Egypt. Acad. J. Biolog. Sci., 12(1): 23- 37 (2020)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
http://eajbsc.journals.ekb.eg

Influence of Thermally Oxidized Palm Oil on Growth Performance and PPAR-α Gene Expression in Broiler Chickens

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ARTICLE INFO

Article History
Received:23/12/2019
Accepted 18/1/2020

***Keywords*:** Thermally oxidized, fresh, oils, PPAR-α gene, broilers

ABSTRACT

The present study was planned to investigate the effect of thermally oxidized palm oil on the broiler performances, liver gene expression, lipid profile and oxidative stress markers. A total number of 150 one d-old Cobb 500 broiler chicks were randomly divided into three treatment groups. The first group was served as control and fed the basal diets with tap water. The second group fed the basal diet supplemented with 5% fresh palm oil, the third group fed the basal diet supplemented with 5% thermally oxidized palm oil. The experiment continued till chicks were 42 d old. Body weight, feed consumption, feed conversion ratio was estimated. Estimation of the effect of different treatments on the liver gene expression, Estimation of the lipid profile in serum, Estimation of the cholesterol level in the liver tissue. Correlation between the cholesterol level in both serum and liver tissue. Determination of oxidative stress markers in serum. The results showed that the supplementation of thermally oxidized palm oil decrease the body weight, reduce feed consumption, and increase the feed conversion ratio. Thermally oxidized oil cause a significant increase in the PPAR-α gene expression in liver tissue, increase the cholesterol concentrations in serum and liver, elevate triglycerides in serum and elevate the oxidative stress markers..

INTRODUCTION

Poultry production is a business which like any other business seeks to generate profit, one of the objectives of any poultry producer is to keep the balance between low coast diets with the least coast and obtain the maximum productivity (Ahiwe, *et al.,* 2018). Lipids are commonly added to the poultry rations as concentrated sources of energy to improve feed efficiency (Pettigrew and Moser, 1991). The most used lipids are subjected to heating and potential oxidative processes before being used in poultry diets (Canakci, 2007).

Lipids commonly added to poultry diets primary to serve as a cheap form of energy because lipids supply about 2.25 times more energy than carbohydrates and proteins (Azain, 2001). Lipids also improve the absorption of fat-soluble vitamins and increase the efficiency of the consumed energy (lower caloric increment) (Baião, & Lara, 2005). Lipids help in Supplying fat-soluble vitamins and essential fatty acids, reduce dust in the facilities, attenuating growth reduction in heat stress conditions, improve the pellet quality, and improve the diet palatability (Pettigrew, and Moser, 1991). In fast food restaurants, fat is heated in fryers about 18 h daily, at temperatures close to 180°C. For coast saving, heated fats are used for up to 1 week before it is discarded and replaced with a fresh one. These fats have high concentrations of lipid peroxides (Sülzle, *et al.,* 2004). Several studies reported that the consumption of oxidized fats affects metabolism in several ways (Corcos, *et al.,* 1987; Blanc, *et al.,* 1992; Hochgraf, *et al.,* 1997; Skufca, *et al.,* 2003). The concentrations of various lipid peroxidation products in heated fats depend on their thermal treatment (Kubow, 1992). The oxidized fat heated at a relatively low temperature over a long period containing high concentrations of primary lipid peroxidation products affect the lipid metabolism of rats more than the oxidized fat heated at a high temperature for a shorter period (Skufca, *et al.,* 2003). Heating of oils at high temperature and in the presence of oxygen resulted in their oxidative deterioration. Oxygen from air and water from food being fried when mixed with heated oil accelerating the rate of its oxidation. The cooked food absorbs this oxidized oil so it becomes a part of our diet (Ammu, *et al.,* 2000). In developing countries, the consumption of highly oxidized fat through the deep fried food is high. Because lipid peroxidation is a free radical producing reaction, consumption of lipid peroxidation products can cause oxidative stress by straining the antioxidant defense system creating an imbalance of free radicals in vivo (Lindblom, 2017). Oxidative stress is caused by the imbalance between prooxidants and antioxidants at the cellular or individual level (Voljc, *et al.,* 2011). Oxidative stress constitutes an important factor of biological damage and is regarded as the cause of several pathological conditions that affect poultry growth and development (Avanzo, *et al.,* 2001; Iqbal, *et al.,* 2002). In poultry, oxidative stress may occur due to several factors such as:1) feed (high concentration of polyunsaturated fatty acids [PUFA], contamination with fungal toxins, prolonged storage, antioxidant deficiency) (Chung, *et al.,* 2005), 2) environmental (heat, high stocking density, transportation, vaccination) (Sahin, *et al.,* 2003; Panda, *et al.,* 2008), and 3) pathological conditions (ascites, fatty liver haemmorrhagic disease syndrome, arthritis, coccidiosis) (Papas, 1999; Iqbal, *et al.,* 2002).

