



Inclusion of vegetable oils in diets of broiler chicken raised in hot weather and effects on antioxidant capacity, lipid components in the blood and immune responses

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ABSTRACT. The aim of this study was to assess the effects of vegetable oils on the antioxidant capacity, blood lipid components and immune responses in chickens raised in hot climate. In a completely randomized design, 300 chicks were assigned to four treatments and five replicates with 15 chicks per each. The highest concentration of malondialdehyde was found in broilers fed palm oil ($p < 0.05$). Chickens fed corn oil had the lowest and those fed flaxseed oil had the highest antioxidant enzymes activities ($p < 0.05$). The highest hepatic Alanine-transferase and Aspartate-transferase was found in chickens fed corn oil and the lowest levels in those received flaxseed and olive oils ($p < 0.05$). The highest hypersensitivity was in chickens fed palm oil and the lowest was for olive oil. The highest antibody titer against sheep red blood cells was found in chickens fed flaxseed oil and the lowest titer was for those received palm oil ($p < 0.05$). The highest triglyceride, cholesterol and very low density lipoprotein and the lowest high density lipoprotein was found in chickens fed palm oil and vice versa for flaxseed and olive oils. Olive and flaxseed oils had beneficial effects on antioxidant activity and lipid attributes and palm oil had detrimental effects on these parameters that related to the poultry health.

Keywords: animal health; antibody titer; heat stress; hepatic enzymes; lipid attributes.

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Introduction

Major poultry production industry has been located in the zones with hot and humid climate (Daghir, 2008). In these areas, producers have to utilize systems or nutritional management for control of housing or body temperature, to ameliorate the harmful effects of heat stress on the performance and health of chickens (Lara & Rostagno, 2013). In many tropical regions, the cost for cooling broiler houses is very high and hence focuses are on the nutritional management (Konca, Kirkpinar, & Çabuk, 2009). The management mainly includes: dietary supplementation of antioxidant (Tawfeek, Hassanin, & Youssef, 2014; Delles, Xiong, True, Ao, & Dawson, 2014), electrolyte (Ahmad et al., 2008), mineral (Ebrahimzadeh, Farhoomand, & Noori, 2012) or vitamins (Ipek, Canbolat, & Karabulut, 2007) and lipids (Sadeghi, Mirmohseni, Shawrang, & Aminafshar, 2013; Taleb, Sadeghi, Shawrang, Chamani, & Aminafshar, 2017; Tari, Sadeghi, & Mousavi, 2020). In some countries, the inclusion of vegetable oils or animal fat in diet of broiler chickens is common. The fatty acids content of oils are very differ, especially the type of poly unsaturated fatty acids (PUFA). The type of Pufa can affect some immune parameters in animals. In this regard, researchers (Sadeghi et al., 2013; Alagawany et al., 2019) reported that high levels of oils containing n-6 PUFA resulted in a decrease antibody response against antigens or immunoglobulin production.

In the literature, there was no report concerning the effect of oils on blood lipid components and immune function, especially in the heat stress condition. It was hypothesized that in the heat stress condition, dietary addition of oil sources with poly unsaturated fatty acids (PUFA) could enhance the health through increase the immune function by changing immune cell membrane composition and fluidity compare to saturated fatty acids (SFA). Therefore, the main objective was to evaluate and compare the effects of different vegetable oils on the antioxidant capacity, blood lipid components and immune responses in broiler chicken raised in hot climate.

Material and methods

This experiment was carried out under the ethical guidelines of Department of Animal Science, Islamic Azad University of Tehran Science and Research Branch (93/987-2014).

Animals and dietary treatments

This study was done in a research farm located in Kianmehr (Alborz Province, Iran) during summer 2019. One-day broiler chicks ($n = 300$, Cobb 500) was purchased from a commercial hatchery and housed equipped with environment control sets. Chicks were assigned to four treatment groups (4 types of vegetable oils) with 5 replicates and 15 chicks per each replicate based on completely randomized design. Cobb 500 requirement recommendations was used for formulation of rations. Dietary treatments were iso-nutritive with the same ingredients, but included one of vegetable oils at the same level (Table 1). Treatments were 1: a saturated oil (palm oil), 2: a source of n-3 fatty acid (flaxseed oil), 3: a source of n-6 fatty acid (corn oil) and a source of n-9 fatty acid (olive oil). Oils were included in the starter, grower and finisher rations as 1.5, 3 and 4%, respectively. Metabolizable energy content of palm oil was lower compared to other oils and the related ration was balanced by higher palm oil (1.6, 3.15, and 4.3%) and little changes in other ingredients. Chicks had free access to fresh water and experimental diet. For inducing heat stress in chickens, house temperature was raised to $34 \pm 1^\circ\text{C}$ for 6 hours per day from day 11 to 41 of age. The increase in temperature in each day was done from 10:00 to 16:00 and then house temperature decreased to $22 \pm 1^\circ\text{C}$. The relative humidity of house was maintained in 60-70%.

