



# Dietary vegetable oils inclusion on the performance, hormonal levels and hsp 70 gene expression in broilers under heat stress

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**ABSTRACT.** The aim of the present study was to compare the effects of unsaturated and saturated oils on the performance, hormonal levels and hsp gene expression in broiler chickens exposed to heat stress. 300 one-day male broiler chicks were assigned to 4 treatments (Diets containing palm, corn, linseed or olive oils) with 5 replicates. At day 28 of age, 2 chickens were removed from each replicate, then blood samples and liver tissue samples were collected for analyses. Feeding linseed and olive oil reduced feed conversion ratio compared to corn and palm oils. The lowest level of insulin was for chickens fed linseed oil and corn oil. The highest level of corticosterone was found in chickens fed palm oil and the lowest level was for those received linseed oil. Chickens received linseed and corn oils had the highest levels of T3 and T4 and those fed palm and olive oils had the lowest levels. The highest HSP 70 gene expression was for chickens fed diet containing olive and linseed oils and the lowest one was for those fed corn and palm oils. It was concluded that olive oil and linseed oil could improve performance and heat tolerance of chickens under heat stress.

**Keywords:** corticosterone; insulin; heat shock protein; heat tolerance.

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## Introduction

Vegetable oils are used in the industrial broiler diets as supplementary energy source and an important source of essential fatty acids (Leeson, Diaz, Gonzalo, & Summers, 1995). In addition, lipids have lower heat increment than carbohydrates (Musharaf & Latshaw, 1999), therefore a part of energy of carbohydrate in broiler rations substituted with energy of lipids during heat stress period (Syafwan, Kwakkel, & Versteegen, 2011).

Some studies speculated that inclusion of vegetable oils in broiler diets could enhance the performance (Ailhaud et al., 2006; He, Yang, & Guo, 2007), increase the function of immune system and antibody titer production (Sadeghi, Mirmohseni, Shawrang, & Aminafshar, 2013) and improve the health status (Moslehi, Sadeghi, Shawrang, & Aminafshar, 2016) in normal and heat stress conditions. In contrast, some reports (Sijben, De Groot, Nieuwland, Schrama, & Parmentier, 2000) rejected mentioned benefits.

During heat stress period, activation of some genes like heat shock protein 70 is important for heat resistance (Raghebian, Sadeghi, & Aminafshar, 2017; Taleb, Sadeghi, Shawrang, Chamani, & Aminafshar, 2017). An interesting study showed that heat stress could up regulate the hepatic expression of heat shock protein in broilers. Also reported that heat stress induced the hepatic lipogenesis in chickens and they mentioned that this effect probably mediated by heat shock protein (Flees et al., 2017). In the literature there was no information concerning the effects of dietary oils inclusion on heat shock proteins and blood lipid attributes. A study speculated that dietary lipid supplementation could improve the heat tolerance in broiler chickens and also performance in heat-stressed broiler chickens (Zulkifli, Liew, Israf, Omar, & Hair-Bejo, 2003).

Therefore, the main objective of present study was to evaluate and compare the effect of vegetable oils inclusion in diet on the performance, blood attributes and hsp 70 genes expression in broiler chickens under heat stress.

## Material and methods

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

### Animals and dietary treatments

The present study was done in a research farm located near Karaj city (Alborz, Iran). Three hundred 1-day old Cobb 500 broiler chicks was provided from hatchery and placed in a environment controlled house. In a completely randomized design, chicks were assigned to 4 treatment groups (4 types of vegetable oils) with 5 replicates and 15 chicks per each replicate. Diets were formulated based on the Cobb 500 requirement recommendations. Dietary treatments were iso-nutritive with the same feeds and, but included one of vegetable oils at the same level. Treatments were 1: saturated oil (palm oil), 2: source of n-6 fatty acid (corn oil), 3: a source of n-3 fatty acid (flaxseed oil) and a source of n-9 fatty acid (olive oil). The experimental vegetable oils were included in the starter, grower and finisher rations as 1.5, 3.0 and 4.0%, respectively. During trial, chicks had free access 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%. At days 28 and 42 of age, chick's weight and feed consumption was measured and feed conversion ratio was calculated for grower and finisher periods.