This study was conducted to investigate the effects of feeding thermally oxidized vegetable oil on hepatic gene expression and through estimation of Growth performance parameters (body weight, feed consumption and feed conversion ratio).Estimation of the effect of different treatments on the PPAR-α gene expression in liver tissues, lipid profile in serum, cholesterol level in the liver tissue, Correlation between the cholesterol level in both serum and liver tissue, Determination of oxidative stress markers in serum.

MATERIALS AND METHODS

Birds and Management:

A total number of 150 one-day-old Cobb 500 broiler chicks of both

sexes, weighing 48- 53 g were purchased from Ismailia-Misr Poultry Company, Egypt. Chicks were left in a good ventilated clean place with temperature range (32- 35^{o} C). Electric bulbs were used as a source of light and electrical heaters were used to adjust the temperature. The light was provided to chicks around the whole day's length (24 hours). All birds were treated in accordance with the bird's use protocol approved by the Faculty of Veterinary Medicine, Suez Canal University.

Experimental Diet:

Experimental birds offered 2 rations (starter, from 1- 3 weeks of age and grower, from 3 – 6 weeks of age). Both diets were formulated to meet the nutrient requirements of broiler chicks according to NRC (1994).

Experimental Design:

Chicks were randomly divided into 3 random groups, each group contains 50 Cobb 500 chicks. The first group: Fed on basal ration without any additives and act as a control group (G1). The 2nd group: Fed on basal ration supplied with 5% fresh palm oil (G2). The 3rd group: Fed on basal ration supplied with 5% thermally oxidized palm oils (G3).

Preparation of Thermally Oxidized Oil:

Palm oil purchased from the local market. The thermal oxidation of the palm oil was done in an uncovered stainless steel pan fryer. The thermal oxidation processes were repeated 15 times at 175 ± 5 °C (15 minutes each) twice daily for 8 successive days. No renewal of oil was done. At the end of the experiment, oil was taken out until it reaches the room temperature then placed in a bottle in the refrigerator (4^{o}C), and then thoroughly mixed with the basal diet freshly day by day (Izaki, & Uchiyama, 1984).

Bodyweight and Body Weight Gain:

Body weight and body weight gain of each bird were determined weekly according to Brady, (1968). The live body weight changes were taken as a measure for growth. Body weight gain was determined by subtraction of 2 successive weights.

Feed Consumption G/ Week:

The feed consumption (g/week) was calculated per group by obtaining a sum difference between the weight of offered feed and the remained portion for 7 days.

Feed Conversion Ratio (FCR):

Feed conversion ratio FCR (g) /bird/week was obtained by dividing food consumption (g)/ week by the number of birds in each group. Body weight gain was calculated by subtracting 2 weekly successive weights. The feed conversion ratio (FCR) was calculated weekly.

$$FCR = \frac{Feed\ consumption(g)/bird/week}{Body\ weight\ gain(g)/bird/week}$$

Blood & Tissue Sampling:

At the age of 21 and 42 days 15 chicks from each group respectively were taken and fasted overnight and then blood samples were collected by slaughtering into plain tubes (non-heparinized tubes) for serum separation. Blood was left for 15 min to clot then kept in the refrigerator for 3 hours then centrifuged at 3000 rpm for 20 min to obtain serum which is stored at -20 ^{o}C for biochemical analysis. Liver samples were taken immediately and kept at RNA-Later Stabilization Solution which stabilizes and protects cellular RNA, and stored at -20°C for PPAR-α gene expression analysis.

