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RESEARCH ARTICLE

The ameliorative effects of L-carnitine and synbiotics on induced hyperlipidemia and oxidative stress in broilers

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Abstract

Background: Hyperlipidemia is a serious problem in broilers as it is associated with an increase in the oxidative biomarkers level. Fasting and refeeding cycles is an important cause of hyperlipidemia. Fasting and refeeding is a special feeding regimen in tropical countries. It is used as a method for decreasing the heat stress.Consequently, it leads to hyperlipidemia and oxidative stress.

Methods: 120 (one day old) broiler chicks were kept at optimal conditions. The chicks were received water & feed ad libitum. At 20th day old, the chicks were divided into 4 groups, 3 replicates of 10 birds each. The first group was reared at optimal conditions till the end of experiment. The second group was subjected to repeated fasting and refeeding cycles and high temperature (35^{o}C). The third and fourth groups were received L-carnitine and synbiotics in drinking water respectively before and during the challenge by repeated fasting and refeeding cycles and high ambient temperature. Lipid profile (total lipids, triacylglycerol, total cholesterol, LDL-C and HDL-C) in serum and oxidative-antioxidant biomarkers (GSH, glutathione reductase, SOD, MDA, NO and TAC) in liver were measured in all groups

Results: birds in the second group showed hyperlipidemia with increased ROS production. L-carnitine and synbiotics received groups showed a significant amelioration in lipid profile and antioxidant parameters as compared to the second group.

Conclusion: The repeated fasting and refeeding cycles in addition to heat stress induce hyperlipidemia and oxidative stress which can be ameliorated by using of L-carnitine or synbiotics.

Introduction

The commercial poultry industry has focused on enhancing bird growth rate, but little attention has been paid to the concomitant abdominal fat deposition. Fat deposition needs to be controlled due to its negative effect on productivity. In meat-type chickens, excess fat deposition could decrease feed efficiency during rearing and the yield of lean meat after processing **(Sanz et al., 2000)**.

Lipid metabolism in poultry differs from that in mammals since the liver is considered the main organ involved in its metabolic activity as compared to adipose tissue in mammals. The synthesis and decomposition of fatty acids are important parts of lipid metabolism. The catabolism of fatty acids (beta-oxidation) is carried out in the mitochondria, whereas fatty acids are synthesized in the cytoplasm of the hepatocyte is reported as the principle location in poultry **(Juan et al., 2012)**.

In general, body fat accumulation may be considered the net result of the balance among dietary absorbed fat, endogenous fat synthesis (lipogenesis) and fat catabolism via beta-oxidation (lipolysis). **(Sanz et al., 2000)**.

In order to improve the feed efficiency and reduce fat deposition, feed restriction has been adopted in broilers production. Feed restriction on early postnatal period leads to adult obesity by inducing a metabolic shift that increases lipogenesis (**Zhan et al., 2007**). Moreover, sever feed restriction is considered one of the main causes of oxidative stress in broilers (**Xiaojing et al., 2010**). The repeated fasting and refeeding cycles are associated with changes in lipid metabolism such as increased serum triacylglycerol , total cholesterol levels and increased hepatic lipogenic enzymes activities (maleic enzyme, acetyl coA carboxylase & fatty acid synthase). Disturbance of lipogenesis occurs as a result of up-regulation of hepatic lipogenic enzymes through an increase in mRNA gene expression level of these enzymes (**Pei et al., 2009**)**.** Consequently, the increased lipogenesis may be a predisposing factor for hyperlipidemia and fatty liver. These metabolic disorders are associated with lipid peroxidation and oxidative stress (**Matsuzawa et al., 2007**)**.**

The reactive oxygen species (ROS) are generated in eukaryotic organelles such as mitochondria (in the electron transfer chains) and peroxisomes (fatty acid degradation). ROS can also be produced extra cellularly, by the action of NADPH oxidases in phagocytes and colon epithelial cells **(Gara et al., 2010)**. An increase in ROS and/or a decrease in the antioxidant defense mechanism lead to an increase in oxidative stress **(Azad et al., 2010)**.

ROS attack various biomolecules (e.g. DNA, RNA, proteins, lipids, cofactors in enzymes) damaging them and disturbing normal cellular metabolism. Consequently, this may lead to cell death **(Van et al., 2011).**

Liver plays a central role in the maintenance of systemic lipid homeostasis and it is highly susceptible to ROS damage (**Zhang et al., 2009**). ROS have detrimental effects on hepatocytes by damaging DNA, lipids, and proteins **(Raval et al., 2006)**.