Blood sampling

On days 28 of age, 3 mL of blood sample was collected from wing vein of two birds in each replicate (8 birds per each treatment), centrifuged at $1500 \times g$ for 10 min. and the serum was separated, then stored at -20°C until analysis.

Enzyme activity measurement

The glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities in the serum of broilers was measured by using commercial kits (Randox Laboratories Ltd. Ardmore, Crumlin, UK) according to the manufacturer guideline.

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were measured using photometric analyzer (Pars Azmoon Co., Iran).

Serum levels of malondialdehyde (MDA) as a product of lipid peroxidation was measured according to the thiobarbituric acid assay. The contents of MDA were expressed as $\mu\text{mol mg-protein}^{-1}$.

Table 1. Feed ingredients (% , as fed) and composition of the basal diets.

	Starter (d 1 to11)	Grower (d 12 to 22)	Finisher (d 23 to 42)
Yellow corn	49.06	53.04	53.44
Soybean meal (44%)	38.31	31.11	30.02
Wheat grain	5.02	7.00	9.00
Fish meal (66%)	2.50	2.50	0.00
Vegetable oil	1.50	3.00	4.00
Di-calcium phosphate	1.53	1.44	1.52
Oyster shell	0.95	0.84	1.02
Salt	0.15	0.14	0.18
Vitamin-mineral premix*	0.60	0.55	0.50
Coccidiostat	0.04	0.04	0.04
DL- Methionine	0.23	0.22	0.20
L-Lysine	0.11	0.12	0.08
Calculated composition			
ME (kcal kg^{-1})	2990	3100	3150
Crude protein (%)	22.2	20.2	18.2
Calcium (%)	1.00	0.97	0.92
Ava. Phosphorus (%)	0.50	0.48	0.45

*Vitamin and mineral provided per kilogram of diet: vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg, vitamin B5, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg; Choline chloride, 200000 mg, Manganese; 40000 mg; Iron, 20000 mg; Zinc, 40000 mg; Copper, 4000 mg; Iodine, 400 mg.

CBH response and SRBC antibody titer

The cell mediated immune response was assessed by a cutaneous basophil hypersensitivity test using Phytohemagglutinin P (PHA-P) as described by Boostani, Sadeghi, Mousavi, Chamani, and Kashan (2015). At days 28 and 42 of age, the interdigital skin of the right foot (2 broilers per replicate) was measured by a Caliper with an accuracy of 0.01 mm. Immediately after measurement, 100 µg of PHA-P (suspended in 0.1 mL of PBS) was injected intradermally in interdigital skin between the second and third digits of the right foot. The left foot was injected with 0.1 mL of physiological saline as a sham control. The interdigital skin swelling was measured 24 hours after injection. The response was measured by subtracting the skin thickness of the first measurement from the skin thickness 24 hours after dermal injection .

On day 28 of age, two birds were randomly selected from each replicate and were injected intravenously by 0.2 mL of 5% sheep red blood cells (SRBC) diluted in PBS and these broilers identified by color. The broilers were bled from the brachial vein at day 35 of age (1 week later) and blood samples (2 mL) from 2 birds per each replicate were centrifuged (2500 × g, 10 min., 17°C) and the serum was stored at -20°C. Antibody titers against SRBC were measured as described by Sadeghi, Safaei, and Aminafshar (2014) and expressed as the log 2 of the reciprocal of the highest serum dilution giving complete agglutination.

Lipid components analyses

The obtained sera were used also for spectrophotometric analysis of serum triacylglycerol, total cholesterol and high density lipoprotein (HDL) measured by routine laboratory techniques (Pars Azmoon Co., Iran). Very low density lipoprotein cholesterol (VLDL) was calculated by division of triacylglycerol by 5 (mg dL⁻¹) while low density lipoprotein (LDL) was calculated (mg dL⁻¹) by subtracting the sum of HDL and VLDL from total cholesterol.

Statistical analysis

The Shaapiro-Wilk test of normality was used in order to define normal distribution of data. If data were not normal the BOXCOX transformation used to normalize them. Normal Data was analyzed based on ANOVA appropriate for completely randomized design to determine the effects of treatment groups on traits using SAS software (version 9.1, SAS Institute, Cary, NC, USA). Mean comparison was done using the Tukey test. Probability values of less than 0.05 were considered significant.

