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Effect of different sources of lipid on heterophil to lymphocyte ratio and serum corticosterone concentration in broiler chickens

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Abstract

The aim of this study was to investigate the effect of different sources of lipid on Heterophil:Lymphocyte (H:L) ratio, as an index of stress, and serum corticosterone concentration 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. The counts of heterophil, lymphocyte and serum corticosterone concentration were measured at days 28 and 42 of age. Chicks fed diet containing sunflower oil showed the highest H:L ratio ($P<0.05$) and those fed diet containing corn oil had the lowest H:L ratio at days 28 and 42 of age ($P<0.05$). Feeding flaxseed oil significantly showed the highest level of blood corticosterone concentration at day 28 of age. In this study, it was concluded that H:L ratio is affected by lipid source and corn oil among used lipids had the lowest H:L ratio, that is useful for wellbeing and health of broiler chicks.

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Introduction

Heterophils to lymphocyte (H:L) ratio is used to determine stress condition and is related to increasing level of glucocorticoid (Davis and Maerz, 2008). Stress or glucocorticoid treatments could increase the numbers of heterophils and decrease the lymphocyte counts. The H:L ratio appears to be a more reliable indicator of stress than corticosteroid levels (Gross and Siegel, 1983) and blood corticosteroids may be effected by many short-term factors. Lymphocytes play an important role in the immune response of the body and heterophil is involved in the inflammatory response. Heterophil numbers increase in response to infection and inflammation. As a result, H:L ratio could be an index of inflammation and stress in birds (Gross and Siegel, 1983).

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 the literature, reports on the effects of fatty acids and different lipid sources on functionality of immune system are numerous; however, no study exists concerning the effect of addition of different lipids to diet of broiler chicks on serum corticosterone concentration and H:L ratio. Feeding diets containing high levels of fish oil were suppressed proliferation of Lymphocytes compared with feeding diets rich in other lipids such as lard, coconut oil, corn oil, safflower oil or linseed oil (Alexander and Smythe, 1988; Fristche *et al.,* 1991; Kelly *et al.,* 1988; Yaqoob *et al.,* 1994; Al-Khalifa *et al.,* 2012). Lymphocyte proliferation was significantly decreased after feeding diets rich in soybean oil to broiler chickens (Sadeghi *et al.,* 2013). The available findings concerning fatty acids or lipid sources on lymphocytes and heterophil counts are very controversial. Therefore this study was conducted to evaluate the effects of the different dietary sources of lipids on H:L ratio and serum corticosterone concentration in broiler chickens.

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. Fatty acid profile of each source of lipid (Table 1) 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 2. As seen in Table 2, the only difference among diets was the source of lipid.

Blood sampling and analysis

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. 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. These tubes transferred to Laboratory for counting heterophil and lymphocyte according to the method of Ye *et al.* (2006). 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.

Statistical Analysis

The chicken (10 determinations per treatment) was the experimental unit for white blood cells and corticosterone concentration. 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 $P \leq 0.05$ in the ANOVA table, means were compared for significant differences using the Tukey test of SAS.

Results and discussion

The results of corticosterone concentration, heterophil, lymphocyte, and H:L ratio are shown in Table 3. 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 ($P<0.05$). There was no significant difference for corticosterone concentration in chicken fed corn oil and sunflower oil ($P<0.05$). These results agreed with a study (Claudia *et al.* 2010). Previously, it was shown that H:L ratio and serum corticosterone had no intact correlation as index of stress, because blood concentration of corticosteroids is effected by many factors (Gross and Siegel , 1983). Among these lipid sources, flaxseed oil has the lowest n-6:n-3 ratio and the highest level of n-3 PUFA. 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, the highest concentration of corticosterone was found in the birds fed flaxseed oil, whereas H:L ratio for these birds was less than birds fed tallow and sunflower.

Table 1. Fatty acid compositions of used lipids.

Fatty acids	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.

No significant difference was found between bird fed flaxseed oil and olive oil in H:L ratio ($P<0.05$). This result showed that corticosterone concentration and H:L ratio have different effects in stress conditions (Davis and Maerz, 2008) and blood corticosterone may be affected by other factors rather than PUFA's (Gross and Siegel, 1983). In addition, this finding indicated that corticosterone is not a suitable index for stress and H:L ratio is less variable and longer lasting than corticosterone (McFarlane and Curtis, 1989; McKee and Harrison,1995).