Hyperlipidemia is associated with constant increase in cholesterol and/ or TG blood levels (**Anderson et al., 1987**). Hyperlipidemia is associated with high level of MDA and subsequent lipid peroxidation. The high blood lipid concentration increases the fat-mediated oxidative stress and decrease antioxidative enzymes activities. Consequently, this imbalance leads to induction of oxidative stress **(Gorinstein et al., 2006).**

Ambient temperature is considered as an important factor in poultry production due to its effects on the performance, carcass characteristics and economic returns. Heat stress can disturb the balance between the production of ROS and the antioxidant systems, and may further stimulate the formation of ROS (**Feng et al., 2008**). The activity of mitochondrial respiratory enzymes, production of ROS and lipid peroxidation were affected significantly by heat stress. The exposure to high temperature may enhance the activity of the mitochondrial respiratory chain. This leads to the over-production of ROS, which ultimately results in lipid peroxidation and oxidative stress (**Azad et al., 2010**). Exposure to heat stress increased lipid peroxidation which is indicated by the increase of MDA concentration (**Lin et al., 2010**).

Lipotropes limit excess hepatic fat deposits by hastening fat removal, limiting fat uptake, increasing fatty acid oxidation and reducing fatty acid and triacylglycerol synthesis **(Gastaldelli et al., 2009)**.

L-carnitine (3-hydroxy-4-N-trimethylaminobutyric acid) is an important cofactor in β-oxidation, as it facilitates the transport of long-chain fatty acids into the inner mitochondrial matrix in the form of acyl carnitines **(Jo et al., 2004)**. The dietary L-carnitine supplements might increase the energy utilization efficiency from lipid oxidation (**Harpaz, 2005**). In addition, dietary L-carnitine supplements can enable the utilization of high-energy diets with less accumulation of fat (**Ozorio, 2001**). Supplementation of L-carnitine in the diets accelerate lipid flux into oxidative metabolism.

L-Carnitine completely abolishes the age-dependent rise of plasma cholesterol & triacylglycerol. The lipid lowering effect of L-carnitine was reported by **Shimura and Hasegawa (1993)**.

L-carnitine decreases free radical formation by acting as a metal chelator and decreasing the concentration of cytosolic iron, which plays an important role in formation of ROS **(Rani and Paneerselvam, 2001).** Also it retards oxidative damage to sulphydryl groups by direct radical scavenging activity **(Gulcin, 2006)** and indirectly reduced oxidative stress by its ability to stabilize mitochondrial membranes and metals chelating properties which have been shown to attenuate toxic cation-induced generation of ROS through the electron transport chain **(Loots et al., 2004).**

Synbiotics are defined as a combination of probiotics and prebiotics, which aimed to increase the survival and activity of beneficial intestinal flora **(Eamonn, 2010)**.

Probiotics are defined as live organisms that, when ingested in adequate amounts, exert a health benefit to the host. The most commonly used probiotics are Lactobacilli and Bifidobacteria **(Eamonn, 2010)**. They inhibit pathogen adhesion **(Laparra and Sanz, 2010),** enhance mineral absorption **(Scholz et al., 2007),** modulate lipid metabolism **(Brighenti, 2007)**.

Chicken fed with probiotics showed a significant decrease in cholesterol concentration **(Alkhalf et al., 2010).** Moreover, **Arun et al. (2006)** found that serum cholesterol and triacylglycerol were significantly reduced by dietary

supplementation of probiotics. It is thought that Probiotics have a hypocholesterolemic effect by inhibiting the synthesis of hydroxyl methyl-glutaryl-coenzyme A (HMG COA) reductase **(Fukushima and Nakano 1995)**.

Also, probiotics can reduce the blood cholesterol level through its bile salt hydrolytic activity which is responsible for deconjugation of bile salts **(Surono, 2003)**. Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and are more likely to excrete in faeces **(Klaver and van, 1993)**. The increased excretion of bile acids is associated with the conversion of cholesterol to bile acids (**Ellegard and Andersson, 2007**).

Prebiotics are defined as selectively fermented ingredients that allow specific changes, both in the composition and/or the activity of the gastrointestinal microbiota that confers benefits upon host well-being and health **(Gibson et al., 2004).**

β -Glucan is a fiber that is made up of a chain of D-glucose molecules linked by (β1-4) bonds to form oligomers that are, in turn, linked by (β1-3) bonds to form longer molecules (**Lazaridou and Biliaderis, 2007**). As a result of β -glucan structure (β-glycosidic linkage) it can't be digested in the stomach &can be utilized by intestinal microflora as a prebiotic.

There is a relationship between the consumption of β -glucan and the reduction of blood cholesterol concentrations. Cholesterol lowering effects may be a result of increased excretion of bile acids, which are then replaced by a conversion of cholesterol to bile acids (**Ellegard and Andersson, 2007**), decreased absorption of dietary cholesterol (**Theuwissen and Mensink, 2007**) and an increased production of short chain fatty acids in the colon. This down regulates cholesterol neosynthesis (**Drozdowski et al., 2010**) alone or by a combination of the three mechanisms.

β-glucan inhibits the in vitro uptake of the long-chain fatty acids particularly at higher fatty acid concentrations. Cholesterol uptake was also reduced. The expression of intestinal genes associated with fatty acid and cholesterol syntheses and fatty acid transport was also down-regulated (**Laurie et al., 2010**).