### Sampling and analyses

On day 28 of age, the blood samples of 2 chicks per replicate were randomly collected directly from heart using vacuum tubes containing EDTA-gel.

The plasma T3 and T4 concentration were determined using relative ELISA kits according to manufacturer directions (Biocheck Inc., Foster City, CA, USA). Plasma corticosterone concentration was measured using autoanalyser (BS-120 model, Minbray Co., USA) and commercial available kit (Pars Azmon Co., Tehran, Iran). Plasma insulin level was measured enzymatically and by chicken antibody against insulin using photometric method and commercial kits (Cusabio Co., TX, USA).

After blood sampling, chicks were sacrificed by cervical dislocation, then liver was removed. Liver sample was collected and frozen in liquid nitrogen and then stored at  $-70^\circ\text{C}$  until analysis of HSP70 gene expression.

### Analysis of HSP70 gene expression

Accuzol reagent ( $10\text{ mL g}^{-1}$  of tissue) was used for total RNA extraction from the grounded liver samples according to the manufacturer directions (Bioneer, Cat. No. K-2102). After extraction, cDNA was synthesized from RNA sample (1 ug) by reverse transcription method using commercial cDNA Reverse Transcriptase kit (Bioneer Co., Seoul, South Korea). The resulting cDNA was placed in freezer at  $-20^\circ\text{C}$  prior to use. Specific primer pairs (*Gallus gallus*, AY, 372 bp-763790, forward: 5'-AGCGTAACACCACCATTCC-3', reverse: 5'-ACGCTCCTGCAAGATAGTGAT-3') was designed and quantitative PCR analysis was done using Quanti Fast SYBER Green PCR kit (QIAGEN, Cat. No. 204052). Reference gene was GAPDH gene (M-32599, 230 bp, forward: 5'-TGAAAGTCGGAGTCAACGGAT-3', reverse: 5'-ACGCTCCTGGAAGATAGTGAT-3'). Amplification of HSP70 gene was performed as described by Aminoroaya, Sadeghi, Ansari-pirsaraei, and Kashan (2016). The relative HSP70 expression ratio as target gene was normalized to GAPDH gene using  $2^{-\Delta\Delta\text{ct}}$  procedure as previously described by Aminoroaya et al. (2016).

### Statistical Analysis

Before ANOVA analysis, the normal distribution of data was evaluated using Kolmogorov-Smirnov test. SAS (2004) software (version 9.1, SAS Institute, Cary, NC, USA) was used for statistical analysis based on ANOVA appropriate for completely randomized design to determine the effects of treatment groups on performance, hormonal levels and HSP70 gene expression. Mean comparison was done using the Tukey test. Probability values of less than 0.05 were considered significant.

### Results and discussion

Table 1 show the dietary oils effects on daily feed intake, daily gain and FCR of broiler chicks at grower and finisher periods. There were significant differences ( $p < 0.05$ ) among treatments for daily feed intake, daily gain and feed conversion ratio of chickens during grower and finisher periods. Lower feed conversion ratio was for chicken fed linseed oil and olive oil ( $p < 0.05$ ).

**Table 1.** Effects of vegetable oils on daily feed intake, daily gain and feed conversion ratio.

Treatments	Grower (days 10-28)			Finisher (days 28-42)		
	Olive oil	87.4	52.3 <sup>ab</sup>	1.67 <sup>b</sup>	161.2 <sup>a</sup>	86.7 <sup>a</sup>
Corn oil	87.1	50.1 <sup>c</sup>	1.73 <sup>a</sup>	164.6 <sup>a</sup>	79.8 <sup>b</sup>	2.05 <sup>a</sup>
Linseed oil	87.2	53.5 <sup>a</sup>	1.63 <sup>b</sup>	160.8 <sup>b</sup>	85.7 <sup>a</sup>	1.87 <sup>b</sup>
Palm oil	87.9	51.4 <sup>bc</sup>	1.72 <sup>a</sup>	160.2 <sup>b</sup>	80.3 <sup>b</sup>	1.99 <sup>a</sup>
SEM	2.37	1.11	0.053	3.08	2.41	0.057

<sup>a, b, c</sup> Means within a row with different superscripts are significantly different ( $p < 0.05$ ).