Determination of Serum Lipid Profile (mg/dl):

Serum levels of total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C), were measured using enzymatic colorimetric kits (Cat. No. 0599, Stanbio Laboratory, USA, Cat. No. 304710050, ELITech Diagnostic, France and (Cat. No. 303113050, ELITech Diagnostic, France) following the instructions of the corresponding reagent kit., respectively, Serum low-density lipoprotein cholesterol (LDL-C) was

measured using enzymatic colorimetric kits (lot no. 990610, QCA Co., Spain), following the instructions of the corresponding reagent kit.

Determination of Liver Total Cholesterol:

Total lipids from the liver were extracted using the modified method of Folch, *et al.* (1957). Briefly, 250 mg of frozen liver tissue from the same region of the liver was weighed and transferred into a 2-mL flat-bottom centrifuge tube containing 0.5 mL methanol. After homogenization, 0.5 mL of chloroform and 0.4 ml of dist. water was added to the liver homogenate and mixed by vortexing. The lipid fraction in chloroform was separated from the aqueous fraction and liver debris by centrifuging for 10 min at 14,000 rpm at 20°C and was then transferred to a new glass tube. After drying the lipid fraction was reconstituted in n-butanol for further analysis of TC. TC concentrations were determined enzymatically by conducting colorimetric assays (Pointe Scientific, Canton, MI) in a 96-well plate reader (SpectraMAX 250, Molecular Devices, Sunnyvale, CA).

Determination of Oxidative Stress Markers in Serum:

Harvested sera were used for the determination of serum oxidative stress markers. Serum catalase **(**CAT) activity was assessed by measuring catalase degradation of H2O2 using a redox dye (ELISA Kit: QuantiChromTM, BioAssay Systems, USA, Catalog No. ECAT-100) according to Cowell, *et al.,* (1994). Superoxide dismutase (SOD) activity was measured by the xanthine oxidase method (ELISA Kit: Cayman Chemical Company, USA, Catalog No. 706002), which monitors the inhibition of nitro blue tetrazolium reduction by the sample (Sun, *et al.,* 1988). Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) forming the MDA-TBA product in acidic conditions and high temperatures (90-100C°) and measured colorimetrically at 540 nm. Sample malonaldehyde concentration was compared to an MDA standard curve (Boler, et al., 2012).

Gene Expression Analysis:

The oligonucleotide primers and probes used in SYBR Green real time pcr are demonstrated at (Table 1)

Extraction of RNA (According to RNeasy Mini Kit Instructions):

Thirty mg of organ sample was weighed and put in 2 ml screw-capped tubes. 2) 600 µl of the Buffer RLT (with 10 µl ß-Mercaptoethanol/ ml Buffer RLT) was added into the tubes. 3) For the homogenization of samples, tubes were placed into the adaptor sets, which are fixed into the clamps of the TissueLyser. Disruption was performed in 2 minutes high-speed (30 Hz) shaking step. 4) The lysate was centrifugated for 3 min at 14000 rpm. 5) One volume of 70% ethanol was added to the cleared lysate and mixed immediately by pipetting. 6) 700 µl of the sample, including any precipitate that may have formed, was transferred to an RNeasy spin column placed in a 2 ml collection tube. Centrifugation was done for 1 min. at 14000 rpm. The flow-through was discarded. 7) Step 6 was repeated again for the excess volume. 8) 700 µl of Buffer RW1 was added. Centrifugation was done for 1 min. at 10000 rpm. The flow-through was discarded. 9) 500 µl of Buffer RPE was added. Centrifugation was done for 1 min. at 10000 rpm. The flow-through was discarded. 10) Step 9 was repeated again, but Centrifugation was done for 2 min. at 10000 rpm. 11) RNA was eluted by adding 50 µl RNase-free water. Centrifugation was done for 1 min. at 10000 rpm. Cycling conditions for SYBR green real time PCR according to Quantitect SYBR green PCR kit are demonstrated at (Table 2).

Analysis of the SYBR Green rt-PCR Results:

Amplification curves and ct values were determined by the Stratagene MX3005P software. To

estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the control group according to the "ΔΔCt" method stated by Yuan, *et al*., (2006).