It has become clear that a group of non-digestible carbohydrates with fiber-like properties (NDCs: fructans, Raffinose Family Oligosaccharides or RFOs, arabinoxylans, b-glucans) and their breakdown products(e.g. fructosyl oligosaccharides or FOS) as well as sugar-sterols and sugar-phenols might act as important ROS scavengers **(Nishizawa et al., 2008)**

The mechanism of (1→3)-β-d-glucan activity as the typical antioxidant may be attributed to the hydroxyl groups present in the carbohydrate molecule. For (1→3)-β -d-glucan derivative the scavenging activity was much stronger than that for d-mannitol **(Yilmaz et al., 2007)**. These findings suggest that the increased antioxidant activity of polysaccharides in comparison with monosaccharides is due to the fact that polysaccharides contain multiple anomeric hydrogen atoms, which are primarily abstracted by the active free radicals, while monosaccharides possess only one such anomeric hydrogen **(Silke et al., 2007)**.

Material and methods

1.Material

Chemicals

The following chemicals were used: L-carnitine as a commercial product (L-carnitine)^{R} 300 mg/ml, Mepaco-Medifood, Egypt. Probiotic was purchased as (Lacteol Fort)^{R} which is composed of lactobacillus delbruekii and lactobacillus fermentum (10 billions /sachet), Rameda, Egypt. Also prebiotic was used as (Betapolo)^{R} β 1,3-glucan 30 mg/ml, Sky pharma, Egypt.

2.Methods

2.1.Experimental design & animal grouping

1.1.Experimental animals

One day- old 120 broiler chickens (cobb) were purchased from El Qahera Company for poultry industry, Egypt. They were reared at (35^0 C) & the temperature decrease (0.5^0 C) per day till reach to optimal temp. (25^0 C$\pm$ 0.5^0 C) & relative humidity (75 %). The chicks were reared until 38 days old.

Ration composition: Ration source: El Qahera Company for poultry industry, Cairo, Egypt.

Ration composition:

	Starter	**Growing**
Ingredients		
Crude protein	not less than 23%	not less than 21%
Crude fat	not less than 8.20 %	not less than 6.14 %
Crude fiber	not more than 3.50 %	not more than 3.44 %
Full energy	not less than 3050 Kcal/ kg	not less than 3146 Kcal/ kg

At the 20th day, the chicks were divided into 4 groups (n=30) three replicates per each group as following

Group 1(Normal):

Chickens in this group were received free water, feed ad libitum and were reared at optimal temp.

Group 2 (FR&HS)

Chickens in this group were received free water &feed ad libitum till 28th day, and then they were subjected to repeated fasting &refeeding cycles (fasting for 9 hours from 10:00 am to 7:00 pm) (**Rosebrough, 2000**) and were kept at high temperature (35^0 C) for 9 hours (**Zulkifli et al., 2003**) till the end of experiment.

Group 3 (FR&HS + LC)

The broiler chickens of this group were received water &feed ad libitum till 28th day. Starting from the twentieth day of the experimental period, L-carnitine was added in drinking water (1 ml/ L). The birds of this group were subjected to both" repeated fasting &refeeding cycles" and heat stress from 28th day till the end of experiment.

Group 4 (FR&HS + Syn)

The broiler chickens of this group were received water &feed ad libitum till 28th day. Starting from the twentieth day of the experimental period, synbiotics were added in drinking water (probiotic 1gm/L, prebiotic 1ml/L). The birds of this group were subjected to both "repeated fasting &refeeding cycles" and heat stress from 28th day till the end of experiment.

2.2. Sampling &tissue preparation

2.2.1. Serum

At the end of experimental period (38 days), where the broiler chickens reached the target weight, they were slaughtered and the blood samples were collected. Blood samples were left at room temperature for 20 minutes to clot. The clotted blood samples were centrifuged at 1000 X g for 15 minutes for serum separation. The serum samples were kept in labeled epindorf tubes at -20 ºC till use.

2.2.2. liver tissue preparation

After slaughtering, the abdomen was opened and rinsed with physiological saline. Liver tissues were quickly collected and then washed by physiological saline to remove any clotted blood. The liver samples were divided into two parts. The first part (0.5 gm) was suspended in 5 ml physiological saline (0.9 % NaCl) for homogenization (Teflon Homogenizer, India). The liver tissue homogenates were centrifuged 1500 X g for 20 minutes at 4 ºC. The supernatants were kept at – 20 ºC till the time of determination of oxidative/ antioxidant parameters (**Lin et al., 2010**). The second part was placed in 10 % formaline solution for histopathological investigations.

2.3. Biochemical parameters:

All kits of the measured parameters were purchased from Biodiagnostic company (Cairo, Egypt). The following parameters are measured using T80 UV/VIS spectrometer (China).

