Effect of vegetable oils supplementation on hepatic pathology and liver PPAR-γ gene expression in broiler chickens exposed to heat stress

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Abstract

The objective of this work was to test the effect of palm oil, flaxseed oil, corn oil and olive oil on liver health, morphology and peroxisome proliferator-activated receptors-γ (PPAR-γ) gene expression in broiler chickens under heat stress. Chicks were divided to four groups (four vegetable oils) with five replicates and 15 chicks per each based on completely randomized design. Treatments were basal containing one of oils: palm oil, corn oil, linseed oil and olive oil. At day 35 of age, 10 chickens were selected from each treatment and the liver tissue sample was used for histology and estimation of PPAR-γ gene expression. Chickens received olive oil and linseed oil had moderate steatosis (lipid droplet accumulation) and those received corn oil and palm oil had severe steatosis. Chickens fed linseed oil and olive oil had no lesions in the liver, but those received corn oil and especially palm oil revealed many lesions. Swelling of hepatocyte was not detected in chickens received corn oil, olive oil and linseed oil, but feeding diet containing palm oil resulted in a mild hepatocyte swelling. The chickens fed diet containing corn oil and palm revealed severe sinusoid dilation, intense hepatic degeneration, and mild focal infiltration of mono nuclear cells. Severe hyperemia was detected in birds fed diet containing palm oil and mild hyperemia in those received corn oil. The highest gene expression was for corn oil and the lowest one for palm oil. It was concluded that dietary source of lipids have various effects on liver cells and structure and PPAR-γ gene expression. Olive oil and then linseed oil in this study had lower hepatic pathological effect than palm oil and corn oil.

Keywords: vegetable oils; hepatic pathology; gene expression; broiler chickens; heat stress

Introduction

Lipids with important roles in energy homeostasis and the source of essential fatty acids, are included in broiler rations (Peck, 1994; Simopoulos, 2002; Yaqoob, 2004). There are numerous studies concerning the benefits of vegetable oil on performance (De Pablo and De Cienfuegos, 2000; Ailhaud *et al*., 2006; He *et al*., 2007), immune system functionality (Sadeghi *et al*., 2013) and blood biochemical parameters (Moslehi *et al*., 2016). There are some reports (van Heugten *et al*., 1996; Sijben *et al*., 2000) that rejected these benefits. It was mainly recommended to include oils in diet during heat stress period as the heat increment of lipids is lower than carbohydrates (Daghir, 1995).

Peroxisome Proliferator Activated Receptor Gamma (PPAR-γ) regulates the storage of fatty acid and metabolism of glucose. Some genes activated by PPAR-γ, of which heat shock protein 70 and also it stimulates the uptake of lipid to cells and adipogenesis by cells (Tyagi *et al*., 2011). During heat stress, glucose metabolism and activation of genes, especially heat shock protein 70, for heat resistance is important (Rhaghebian et al., 2017; Taleb et al., 2017). It was reported that inclusion of flaxseed oil (Hashemzadeh *et al*., 2017) and palm oil (Royan *et al*., 2011) in broiler diet regulated the gene expression of PPAR-γ.

In the literature, study concerning the effect of dietary supplementation of vegetable oil on liver health, morphology and gene expression of factors related to heat tolerance was not found. Lin et al. (2006) reported that heat stress affect negatively broilers performance and health. Some evidences exist that show dietary lipid supplementation could improve the heat tolerance in broiler chickens (Daghir, 1995) and performance (Zulkifli *et al*., 2003) in heat-stressed broiler chickens. Therefore, the objective of this work was to test the effect of palm oil, flaxseed oil, corn oil and olive oil on liver health, morphology and PPAR-γ gene expression in broiler chickens under heat stress.