Whereas ΔΔCt = ΔCtreference - ΔCttarget

Δ*Ct target = Ct control – Ct treatment* and Δ*Ct reference = Ct control- Ct treatment*

Table 1: Oligonucleotide primers and probes used in SYBR Green real-time PCR.

Gene	Primer sequence (5'-3')	Reference
PPAR-α	TGGACGAATGCCAAGGTC	Zhou, *et al.* 2016
	GATTTCCTGCAGTAAAGGGTG	
ß. actin	CCACCGCAAATGCTTCTAAAC	Yuan, et al., 2007
	AAGACTGCTGCTGACACCTTC	

Table 2: Cycling Conditions for SYBR green real-time PCR according to Quantitect SYBR green PCR kit.

Gene	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)		
			Secondary denaturation	Annealing (Optics on)	Extension	econdary denaturation	Annealing	Final denaturation
PPARα	50°C 30 min	94°C 15 min.	94°C 15 sec.	60.3°C 30 sec.	72°C 30 sec.	94°C 1 min.	60.3°C 1 min.	94°C 1 min.
ß. actin	50°C 30 min.	94°C 15 min.	94°C 15 sec.	51°C 30 sec.	72°C 30 sec.	94°C 1 min.	51°C 1 min.	94°C 1 min.

Statistical Analysis:

Data collected from treated groups were statistically analyzed in comparison to the control group and each other for the mean and standard error. Data were expressed as means ± SE. Differences between means of different groups were carried out using one way ANOVA followed by Duncan multiple comparison tests using a statistical software program (SPSS for Windows, version 16, USA). Differences were to be significant at ($P<0.05$) and highly significant at ($P<0.01$) according to (Coakes, *et al.*, 2010). In addition, relationships between measures of the TC level in both serum and liver tissue variables were evaluated by simple linear correlation (Pearson correlation coefficients) analysis using a statistical software program (SPSS for Windows, version 16, USA). Treatment effects were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Table 3 demonstrated that LBW was non-significantly altered in 1st, 2nd and 3rd week of treatments. While in the 4th, 5th and 6th weeks of treatment (G3) showed a significant decrease in LBW than the other groups of treatment. Our study results agree with the results of (Dong, *et al.*, 2011; Tavarez, *et al.*, 2011; Wang *et al.*, 2015), who reported that dietary oxidized oils impaired animal performance. While (Anjum, 2004) reported that the highest weight gain was found in chicks fed fresh oils than those fed thermally oxidized oil, This may be related to differences in dietary oil content and degree of oil oxidation (Zhang, *et al.*, 2011). Overall, the decreased growth performance observed in animals fed thermally oxidized lipids may be related to the activation of PPAR-α pathway by the thermally oxidized lipids, as the PPAR-α mediated upregulation of catabolic metabolism, such as fatty acid oxidation lead to decreased lipid availability for adipose tissue accretion and protein synthesis (Liu, *et al.*, 2014). Reduced growth rate in the group that fed thermally oxidized oil lipids could be a result of rancidity of thermally-oxidized lipids, which can reduce diet palatability, and thereby

decrease feed intake leading to a poor growth rate (DeRouchey, *et al.,* 2004), also, it may be a consequence of the toxic effects of the lipid peroxidation products such as α, β-unsaturated hydroxy aldehydes, are of particular interest because some of them are highly toxic and readily absorbed (Grootveld, *et al.,* 1998; Kim, *et al.,* 1999).

In the first week no significant difference was observed in chicks feed consumption among groups, While in the 2nd week of treatment, feed consumption showed a significant ($p<0.05$) increase in (G2) group than other groups. At the 3rd, 4th, 5th and 6th week of treatment, group (G3) revealed a significant ($P<0.05$) reduction in food consumption than other groups (Table 3). Kishawy, *et al.,* (2016) results agree with our study findings as they reported significant ($p<0.05$) reduction in feed intake in birds fed rations having a rancid feed. While Diaz, (1977) reported a non-significant difference in feed intake among broiler chicks fed on ration having oxidized oil with or without added antioxidant this in contrast with the present findings. Also (Chae, *et al.* 2002) reported that chicks fed diet containing fresh or oxidized soybean oil had no difference in feed intake. L'Estrange, *et al.* (1966) reported no differences in feed efficiency in broilers fed oxidized beef tallow compared with control. This might be due to the use of oil of a lower degree of rancidity compared to the oil used in our study.