2.3.1. Determination of lipid profile

Total serum lipids were measured by a biodiagnostic test kit for total lipids which is based on sulphospho-vanillin reaction described by **Zollner and Kirsch, 1962**. Serum triacylglycerol was determined by biodiagnostic test kit for triacylglycerol which is based on the enzymatic colorimetric method described by **Fassati and Prencipe, 1982**. Total serum cholesterol was measured by biodiagnostic test kit for total cholesterol which is based on enzymatic colorimetric method described by **Richmond, 1973.**

LDL-Cholesterol measurement occurred according to **Wieland and Seidel, 1983**. HDL-Cholesterol determination based on **Burstein et al., 1970**.

2.3.2. Determination of antioxidant parameters

Nitric oxide (NO) was measured according to **Montgomery and Dymock, 1961.** Total antioxidant capacity (TAC) determination was based on **Koracevic et al., 2001.** Malondialdehyde (MDA) was measured according to **Satoh, 1978**. Superoxide dismutase (SOD) activity measurement was based on **Nishikimi et al., 1972.** Reduced glutathione (GSH) content was measured according to **Beutler et al., 1963.** Glutathione Reductase (GR) activity was determined according to **Goldberg and spooner, (1983).**

2.4. Histopathological examination.

Liver samples were fixed in 10% formalin solution for 48hrs. Then they were processed (washed by water, dehydrated in graduated ethyl alcohol, cleared in xyline and embedded in paraffin wax at 70 ^{0}C) according to **Bancroft and Gamble (2008)**. Five microns tissue thickness were mounted on clean glass slides and stained by Heamatoxylin and Eosin.

2.5. Statistical analysis.

All data were expressed as means± SEM. Differences between the groups were determined by one-way ANOVA followed by least square difference (LSD) post hoc, using SPSS software version 15.0. and results were considered significant when $P < 0.05$ (**Wei et al., 2009**).

Results

Tables (1): changes of total lipids and TAG concentrations in Normal, FR&HS, FR&HS +LC and FR&HS+Syn groups.

This table showed a significant increase in the serum levels of total lipids and TAG of FR&HS groups as compared to normal. On the other hand, There was a significant decrease in the level of these parameters in FR&HS+LC and FR&HS+Syn groups in comparison with FR&HS group. Moreover, L-carnitine administration led to non significant changes in these parameter levels as compared to normal.

Parameters / Group	Total lipids(mg/dl)	TAG(mg/dl)
Normal	**200.81 ± 52.40^{a}**	**34.83 ± 7.20^{a}**
FR&HS	**643.44 ± 106.13^{b}**	**133.97 ± 6.50^{b}**
FR&HS+LC	**389.34 ± 38.27^{a}**	**56.10 ± 19.80^{a}**
FR&HS+Syn	**398.36 ± 19.58^{c}**	**68.75 ± 13.39^{c}**

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups in the same column.

Tables (2): changes of total cholesterol, LDL-C and HDL-C concentrations in Normal, FR&HS, FR &HS+LC and FR&HS+Syn groups.

Measurement of serum levels of total cholesterol, LDL-C and HDL- C of tested birds' groups relvealed that, there was a significant increase in serum levels of total cholesterol and LDL-C of FR&HS group as compared to those of normal group. However, there was a significant decrease in serum level of HDL-C of FR&HS group in comparison with that of normal one. Administration of L-carnitine and synbiotics led to a significant decrease in both total cholesterol and LDL-C levels in FR&HS+LC and FR&HS+Syn groups as compared to FR&HS groups. Moreover, synbiotics administration resulted in a significant increase in serum HDL-C level when compared to FR&HS group.

Parameters / Group	Total cholesterol (mg/dl)	LDL-C(mg/dl)	HDL-C(mg/dl)
Normal	**117.19± 2.16^{a}**	**16.59 ± 6.20^{a}**	**93.63 ± 1.84^{a}**
FR&HS	**305.21 ± 6.31^{b}**	**221.99 ± 6.88^{b}**	**56.43 ± 2.70^{b}**
FR&HS+LC	**124.50 ± 2.36^{c}**	**51.48 ± 4.82^{a,c}**	**61.80 ± 2.06^{b}**
FR&HS+Syn	**155.47 ± 9.10^{c}**	**67.75 ± 4.64^{c}**	**73.97 ± 1.55^{d}**

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups in the same column.

Table (3): changes of hepatic GSH concentration, GR and SOD activities in Normal, FR&HS, FR&HS+LC and FR&HS+Syn groups.

Results in this table showed a significant decrease of hepatic GSH content, GR and SOD activities of FR&HS group as compared to those of normal group. Moreover, there was a significant increase of hepatic GSH content, GR and SOD activities of FR&HS +LC, FR&HS+ Syn as compared to those of FR&HS group.

Parameters / Groups	GSH (mg /gm tissue)	GR (U/ L)	SOD (U/gm tissue)
Normal	**47.00 ± 4.12^{a}**	**562.66 ± 78.34^{a}**	**327.69 ± 79.32^{a}**
FR&HS	**14.98 ± .91^{b}**	**99.67 ± 37.90^{b}**	**80.11 ± 6.90^{b}**
FR&HS+LC	**45.43 ± 3.05^{c}**	**559.44 ± 46.96^{a}**	**291.56 ± 9.92^{a}**
FR &HS+ Syn	**32.95 ± 1.62^{a}**	**517.64 ± 20.31^{a}**	**279.54 ± 5.86^{a}**

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups in the same column.