In this study, the broiler chickens were fed iso-nutritive diets and the oils supplemented at the same level in grower and finisher periods. Significant differences for feed intake, gain and feed conversion ratio among chicks fed diet containing different vegetable oils were observed that is in line with the results of Nobakht, Tabatbaei, and Khodaei (2011), who found that when supplemented different oil sources in broiler diets, significant differences were found for growth performance of broilers. Our finding is incontinence with finding of El-Deek *et al.* (2005) who reported that different source of vegetable oils had no significant effect on performance of broiler chickens under heat stress. Higher performance of chicks fed olive and linseed oils may be related to beneficial effects of these oils on plasma hormonal levels (Table 2) and gene expression of hsp 70 (Figure 1).

Plasma hormonal concentrations of chickens fed dietary oils were presented in Table 2. The lowest level of insulin was for chickens fed linseed oil and corn oil. Glucose and insulin levels were the highest in plasma of chickens fed palm oil ( $p < 0.05$ ). In fact, insulin resistance occur in chicks fed diet containing palm oil. Some animal and human studies have shown that saturated fatty acid especially palmitic acid could increase the insulin resistance (Saidpour *et al.*, 2011).

**Table 2.** Effects of feeding diet containing vegetable oils on hormonal level

	Insulin pg mL <sup>-1</sup>	Glucose mg dL <sup>-1</sup>	Corticosterone ng mL <sup>-1</sup>	T3 ng mL <sup>-1</sup>	T4 ng mL <sup>-1</sup>
Olive oil	175 <sup>b</sup>	169 <sup>d</sup>	5.35 <sup>b</sup>	1.01 <sup>ab</sup>	5.23 <sup>b</sup>
Corn oil	143 <sup>c</sup>	209 <sup>b</sup>	5.88 <sup>b</sup>	1.11 <sup>a</sup>	6.77 <sup>a</sup>
Linseed oil	140 <sup>c</sup>	181 <sup>c</sup>	4.74 <sup>c</sup>	1.10 <sup>a</sup>	7.33 <sup>a</sup>
Palm oil	210 <sup>a</sup>	229 <sup>a</sup>	6.49 <sup>a</sup>	0.91 <sup>b</sup>	5.74 <sup>b</sup>
SEM	8.6	4.5	0.204	0.048	0.408

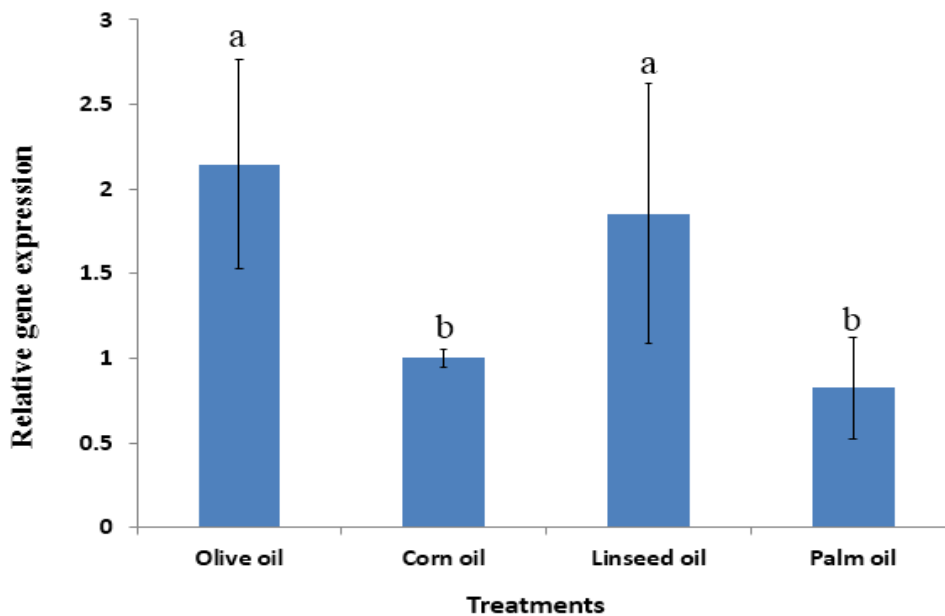
<sup>a, b, c</sup> Means within a row with different superscripts are significantly different ( $p < 0.05$ ).