Feed conversion ratio demonstrated a significant ($P<0.05$) reduction in G2 groups as compared with G1 and G3 after the 1st week of treatments. However, at 2nd, 3rd, 4th, and 5th week, there was a significant ($P<0.05$) increase in G3 group than other treatment groups (Table 3). These results are in line with the previous findings (Takigawa and hyama, 1983; Miyzawa and Knobb, 1986; Anjum, (2004). This might be due to the destruction of fat-soluble vitamins in rancid oil that leads to reduced availability of nutrients as well as immunity, and consequently depressed growth performance (Lin, *et al.,* 1989; Cheeke, 1991). While (Dorra, *et al.,* 2014) reported that thermally oxidized oil causes no significant change in FCR.

Table (3): Effect of thermally oxidized vegetable oil on LBW (g), feed consumption (g) and FCR of Cobb broiler chicks.

	Age/ w	G1	G2	G3
live body weight (LBW)	**1st w**	77.00 ± 5.14^{a}	82.00 ± 2.55^{a}	82.00 ± 2.55^{a}
	2nd w	301.00 ± 12.70^{a}	304.00 ± 19.68^{a}	249.00 ± 17.10^{a}
	3rd w	526.00 ± 10.40^{b}	591.00 ± 13.79^{ab}	509.60 ± 6.85^{b}
	4th w	1305.00 ± 8.79^{a}	1188.80 ± 10.27^{ab}	941.00 ± 9.43^{c}
	5th w	2062.00 ± 19.91^{b}	2182.00 ± 18.60^{ab}	1665.00 ± 14.90
	6th w	2473.00 ± 17.12^{ab}	2526.00 ± 11.22^{ab}	1880.00 ± 14.60^{c}
Feed Consumption (g/week)	**1st w**	163.00 ± 0.29^{a}	163.00 ± 0.21^{a}	155.00 ± 0.29^{a}
	2nd w	365.00 ± 0.29^{b}	416.70 ± 0.17^{a}	151.70 ± 0.17^{c}
	3rd w	670.30 ± 2.05^{a}	695.00 ± 7.64^{a}	560.00 ± 1.73^{b}
	4th w	1061.6 ± 20.74^{a}	1064.4 ± 16.67^{a}	9640.0 ± 28.87^{b}
	5th w	1200 ± 9.87^{a}	1230 ± 10.41^{a}	1120 ± 16.67^{b}
	6th w	1399.70 ± 6.06^{bc}	1413.30 ± 8.82^{ab}	1303.30 ± 3.33^{d}
Feed conversion ratio (FCR)	**1st w**	1.33 ± 0.21^{a}	0.93 ± 0.06^{c}	1.04 ± 0.17^{b}
	2nd w	$1.05 \pm .016^{c}$	1.07 ± 0.02^{b}	1.10 ± 0.02^{a}
	3rd w	1.29 ± 0.02^{c}	1.45 ± 0.11^{b}	2.25 ± 0.39^{a}
	4th w	1.29 ± 0.02^{c}	1.46 ± 0.12^{b}	2.25 ± 0.39^{a}
	5th w	1.96 ± 0.06^{b}	1.74 ± 0.09^{c}	2.39 ± 0.10^{a}

Values are means ± standard error (SE); Values within the same row with different superscripts (a, b &c) indicate significant difference at ($P<0.05$).

The gene transcripts (mRNAs) of the PPAR-α gene were successfully detected in all liver tissues within all treated groups. The gene expression was normalized with the expression values of the β-Actin gene. At 3rd and 6th w the results revealed PPAR-α mRNA expression in the liver tissues of the group fed the thermally oxidized oil diet (G3) was significantly higher (P<0.05) than the other groups (Table 4). Liu, *et al.,* (2014) suggested that thermally oxidized lipids caused activation of PPARα in the liver tissue. Considering the central role of PPARα in fatty acid transportation, uptake, oxidation, and ketogenesis, Activation of PPAR-α caused by feeding oxidized lipids had been reported from studies in both rats and pigs (Chao *et al.,* 2005; Luci, *et al*., 2007). This activation of PPAR-α is possibly due to the presence of hydroxyl and hydroperoxy fatty acids in the thermally oxidized fat, which can act as potent activators of PPAR-α (Delerive, *et al.,* 2000; Luci, *et al.,* 2007). Luci, *et al.,* (2007) reported an upregulation effect of oxidized sunflower oil on lipogenic pathways in pigs, while (Skufca, *et al.,* 2003) reported downregulation of lipogenic enzymes in rats fed oxidized soybean oil or a mixture of sunflower oil and lard**.**