Table 2. Nutritional compositions of the diets.

Ingredients (%)	starter	grower	Finisher
Corn	58.62	64.26	63.48
Soybean meal	35.49	31.31	29.66
Lipid 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	-	-
Nutrient content			
ME kcal/kg	2950	2950	3100
CP (%)	21.7	19.5	18
Ca (%)	1	0.9	0.8
Total P (%)	0.68	0.68	.64
Available P (%)	0.45	0.45	0.4
CF (%)	2.53	2.54	2.54
Met + Sys (%)	0.9	0.79	0.67
Lys (%)	1.4	1.27	1.1
Na (%)	0.2	0.15	0.12
Cl (%)	0.29	0.2	0.15

Table 3. Serum corticosterone, heterophil and lymphocyte counts of chicks fed different sources of lipids.

	Day 21 of age	Day 42 of age
Corticosterone (µg/dl)		
T1*	0.85^{b}	0.74^{a}
T2	0.75^{c}	0.65^{b}
T3	0.75^{c}	0.62^{b}
T4	0.95^{a}	0.72^{a}
T5	0.64^{d}	0.77^{a}
SEM	0.06	0.059
Heterophils (%)		
T1	24.2^{ab}	18.2^{cd}
T2	17.7^{c}	16.2^{d}
T3	25.7^{a}	25.2^{a}
T4	22.0^{b}	20.0^{bc}
T5	23.2^{ab}	21.0^{b}
SEM	4.73	2.81
Lymphocyte (%)		
T1	69.2^{bc}	76.7^{a}
T2	77.0^{a}	80.0^{a}
T3	65.7^{c}	68.5^{b}
T4	76.0^{a}	75.0^{a}
T5	73.7^{ab}	75.2^{a}
SEM	13.08	16.83
H:L ratio		
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	0.0013	0.0010

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

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). Variation in serum corticosterone at day 42 of age was obviously less than day 28 of age. Birds fed corn oil and sunflower oil had the lowest corticosterone concentration and those fed tallow, flaxseed oil and olive oil had the highest concentration (P<0.05). There was no significant different in birds fed tallow, flaxseed oil and olive oil (P<0.05).

Flaxseed oil at day 28 either day 42 of age showed the highest level of corticosterone and count of Lymphocytes. Although feeding long-chain n-3 fatty PUFA was shown to has immunosuppression effects and could decrease the production of cytokines, som studies (Hinds and Sander, 1993; Wander *et al.,* 1997) have shown that feeding more amount of n-3 PUFA in diet is not immunosuppressive and sometimes can increase immune function such as lymphocyte proliferation (Robinson and Field, 1998; Wu *et al.* 2011). In addition, high level of corticosterone in blood and it's reverse relation to H:L ratio in our study may be related to handling the chicks during sampling and secretion of corticosterone after stress.
Considering the birds fed sunflower oil and the level of n-6:n-3 PUFA in diet can found that it directly affect H:L ratio that agreed with other studies (Hill and Sander,1993; Wang *et al.,* 2011). The linoleic acid a n-6 PUFA in sunflower oil converted to arachidonic acid in the body. It is a precursor of inflammatory compounds such as prostaglandins and leukotrienes that have important roles in the regulation of immunity. High n-6:n-3 PUFA ratios could increase heterophil migration to peripheral blood, resulting in a high H:L ratio (Al-Murrani *et al.* 1997; Calder, 2005). Feeding n-3 PUFA in diet such as flaxseed oil, lead to replacement of arachidonic acid with EPA and DHA in cells membrane including immune cells. Because of these events, arachidonic acid inflammatory derived mediators (Eicosanoids) like protaglandins and leukotriens decrease and then H:L decline. Probable mechanism is the decline in arachidonic acid and competition of EPA and DHA with arachidonic acid for cyclooxygenase and lipoxygenase. Corn oil with higher level of n-6:n-3 PUFA showed lower H:L ratio than flaxseed oil and olive oil. Probably it is because of fatty acid profile of corn oil, it's specific n-6:n-3 ratio, higher bioavailability of corn oil and higher pureness of this lipid source.

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