and olive oil in H:L ratio (P<0.05). At day 42 of age, heterophil count in birds fed diet containing corn oil was lower than birds fed sunflower oil, flaxseed oil and olive oil. There were no significant different for this measured parameter between birds fed corn oil and tallow (P<0.05). Lymphocyte count at day 42 of age was the same among treatments in except for bird fed corn oil that had the lowest Lymphocyte count (P<0.05). Birds fed

sunflower oil significantly had the highest H:L ratio and those fed diet containing corn oil had the lowest ratio (P<0.05). There was no significant different in H:L ratio in birds fed diets containing tallow, flaxseed oil and olive oil at this age (P<0.05).

Corticosterone has been found to be immunosuppressive (Gross 1992; El- Lethy 2003), inhibiting the production and actions of antibodies, lymphocyte function, and leukocyte population (Post *et al.* 2003; Siegel 1995). Based on the results in Table 10, chicks fed diet containing sunflower oil may be experienced physiological stress. The heterophil to lymphocyte ratio has been accepted as a reliable index for determining stress in poultry (Post *et al.* 2003). The increases in heterophil to lymphocyte ratio in chicks fed diet containing sunflower oil may be attributed to increased corticosterone secretion, which finally resulted in decrease the antibody titers (Vleck *et al.,* 2000)) in day 28 of age.

**Conclusion**

The results of this study indicated that Sunflower oil and flaxseed oil had negative effect on immune response and performance through high n-6 to n-3 ratio and low efficient energy production respectively. Corn oil, having better relation of its fatty acids, better interaction of corn oil and diet lipid, and higher availability of this fat source showed the best results in performance and immune responses.

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