Results and discussion

The effects of vegetable oil inclusion in diet on MDA level, antioxidant capacity and hepatic enzymes (ALT and AST) released in blood in Table 2. The highest concentration of MDA was found in broilers fed palm and flaxseed oils ($p < 0.05$). This result is consistent with finding of Bobadoye, Onibi, and Fajemisin (2009) who found malondialdehyde concentration was the lowest for birds on the control and increased significantly with increasing levels of palm oil sludge. There was no differences for MDA level among chickens fed oils containing Pufa ($p > 0.05$). All broilers in this study was in heat stress and serum MDA concentration is relatively high. Malonaldehyde is formed as an end product of lipid peroxidation. Palm oil was solid in environment temperature and preserved in cold condition, but flaxseed, corn and olive oils used in this study was prepared freshly and protected by antioxidants, hence, there was no difference for serum MDA level among them. There is an evidence that showed n-3 PUFA can scavenge H₂O₂ and lipid peroxides and thus can enhance the activities of the hepatic antioxidant enzymes and decreased MDA concentration (Bhattacharya et al., 2003). A report showed that n-3 PUFA supplementation results in a lower MDA concentration by activation the enzymatic and non-enzymatic antioxidant systems (Delles et al., 2014). Swanson, Block, and Mousa (2012) stated that flaxseed oil supplementation had a low but significant reducing effect on the malondialdehyde concentration in broilers.

Chickens fed diet containing corn oil had the lowest SOD and GPX activities and those fed flaxseed had the highest antioxidant enzymes activities in serum ($p < 0.05$). There was no difference between olive oil and palm oil for antioxidant enzyme activities. The activities of antioxidant enzymes in these oils were lower than flaxseed oil and higher than corn oil ($p < 0.05$). An interesting study exist concerning fresh or oxidized oil sources and antioxidant activities in broiler, which indicate oil sources had effect on SOD and GPX activities (Lindblom, Gabler, Bobeck, & Kerr, 2019). Available data concerning the oil source on these enzymes activity is limited.

Table 2. The effects of oils inclusion in diet on antioxidant and liver enzymes.

Treatment	MDA ($\mu\text{m mg}^{-1}$ proteins)	SOD (Units per mg of proteins)	GPX (Units per mg of proteins)	ALT (IU L^{-1})	AST (IU L^{-1})
Palm oil	9.78a	5.66b	4.36b	41.85b	81.41b
Flaxseed oil	10.45a	6.46a	4.85a	32.03c	63.29c
Corn oil	8.06b	4.08c	2.84c	45.74a	85.39a
Olive oil	8.32b	5.31b	4.44b	29.85c	64.31c
SEM	0.157	0.149	0.146	1.929	2.965

MDA: malondialdehyde; SOD: superoxide dismutase; GPX: glutathione peroxidase; ALT: Alanin aminotransferase; AST: Aspartate transaminase. Abc: Within columns, values with different superscripts differ significantly ($p \leq 0.05$).

The highest ALT and AST was seen in chickens fed corn oil and the lowest levels in those received flaxseed and olive oils ($p < 0.05$). This impact of corn oil may be related to n-6 fatty acids that induce inflammation processes. Inflammation could increase the tissue sensitivity to damages and elevation of hepatic enzymes in the serum (Aminoroaya, Sadeghi, Ansari-Pirsaraei, & Kashan, 2016). The finding of this study is in agreement with El-Bahra and Ahmed (2012) who reported olive oil supplement resulted in the lower enzymatic activity of ALT compared to control.

Cutaneous basophil hypersensitivity reaction to phytohemagglutinin P injection and antibody titers presented in Table 3. Cutaneous basophil hypersensitivity elicited in broilers by an intradermal injection of Phytohemagglutinin-P (PHA-P) is a thymus dependent response mediated by thymic cells (Boostani et al., 2015). The highest hypersensitivity at days 28 and 42 of age was seen in chickens fed palm oil and the lowest was for olive oil. Corn and flaxseed oils showed moderate hypersensitivity. In the literature, report not found concerning effects of lipids on this parameter in poultry.

The highest SRBC titer was found in chickens fed flaxseed and olive oils and the lowest titer was for those received palm oil ($p < 0.05$). This result was inconsistent with the findings of Das et al. (2014), who reported that chicken received palm oil had higher antibody production than those fed n-3 or n-6 fatty acids sources. It seems that, these discrepant resulting might be associated with the types and dose of oil used.