Material and Methods

Animal and diets

This study was done in Mary Research Farm located at Karaj (Alborz, Iran) from June to September 2017. One-day-old broiler chicks (Ross 308, n=300) was prepared and housed in an environment controlled condition. Healthy chicks were divided to four groups (four vegetable oils) with five replicates and 15 chicks per each based on completely randomized design. Treatments were as: 1) palm oil as saturated oil; 2) corn oil as a source of n-6 fatty acid; 3) flaxseed oil as a source of n-3 fatty acid and olive oil as a source of n-9 fatty acid. Oils were added to soybean-corn based diets as 1.5, 3 and 4% in the starter, grower and finisher periods, respectively. Throughout the feeding trial, broilers had free access to feed and water. Lighting program was 23 h light/1 h dark. From day 11 to 41 of age, chickens were exposed to temperature of 34±1 °C and relative humidity of 60-70% for 6 hours per day from 10:00 to 16:00 and then lowered at 22 ± 1 °C.

Liver tissue sampling and histology

At day 35 of age, 10 chickens were selected from each treatment and killed by cervical dislocation. The liver tissue sample was immediately dissected out and stored in liquid nitrogen and then freezer -70 ˚C until analyzed for PPAR gene expression. A section of liver tissue was placed in 10% neutral buffered formalin for histological examination. After tissue fixation (72 h in buffered formalin), the sub-sections of liver tissue were fixed in paraffin and sliced by a microtome with a thickness of 7 µm. The procedure of hematoxylin and eosin stain was used for staining of slices and then mounted by entellan. Slides were evaluated by pathologist and changes in the liver cells were categorized as not detected (ND), mild (+), moderate (++) and severe (+++). Histological analysis was done according to the method described by Raghebian *et al.* (2017).

Estimation of liver PPAR-γ gene expression

The Trizol reagent was used to extract the total RNA from the chicken liver tissue. The extracted RNA of each sample was evaluated for concentration and quality by using Ultra Violet absorbance at 260 and 280 nm also by 1% agarose gel electrophoresis. Extracted RNA samples were stored at -70 °C until further analysis. Ambions DNA-free kit (Fermentas/Life Science/Isogene Co., USA) was used to eliminate any possible DNA impurity from extracted RNA samples. Reverse Transcription Plymerase Chain Reaction was done using RT-PCR kit (Fermentas/Life Science/Isogene Co., USA) to synthesis the cDNA. Chicken PPAR primer sequences were designed by windows based Oligo software and β-actin primers as an internal control gene. The PCR ABI step-one apparatus (Applied Biosystems Co., USA) was used for amplification. Melting curve were performed by using PCR ABI step-one apparatus (Applied Biosystems Co., USA). Gene expression profile was plotted using Graph Pad (Graph Pad Software, Inc.). Gene expression analysis of PPAR-γ was carried out based on the method explained by Hashemzadeh *et al.* (2017).

Statistical analysis

Data analyses were done using general linear model of SAS software appropriate for a completely randomized design. Significant differences at $P \leq 0.05$ were considered and compared by Duncan's multiple range tests.

Results and Discussion

The histological changes occur in the liver of chickens fed rations containing different vegetable oils are presented in Table 1. Chickens received palm oil had mild, those fed olive oil and linseed oil had moderate and those received corn oil had severe steatosis (lipid droplet accumulation). Our result is consistence to the finding of Badry *et al*. (2007) who reported an increase in the ratio of n-6:n-3 and saturated lipids in diet could lead to steatosis. Corn oil with higher n-6 to n-3 ratio and palm oil with saturated triglyceride resulted in lipid droplet accumulation in the liver and in fact these oils favors lipid synthesis over oxidation and secretion of lipids from the liver. In an interesting study, Ide (2005) showed that including a source of n-3 fatty acid to ration ameliorate steatosis via increase in the secretion of adiponectin from adipose tissue. Chickens fed linseed oil and olive oil had no lesions in the liver, but those received corn oil and especially palm oil revealed many lesions. Swelling of hepatocyte was not detected in chickens received corn oil, olive oil and linseed oil, but feeding diet containing corn oil resulted in a mild hepatocyte swelling.

Table 1. Hepatic histopathology of chickens fed different vegetable oils in the diet.