Table (4): Estimation of the effect of thermally oxidized palm oil on the PPAR-α gene expression in hepatic tissue at 3rd and 6th week of age.

PARAMETERS		G2	G3
PPAR- α	3RD W	8.19 ± 0.29b	12.30 ± 0.38a
	6TH W	8.21 ± 0.37b	12.45 ± 0.37a

Values are means ± standard error (SE); Values within the same row with different superscripts (a, b & c) indicate significant difference at (P<0.05)

Total cholesterol (TC) revealed non-significant increase in G3 group at 3rd w of treatment, the, while at the 6th w of treatment (G3) group revealed a significant (P<0.05) increase in TC concentration in comparison with other groups of treatment (Table 5). Triglycerides (TG) showed non-significant (P>0.05) changes between the different groups at the 3rd w. While at the 6th week of treatment the G3 group showed a significant (p<0.05) increase than all other groups (Table 5). High-density lipoprotein cholesterol (HDL-c) showed significant (p<0.05) decrease in the G3 group when compared to the other experimental groups at the 3rd and 6th w of treatment, (Table 5). Low-density lipoprotein cholesterol (LDL-c) revealed a significant increase in the G3 group than the other groups at the 3rd and 6th W of treatment (Table 5). The increase in serum TC in the study groups is probably due to the high cholesterol level in the diet. This finding is in agreement with Adam, *et al.,* (2008) who reported that feeding cholesterol-enriched diet caused a significant increase in TC and LDL-c levels in the blood. Similarly, Ali, *et al.,* (2000) and Bennani, *et al.,* (2000) reported that rats fed with cholesterol-enriched diet showed severe hypercholesterolemia, elevated plasma LDL and VLDL cholesterol compared to the control group of rats fed on a normal diet. Elevation of TC may be explained by the finding of Temme, *et al.,* (1996) that a palmitic acid-rich diet leads to a higher TC concentration than an oleic-rich diet and it is well known that palm oil is a rich source of palmitic acid (Farag, *et al.*, 2010).

Table 5: Effect of thermally oxidized palm oil on the serum Lipid profile parameters at 3rd and 6th week of treatment.

PARAMETERS		G1	G2	G3
TC MG/DL	3W	143.33 ± 7.17[a]	155.00 ± 13.89[a]	167.00 ± 8.14[a]
	6W	137.00 ± 8.50[c]	167.00 ± 10.69[b]	239.67 ± 3.76[a]
TG MG/ DL	3W	144.42 ± 3.38[a]	151.33 ± 6.19[a]	199.15 ± 4.47[a]
	6W	137.00 ± 8.50[c]	167.00 ± 10.69[b]	240.00 ± 3.76[a]
HDL-C MG/ DL	3W	69.00 ± 4.00[a]	82.67 ± 7.36[a]	49.67 ± 3.87[b]
	6W	78.67 ± 4.10[a]	77.67 ± 9.06[a]	38.33 ± 9.53[b]
LDL-C MG/ DL	3W	42.38 ± 15.26[b]	42.07 ± 9.30[b]	69.78 ± 23.84[a]
	6W	36.93 ± 2.12[b]	41.77 ± 4.12[b]	109.47 ± 3.16[a]

Values are means ± standard error (SE); Values within the same row with different superscripts (a, b &c) indicate significant difference at (P<0.05)