The result of this study was consistent with studies that reported that n-3 fatty acids was shown to enhance the antibody response of chicks to sheep red blood cells than the birds that were not treated with them (Sadeghi et al., 2014). Inclusion of flaxseed oil in diet improved immune response, probably because of the effects on long chain n-3 PUFA on eicosanoid levels (Swanson et al., 2012). Also, n-3 PUFAs have shown beneficial immune responses in infectious bursal disease challenged broilers (Yang, Zhang, Guo, Jiao, & Long, 2010).

Serum lipid profile of broilers at day 28 of age reported in Table 4. The highest triglyceride level was found in chickens fed palm oil. Our finding agreed with previous report that showed palm oil produced a significant rise in serum triglyceride, cholesterol and VLDL (Adeyemi et al., 2016). High saturated fatty acids (SFAs) and low PUFAs contents in palm may be the reason of these results.

Lower concentration of serum triacylglycerol was found in broilers fed diets containing flaxseed oil, corn oil and olive oil as dietary n-3, n-6 and n-9 fatty acid sources. Consistence with our result, Moslehi, Sadeghi, Shawrang, and Aminafshar (2016) showed that dietary Pufa may reduce hepatic fatty acid and triacylglycerol synthesis. Moreover, an inhibition of the activity of Δ^9 -desaturase, which leads to a limited triacylglycerol secretion from the liver to the blood reported, also, PUFAs may show a higher rate of β -oxidation and as a result a higher rate of uptake of triacylglycerol from blood stream to tissue compared with saturated fatty acids such as those found in palm oil.

Concerning dietary inclusion of olive oil, our finding are similar to previous reports showing that chicks or rats fed an olive oil-rich diet had lower values of serum triglyceride and significantly higher level of serum HDL (Namayandeh, Kaseb, & Lesan, 2013). Also, feeding rats on diet containing olive oil significantly improved lipid profile as it reduced serum triglyceride, cholesterol and lipoproteins.

Cholesterol level was the highest in chickens fed palm oil and the lowest in those received olive oil. In contrast to our finding, researchers who found that diets containing different lipid sources did not affect blood cholesterol values (Sadeghi et al., 2014; Moslehi et al., 2016). In general, these results agree with the findings of other authors who reported changes in the concentration of serum lipids of chickens with the dietary lipid sources differing in their degree of saturation (Adeyemi et al., 2016; Tari et al., 2020). The discrepancies between studies on the lipid content of serum maybe attributed to the genetic, sex and dietary factors.

The highest HDL was for olive oil and the lowest was for palm oil and vice versa for LDL. Chickens received palm oil had the highest VLDL. There was no differ for VLDL among other oils. In experiments on rats and humans, the researchers concluded that diets containing different amounts of PUFAs reduce triglycerides, serum cholesterol and LDL, while they increased blood HDL (Ortiz-Munoz et al., 2009).

Table 3. The effects of oils inclusion in diet on hypersensitivity reaction and antibody titer.

		Palm oil	Flaxseed oil	Corn oil	Olive oil	SEM
CBH ² Test (mm)	28d	0.775a	0.702b	0.750b	0.555c	0.046
	42 d	0.722a	0.612b	0.610b	0.482c	0.052
SRBC (log 2)		6.95c	10.04a	8.20b	9.60ab	0.163

a-d: Within rows, values with different superscripts differ significantly ($p < 0.05$). ²Cutaneous basophil hypersensitivity reaction to phytohemagglutinin P injection into the interdigital skin, and measured as skin swelling before and 24 hours after injection; SRBC: sheep red blood cells.

Table 4. The effect of different lipid sources in diets on serum lipid profile of broilers at day 28 of age.

Treatment	Triglyceride mg dL ⁻¹	Cholesterol mg dL ⁻¹	HDL mg dL ⁻¹	LDL mg dL ⁻¹	VLDL mg dL ⁻¹
Palm oil	50.71a	134.08a	55.44c	68.49a	10.14a
Flaxseed oil	38.04b	120.05b	64.71ab	47.73c	7.60b
Corn oil	41.04b	123.08b	61.65b	53.22b	8.20b
Olive oil	40.64b	114.08c	68.06a	37.81d	8.12b
SEM	3.235	4.081	2.666	3.53	1.86

HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein. ab: Within columns, values with different superscripts differ significantly ($p \leq 0.05$).

Conclusion

It was concluded that olive and flaxseed oils had beneficial effects on antioxidant activity and lipid attributes and palm oil had detrimental effects on these parameters that related to the animal health.

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