Signs	Olive oil	Corn oil	Palm oil	Linseed oil
Steatosis	++	+++	+	++
Swelling	ND	+	ND	ND
Sinusoid dilation	ND	+++	+++	ND
Hyperemia	ND	+	+++	ND
Infiltration of mononuclear cells	ND	+	+	ND
Hepatic degeneration	+	++	++	++

As shown in Table 1, the chickens fed diet containing corn oil and palm revealed severe sinusoid dilation, moderate hepatic degeneration, and mild focal infiltration of mono nuclear cells. Severe hyperemia was detected in birds fed diet containing palm oil and mild hyperemia in those received corn oil. Based on the study of Olkowski *et al*. (2005), the reason of degenerative changes in broiler chicks is a high level and prolonged state of hypoxia. High demand for oxygen and continuous situation in fast growing broilers lead the liver tissue to regressive lesions. Hence some regressive lesions such as vacuolar degeneration, fatty degeneration, necrosis an also paranchymatous in hepatocyte developed (Madej *et al*., 2007). The liver of the chickens had steatosis also showed hepatic degeneration. This result was in agreement with finding of McLean and Dutton (1995), who showed impaired lipid transport terminated to hepatocyte degeneration rather than an increase in lipid biosynthesis.

The relative gene expression of PPAR is presented in Figure 1. Olive oil was selected as external control and gene expression of PPAR of other oils was compared with olive oil. There were significant differences among treatments for gene expression. The highest gene expression was for corn oil and the lowest one for palm oil. The peroxisome proliferator-activated receptors act as a transcription factors regulating the expression of many genes (Ailhaud *et al*., 2006). These proteins are a group of nuclear receptors and play many roles in the cellular regulation, differentiation, and development. They have an essential function on cellular metabolism (carbohydrate, lipid, protein), especially lipid metabolism (Albert and Stampfer, 2002). PPAR-γ is present in adipose tissue and its level increased in fatty livers and confirmed that it act as a prosteatotic factor in fatty liver disease (Morán-Salvador *et al*., 2011).

Hepatic PPAR-γ regulates triglyceride homeostasis and contributing to hepatic steatosis (Gavrilova *et al*., 2003).

The expression of this protein is highly linked to lipogenesis and its target gene expression is SREBP-1c. PPAR-γ increases the transcription of SREBP-1c, which finally leading to higher lipogenesis (Souza-Mello *et al*., 2015). The results of PPAR gene expression and hepatic histopathology in this study are in line. The severe steatosis and high PPAR gene expression was seen in corn oil and then linseed oil.

It was concluded that dietary source of lipids have various effects on liver cells and structure and PPAR gene expression. Olive oil and then linseed oil in this study had lower hepatic pathological effect than palm oil and corn oil. Chemical residues in oils may be the reason of different results, as oil was extracted from olive and linseeds by mechanical apparatus and from corn and palm seeds by chemical extraction methods. Further study are needed to declare this hypothesis.

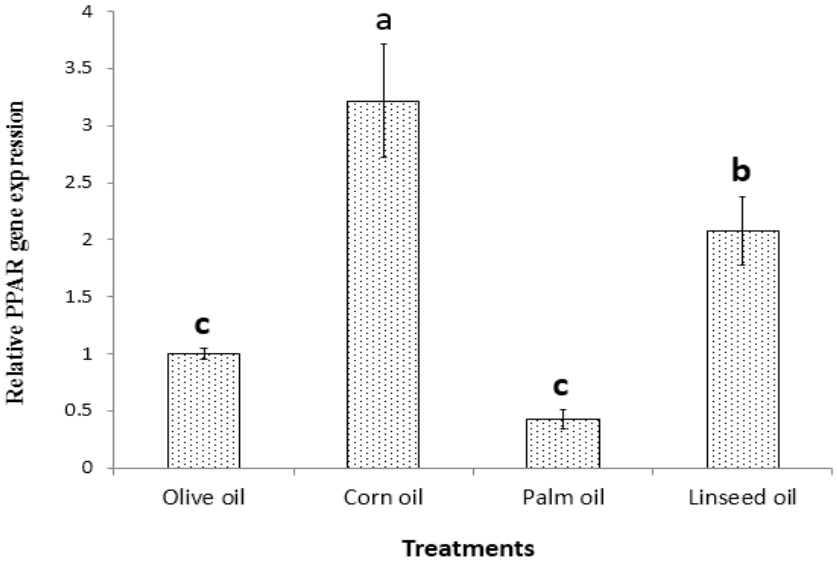

Figure 1: The relative gene expression of PPAR in the liver of broiler chicks

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