At the 3rd and 6th w of treatment, the cholesterol level in the liver tissue showed a significant increase (P<0.05) in (G3) group than all other groups (Table 6). At 3rd W of age there was a significant (P<0.05) positive correlation relationship between cholesterol in both serum and liver tissue. And at 6th W of age, there was a highly significant (P<0.01) positive correlation between cholesterol in both serum and liver tissue as shown in figures 1,& 2 respectively. In previous studies to determine the effect of polyunsaturated fat feeding in man (Spritz, *et al.*, 1965; Grundy, *et al.*, 1970), non-human primates (Corey, *et al.*, 1976) and rabbits (Bieberdorf, and Wilson, 1965) have in part led to the hypothesis that unsaturated fats cause a redistribution of cholesterol between plasma and tissue pools. Increases in liver cholesterol concentrations (Avigan, and Steinberg, 1958; Reiser, *et al.*, 1963) have been reported in rats fed unsaturated fat with minimal changes in plasma cholesterol. Based on one early report (Frantz, and Carly, 1961) it appears that liver cholesterol in humans is not altered by unsaturated fat feeding. However, the fact that human fibroblasts grown in medium supplemented with linoleic acid degrade more LDL than cells grown in the presence of palmitic acid (Gavigan, and Knight. 1981) implies that peripheral cells such as adipocytes, which also bind and degrade LDL, may store increased amounts of cholesterol during unsaturated fat feeding.

At 3rd week of age, MDA showed a significant increase (P<0.05) in (G3) group in comparison with the control group (G1), while at 6th week of age MDA showed a significant increase (P<0.05) in (G3) group in comparison with (G1) & (G2) groups. Although (G2) groups showed a significant increase (P<0.05) concentration when compared with the control (Table 7). MDA is a lipid peroxidation marker, and increasing levels are related to lipid peroxidation and oxidative stress (Aytekin, *et al.*, 2015, Esgalhado *et al.*, 2015). Similar results were obtained in broilers (Zhang, *et al.*, 2011; Liang, *et al.*, 2015, Tan, *et al.*, 2018)) and pigs (Ringseis, *et al.*, 2007). MDA level changes observed in the birds fed oxidized oils may be triggered by free radicals of lipid peroxidation.

Table 6: Effect of thermally oxidized palm oil on the serum Lipid profile parameters at 3rd and 6th week of treatment:

PARAMETERS		G1	G2	G3
CHOLESTEROL	3RD W	173.33± 4.41^{b}	198.33± 4.41^{a}	208.33± 6.01^{a}
MG/ DL	6TH W	197.7 ± 6.74^{b}	236.67± 6.0^{a}	241.67± 3.84^{a}

Values are means ± standard error (SE); Values within the same row with different superscripts (a, b &c) indicate significant difference at (P<0.05)

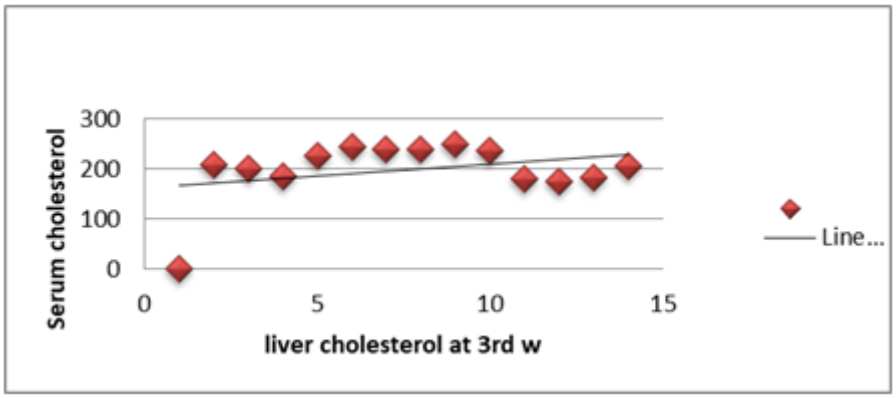

Fig.1: Correlation between the cholesterol level in both serum and liver tissue at 3 weeks age.