A study showed that high levels of palmitic acid in the diet could increase the demand for insulin secretion (Keita, Ramírez-San Juan, Paniagua-Castro, Garduño-Siciliano, & Quevedo, 2013). Another study has shown a relationship between saturated lipid, found in palm oil, and high plasma insulin level (Marshall *et al.*, 1997). Some studies showed that palmitic acid, found in palm oil, impaired the function of  $\beta$ -cell and also insulin sensitivity, finally resulted in insulin resistance. In contrast to palmitic acid, oleic acid found in olive oil could control the blood glucose level by optimizing insulin production in pancreas and improving body glucose uptake and use, resulted in lowering of blood glucose levels (Keita *et al.*, 2013). A report indicated that olive oil inclusion in diet significantly reduced the fasting plasma glucose due to the presence of a beneficial compound in olive oil named Oleuropein (Al Jamal & Ibrahim, 2011). It is known that unsaturated fatty acids may affect phospholipids membranes and modulate the insulin sensitivity in these membranes (Abbott, Else, Atkins, & Hulbert, 2012). Animal studies have demonstrated that n-6 PUFA, found in corn oil, in comparison to n-3 PUFA, found in linseed oil, could decrease the insulin sensitivity (Jucker, Cline, Barucci, & Shulman, 1999; Saidpour *et al.*, 2011). In contrast to our results, a study showed that linseed oil had no effect on fasting blood glucose and insulin levels (Brostow *et al.*, 2011).

In comparison to previous our study (Sadeghi *et al.*, 2013), the levels of corticosterone in the present study was high, which shows that chickens are exposed to heat stress. The highest level of corticosterone was found in plasma of chickens fed palm oil and the lowest level was for those received linseed oil ( $p < 0.05$ ). A study showed that dietary fatty acid composition had significant effect on corticosterone level (Stachoń, Fürstenberg, & Gromadzka-Ostrowska, 2006). It was speculated that inclusion of olive oil in diet could increase adrenal corticosterone secretion (Pál *et al.*, 2015). Despite the studies demonstrated the impact of fatty acids on the hypothalamic–pituitary–adrenal axis, the exact mechanism is still not clear.

Chickens received linseed and corn oils had the highest levels of T3 and T4 and those fed palm and olive oils had the lowest levels ( $p < 0.05$ ). An interesting study showed that lipid source and level may have opposite effects on thyroid hormone levels (Lachowicz, Koszela-Piotrowska, & Rosołowska-Huszcz, 2009). The increase in T3 and T4 levels related to the effects of fatty acids on thyrotropin secretion (Clandinin, Claerhout, & Lien, 1998).

Figure 1 shows the relative gene expression of HSP 70 for chickens fed different dietary oils. There were differences ( $p < 0.05$ ) among treatments for gene expression. The highest HSP 70 gene expression was for chickens fed diet containing olive and linseed oils and the lowest one was for those fed corn and palm oils.



**Figure 1.** The relative gene expression of hsp 70 in chickens fed diet containing different vegetable oils.

It has been reported that lipids could directly and indirectly impact on the expression of genes. The direct is fast and has cute control on the expression levels and indirectly impact on the cell membrane composition and intracellular signaling (Kaput & Rodriguez, 2004). In the literature there was no report concerning the impact of lipids on gene expression of hsp 70. The hsp 70 proteins are involved in the heat tolerance development (Zhao et al., 2014); therefore, our results show that olive oil and linseed oil could influence on the heat tolerance compared to corn and palm oils.

## Conclusion

It was concluded that olive oil and linseed oil could improve performance and heat tolerance of chickens under heat stress.

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