Table (4): changes of MDA, TAC and NO in Normal, FR&HS, FR&HS+LC and FR&HS+Syn groups.

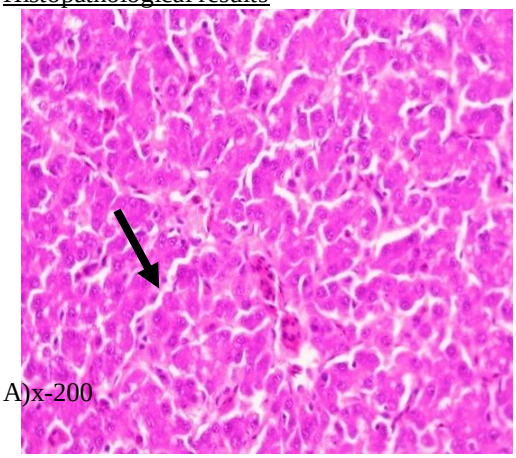
This table showed a significant increase in hepatic MDA and NO contents and a significant decrease in hepatic TAC of FR&HS group in comparison with normal group. Administration of L-carnitine or synbiotics resulted in a significant

decrease in hepatic MDA and NO contents and a significant increase in hepatic TAC of FR&HS+LC and RF&HS+Syn. groups respectively as compared to those of FR&HS group.

Parameters / Groups	MDA (nmol/ gm tissue)	TAC (mM / L)	NO (µmol/ L)
Normal	**2.18 ± .44^{a}**	**20.62 ± .74^{a}**	**39.75 ± 3.98^{a}**
FR&HS	**30.40 ± .67^{b}**	**1.97 ± .07^{b}**	**125.34 ± 15.48^{b}**
FR&HS+LC	**9.64 ± .83^{c}**	**5.20 ± .44^{c}**	**55.53 ± 9.96^{a,c}**
FR& HS+ Syn	**11.01 ± 1.08^{c}**	**5.58 ± .21^{c}**	**83.72 ± 4.27^{c}**

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups in the same column.

Histopathological results



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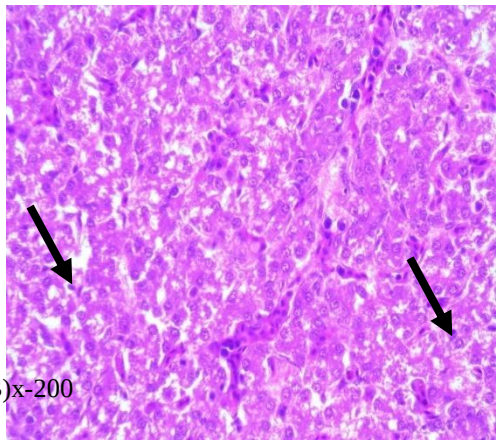

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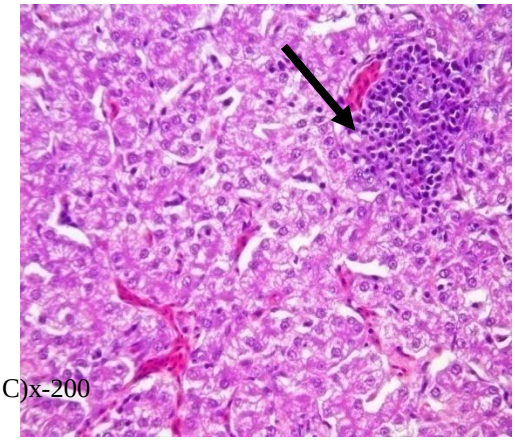
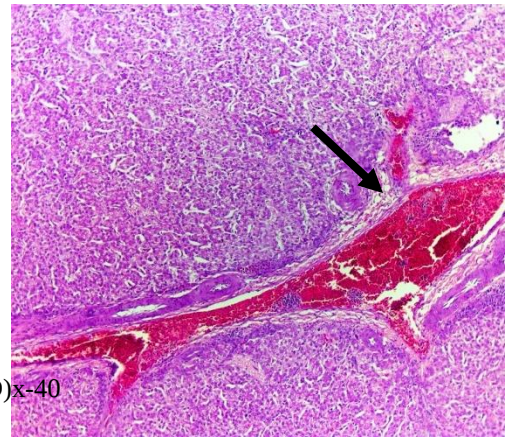