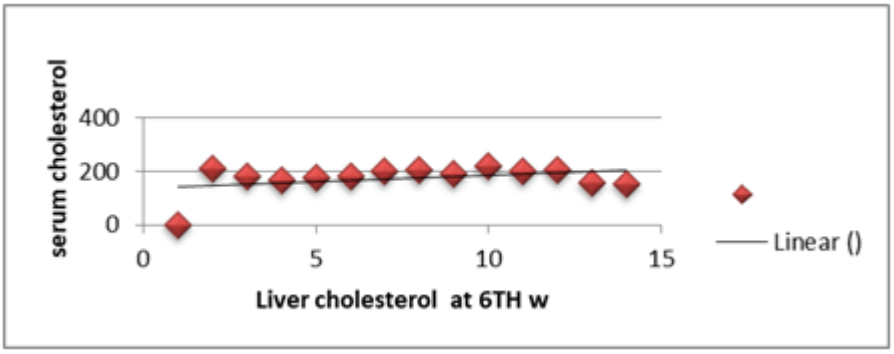

Fig. 2: Correlation between the cholesterol level in both serum and liver tissue at 6 weeks age.

In the 3rd week of age, SOD showed a significant increase (P<0.05) in (G3) group in comparison with the control. At the 6th week of age, SOD showed a significant increase (P<0.05) in (G3) group in comparison with the (G1), (G2) groups (Table 7). Lindblom, (2017) reported that SOD of the broilers fed peroxidized oils had a 19.7% increase in activity in comparison to broilers fed fresh oils. This is in agreement with our results and also with the results recorded by Altan, *et al.*, (2003) who reported a 47.4% increase in plasma SOD in broilers that were exposed to heat as an inducer of oxidative stress in comparison to broilers under thermal neutral conditions. This is in contrast to Lin *et al.*, (2006) who induced oxidative stress via heat exposure to broilers and reported no differences in SOD.

At 3rd week of age, CAT showed a significant increase (P<0.05) in (G3) group in comparison with the (G1), (G2) groups. While at the 6th w of age CAT showed a significant increase (P<0.05) in (G3) group in comparison with the (G1) & (G2)

(Table 7). Increased catalase activity in the thermally oxidized group may probably due to increased formation of H2O2. The results are in agreement with reported findings of Kok *et al.* (1988) and Narasimhamurthy and Raina (1999). It is probable that oxidized oil may lead to an escalation of O2. – generation by cytochrome p450 and NADPH dependent microsomal source. It has also been shown that the destruction of H2O2 by catalase appears to be important only at higher concentrations of H2O2 (Nagel Ranner, 1973).

Table 7: Effect of thermally oxidized palm oil on the Oxidative stress markers at 3rd and 6th weeks of age:

PARAMETERS		G1	G2	G3
MDA (NMOL/ML)	3W	1.45 ± .038^{b}	1.72 ± .097^{ab}	2.00 ± .053^{a}
	6W	1.55 ± .07^{c}	2.07 ± .09^{b}	3.67 ± .049^{a}
SOD (U/ML)	3W	138.02± 3.26^{b}	142.82± 1.65^{ab}	150.03± 2.77^{a}
	6W	146.99± 2.02^{c}	172.69± 2.45^{b}	189.62± 2.41^{a}
CAT (U/ML)	3W	33.51 ± 1.43^{b}	41.22 ± 3.96^{ab}	47.49 ± 2.36^{a}
	6W	35.64 ± 1.05^{c}	53.92 ± 2.00^{b}	73.72 ± 4.85^{a}

The increase in oxidative stress markers in liver tissue of the birds that consumed oxidized oil may be due to oxidative deterioration of longer chain n-3 fatty acids (Panda, and Cherian, 2013) and fat-soluble vitamins (Cheeke, 1991). The function of CAT is to metabolize H2O2 to water, but if this cannot be done effectively, H2O2 is rapidly converted to the OH free radical, which is reactive with lipids, proteins, and DNA (Lindblom, 2017). Catalase and GPx have been credited with the ability to detoxify peroxides and hydroperoxides and prevent lipid peroxidation (Purushothama, *et al.*, 2003).

Conclusion:

From the results of our study we can conclude that using thermally oxidized oil as a feed additive because it is a cheap source of energy cause poor growth performance as it decrease LBW, feed consumption and bad FCR. Using thermally oxidized oil as a feed additive cause increase in PPAR-α gene expression in the liver tissue, increase lipid profile parameters as TC , TG in serum, it also increase TC in liver tissue and increase the levels of oxidative stress markers in serum as MDA, SOD & CAT.

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