C)x-200



D)x-40

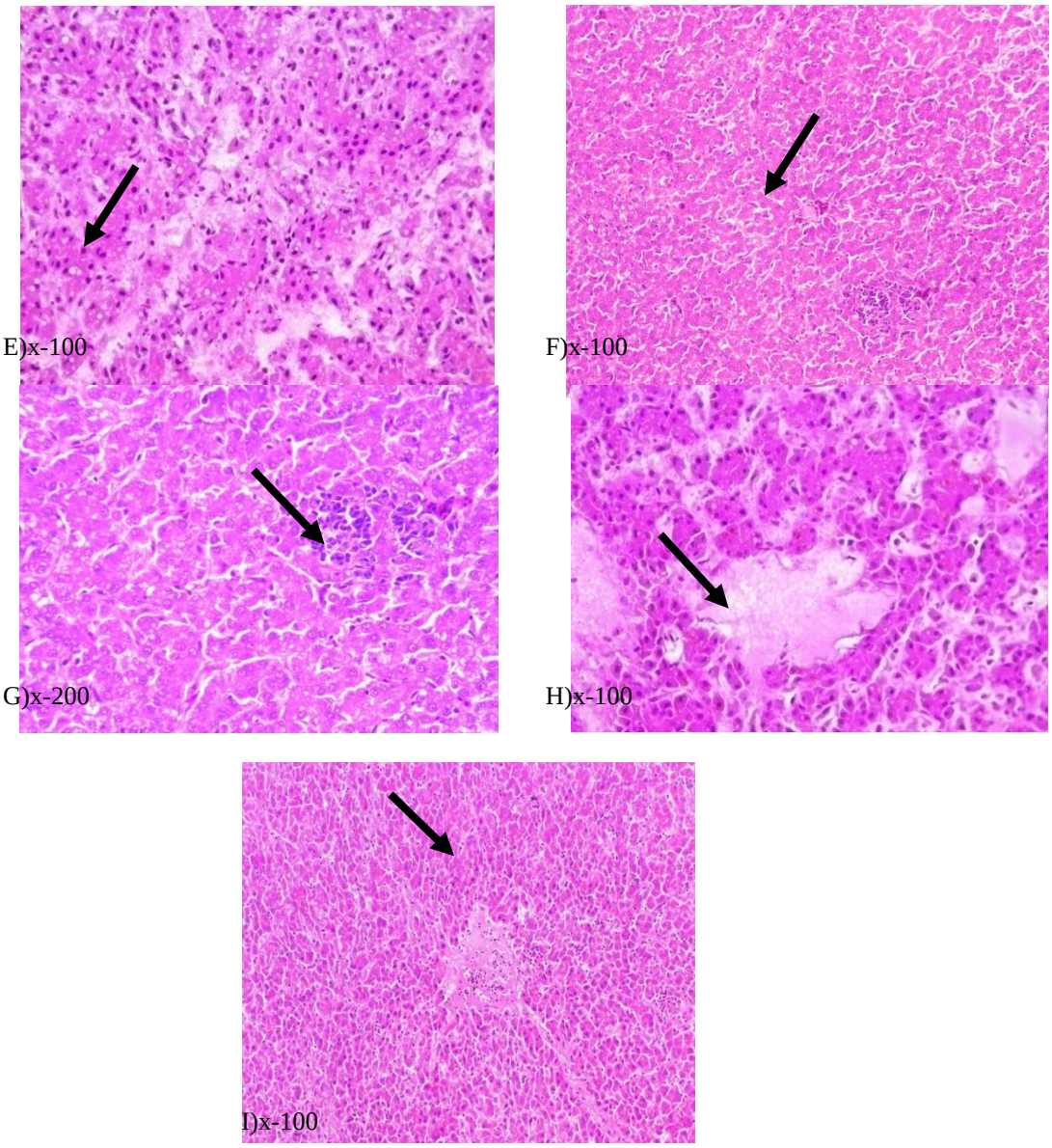


Figure (1): Figure (1,A) the liver of control group appeared histologically normal. The hepatic cells were polyhedral and had 1 to 2 nuclei. Figure (1,B) the liver of FR&HS group showed degenerative changes represented in hydropic degeneration. Figure (1,C) multifocal leucocytic infiltration were prominent in the hepatic parenchyma and portal areas in liver of FR&HS. liver of FR&HS had congestion of blood vessels (figure 1,D). Fatty changes (figure 1,E) were seen in the liver of FR&HS. L-carnitine treated group showed mild hepatic degenerative changes (figure 1,F) and mild leucocytic infilteration (figure 1,G). Synbiotics treated group showed mild hepatic oedematous changes (figure 1,H) and mild vacuolar degeneration (figure 1,I). Lesions were pointed by a head of an arrow.

Discussion

The obtained results as shown in **table (1)** revealed that there was a significant increase in serum TAG and total lipids levels during fasting &refeeding cycles and heat stress. These results come in agreement with data obtained by **Pei et al. (2009**) who demonstrated that fasting and refeeding is associated with increase lipogenesis and this could be attributed to increase in the gene expression of hepatic lipogenic enzymes consequently, leads to hyperlipidemia.

Added to that, there was a significant increase in serum total cholesterol level in FR&HS group as compared to normal group **(table 2)**. This increase of total cholesterol level could be attributed to the FR&Hs induced hyperlpidienmia as the hyperlipidemia is associated with constant increases in total cholesterol and/or triacylglycerol

blood levels **(Ramin et al., 2012**). Moreover, there was a significant increase in serum LDL-C and a significant decrease in serum HDL-C levels of FR&HS group **(table 2)** this agreed with data obtained by **Chi (2003)**. **Fernanda et al. (1995**) demonstrated that the imbalance in the antioxidant defense system seems to be a result of the accumulation of LDL in the course of hyperlipidemia.

Our results showed a significant decrease in hepatic GSH content and GR activity **(Table 3)**. GSH concentration was significantly decreased in hypercholesterolemia (**Esra et al., 2009**). when chickens were exposed to heat stress, their liver GSH concentratin decreased **(Kamel and Edens 2003)** and this declaired by **Song et al. (2000)** who demonstrated that mobilization of the liver GSH storage pool increased when broiler chickens were subjected to heat stress. There was a significant decrease in SOD activity. GSH and SOD in the living tissues prevent cells from the free radical-induced damage and provide a repair mechanism for oxidized membrane components. The activity of SOD was inversely correlated with total cholesterol and triacylglycerol levels **(Fernanda et al. 1995).**

In the current study, lipid peroxidation level was estimated by the measurement of MDA where there was a significant increase in hepatic MDA and a decrease in TAC in FR&HS group as compared to normal group. These changes could be attributed to FR&HS induced hyperlipidemia and oxidative stress. TAC is tightly regulated in conditions of increased exposure to ROS and have been measured in different disease conditions as reliable indices of oxidative stress (**Duarte et al., 2010**).

Our data were in agreement with **Enkhtaivan et al. (2011)** who revealed that hyperlipidemia causes a significant decrease of TAC and a significant increase of serum MDA. The serum total cholesterol level was positive correlation with MDA. Hypercholesterolemia induces the production of ROS such as superoxide anion, through enzymes like NADPH oxidase and xanthine oxidase as well as other mitochondrial ROS sources (**Munzel et al., 2010**).

Exposure to HS increased lipid peroxidation as a consequence of the increased generation of ROS which indicated by the concentration of MDA (**Lin et al., 2010**). Therefore, the content of MDA in serum and tissue can reflect the extent of lipid peroxidation and over production of ROS in the body.

Our results showed a significant increase in NO level in FR&HS group as compared to normal group and the increase in NO production could be attributed to oxidative stress (**Gonzalez et al., 2004**). This finding can be declared by **Dai et al. (2011**) who reported that HS increased NO level by increasing the activity of NOS (nitric oxide synthase).

NO and ROS can react to form peroxynitrite anion, which rapidly decomposes to hydroxyl anion and nitrogen dioxide, the former being a highly reactive and toxic compound. Thus, a complex interaction between NO and ROS may exist (**Ahmet et al., 2001**).

Our biochemical results were confirmed with histopathological examination as shown in figure (1,B) the liver of FR&HS group showed degenerative changes represented hydropic degeneration as compared to the liver of normal group figure (1,A). Also there was multifocal leucocytic infiltration were prominent in the hepatic parenchyma and portal areas(figure 1,C). Moreover, congestion of blood vessels (figure 1,D) and fatty changes (figure 1,E) were seen.

L-Carnitine is a vital endogenous component of lipid metabolism necessary for the production of ATP through the β-oxidation of long-chain fatty acids in the mitochondrial matrix (**Vanella et al., 2000**). L-carnitine has a hypolipidemic effect and this is declared by **Shuenn et al. (2012)** who reported that supplementation of L-carnitine in the diets accelerate lipid flux into oxidative metabolism, and consequently reduce the body lipid accumulation. Also it has been reported that L-carnitine has antioxidant activities. Marked improvement in the antioxidant cellular status has been demonstrated using L-carnitine (**Dayanandan et al., 2001**)

The present study demonstrated the ability of L-carnitine to decrease the serum total lipids and TAG levels when compared to FR&HS group **(table 1)**. **So et al. (2010)** reported that L-carnitine administration lead to decrease in serum TAG level and circulating fatty acids during fasting.

L-carnitine administration decreased serum cholesterol and LDL-C levels **(Table 2)** and these results were in agreement with that obtained by **Shimura and Hasegawa (1993)** who reported the lipid lowering effect of carnitine. This effect of L-carnitine seems to be related to the changes in hepatic fatty acid handling. Also this table showed that administration of L-carnitine caused a little, non significant increase of serum HDL-C. L-Carnitine causes a remarkable decrease in the content of saturated fatty acids in vLDL + LDL fraction and increases this content in the HDL particle. This response is consistent with an increase in fatty acid β-oxidation and/or an enhancement in

lipoprotein metabolism. Therefore, carnitine supplementation may be needed to support lipid metabolism **(Diaz et al. 2006)**.

In our study, L-carnitine led to a significant increase in hepatic GSH level, GR and SOD activities (**table 3**). These results were in agreement with results which were obtained by **Agnieszka and Elzbieta (2009)** who observed that L-carnitine caused an increase in the liver GSH level. An elevation of GSH level on L-carnitine therapy may be due to increase in NADPH generation through the increase in the activity of G6PDH. NADPH is used by glutathione reductase to reduce GSSG to GSH (**Kumaran et al., 2003**).

There were a significant decrease in hepatic MDA and NO levels and a significant increase in TAC in the group treated L-carnitine when compared to FR&HS group **(Table 4)**. These results agreed with results which were detected by **Graziela et al. (2010**) who reported that L-carnitine reduces lipid oxidative damage. They also observed that L-carnitine was inversely correlated to MDA level, what may possibly reflect an antioxidant activity for L-carnitine.

Antioxidant effects of L-carnitine has been associated with its capacity to act as a metal chelator, decreasing free Fe^{+2} ions, which have a vital role in free radical production (**Muthuswamy et al., 2006**). L-carnitine has been also shown to scavenge the superoxide radical (**Gulcin, 2006**), hydrogen peroxide and hydroxyl radical (**Derin et al., 2004**) which induce oxidative damage in lipids, proteins and DNA (**Halliwell, 2006**).

L-carnitine has also been reported to decrease lipid peroxidation and protect tissues from damage by repairing oxidized membrane lipids (**Rani and Panneerselvam, 2002**) and maintaining the levels of antioxidants (**Augustyniak and Skrzydlewska, 2009**) and increasing the activity of enzymes which are involved in the defense mechanism against oxidative damage (**Rani and Panneerselvam, 2002**).

These biochemical results were confirmed with histopathological examination as shown in figure (1, F) the degenerative changes were less commonly observed in the liver of FR&HS+LC group when compared with the liver of FR&HS group. In FR&HS+LC group the liver had mild infiltration with leucocytes (figure 1,G).

Synbiotics is a mixture of probiotics and prebiotics which has a hypolipidemic effect through the decrease of hepatic de novo lipogenesis (**Cani et al., 2009**). Moreover, the antioxidant properties of probiotic strains given to broiler chickens in drinking water have been stated by **Marcela et al. (2011).**

The results illustrated in **table (1)** proved that synbiotics administration caused a significant decrease in serum total lipids and TAG levels when compared to FR&HS group. These results were in accordance with that of **Kurtoglu et al. (2004)** who stated that serum TAG level were reduced by using probiotics supplementation. The use of prebiotics significantly reduce the hepatic triacylglycerol accumulation (**Delzenne et al., 2008**).

There were a significant decrease in serum total cholesterol and LDL-C levels in synbiotics treated group as compared to FR&HS group **(table 2)**. Also synbiotics increased the serum level of HDL-C. **Kerckhoffs et al. (2003**) reported that β-glucans have been shown to reduce serum cholesterol level. When probiotic was administered to broilers at day 21 of age, this birds at day 42 of age showed higher concentration of serum HDL-C in comparison to those fed the control diet (**Daneshmand et al., 2011**). β-glucan was effectively improving the serum lipid profile (**In et al., 2009**).

The gut microbiota exchange metabolites with the host and interact with host signaling pathways to modulate host bile acid, lipid and amino acid metabolism as well as host gene expression (**Velagapudi et al., 2010**). A key concept is that products of gut microbial metabolism, in particular short chain fatty acids (SCFAs) function via diverse host molecular mechanisms to regulate host energy intake, energy expenditure and storage (**Jian et al., 2012**).

The obtained results as shown in **table (3)** revealed that synbiotics administration caused a significant increase hepatic GSH content, GR and SOD activities as compared to FR&HS group. The anti-oxidant activities of probiotics may be attributed to proteolytic fermentation which generates polyphenols that induce anti-inflammatory and anti-oxidative effects **(Compare et al., 2012)**. Moreover, the depletion of tissue GSH stores was inhibited by β-glucan **(Goksel et al., 2006)**.

The antioxidant capacity of synbiotics was detected by a significant decrease of hepatic MDA and NO levels in synbiotics treated group when compared to FR&HS group **(table 4)**. Moreover, the high level of TAC of FR&HS+Syn group in comparison with FR&HS group **(table 4)**. These results were in agreement with that

observed by **Goksel et al. (2006)** who declared that the treatment with β-glucan prevented the elevation in tissue MDA level. **Yilmaz et al., (2007)** explained that the mechanism of β-glucan activity, as the typical antioxidant, may be attributed to the hydroxyl groups present in the carbohydrate molecule. **Marcela et al. (2011)** also showed that probiotics administration is associated with an increase of total antioxidant status and an improved bioquality of LDL particles of sera.

Our biochemical results were confirmed with histopathological examination as shown in figure (1,H) the liver of synbiotics treated group showed mild oedematous changes and mild vacuolar degeneration (figure 1,I) when compared with the liver of FR&HS group.

Conflict of interest: we would like to prove that all authors have no conflict of interest.

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