OXIDATIVE STATUS AND THE INCIDENCE OF FATTY LIVER HEMORRHAGIC SYNDROME IN LAYING HENS FED FLAX SEEDS

Sara. A. M[*1], M. E. BARRI[2], SALWA. M. E[1], SAMIA. H. A[1] and HALA, E.A AND FATIMA. El sheikh. El sammani[2]

[1]Veterinary Research Institute
[2]Africa International University

*Corresponding Author: E-mail: shambat@yahoo.com
Tel: 00249912152945

Abstract - **The current trial was performed to investigate the influence of flax seeds on the oxidative status and incidence of FLHS in laying hens. Fifty (Hisex) bird, 20 week old, were obtained from Animal Production Research Center (kuku), were divided into two groups (n=25) for each one. The control group (A) was under control diet based on corn, maintains the (NRC, 1994) requirements for laying hens, the experimental group, supplemented by 10% flax seeds added to the diet. The trial lasted for eight weeks, blood samples were collected once per month (week 4 and 8), in EDTA coated vials, immediately placed into iced-container, centrifuged at 3000 rpm/20 min, samples were separated in aliquot, and stored at -20ºC and -80ºC until analysis.**

No incidence of fatty liver hemorrhagic syndrome was noticed after feeding laying hens diet containing 10% flax seeds for two months.

Plasma analysis for enzymatic and non-enzymatic antioxidants, revealed no significant different levels of uric acid, malondialdehyde (MDA), vitamin C, and vitamin E between the control group and the treated one, while catalase (CAT), superoxide dismutase (SOD), and vitamin A, levels were significantly enhanced by the addition of 10% flax seeds.

Index Terms - **FLHS, liver enzymes, Oxidative status, Flax seeds, Layers, SOD, CAT, MDA.**

I. INTRODUCTION

Fatty liver-hemorrhagic syndrome occurs in commercial layers in high production and is frequently the major cause of death in healthy flocks causing up to 5% mortality during the laying cycle. Hemorrhage occurs from a ruptured liver. The liver capsule frequently ruptures as well so that a large blood clot is found in the ventral hepatoperitoneal sac of the affected lobe. The liver in high production hens is fragile because of the large amount of lipid present to supply lipid for the developing ova. Rupture and death frequently occur during the increased abdominal pressure of egg-laying. If the liver capsule does not rupture the hen may survive and a large hematoma remains in the liver. These hens may cease production, at least temporarily. There has been extensive research into the cause and prevention of FLHS, with a higher incidence being reported in birds on a high-energy ration in hot weather **(1), (2), (3)** and **(4)** that could increase the fat content of the liver. Analysis of the fatty acid composition of plasma phospholipids showed a difference between normal and FLHS-susceptible laying hens. However the composition of dietary lipids may be more important than total dietary lipids **(5).** This could influence the structural properties and integrity of cell membranes or have an anti-inflammatory effect. The coagulation profile is also different. High levels of plasma estradiol increase the risk of FLHS **(6),** and hens in high production have high levels of estradiol. Rapeseed meal in the ration increases the incidence of FLHS because erucic acid or other toxic products affect the strength of the connective tissue in the liver **(7)** and **(8).** FLHS has also been reported in quail **(9).** Histopathological evidence of a greater hepatocellular lipid infiltration in hens fed 3% Menhaden oil (MO) vs. diets with no supplemented fat was reported by **(10)** suggesting a role of n-3 FA. Nevertheless, no difference in gross liver rank (assessed by liver integrity and fragility) was found.

In a further experiment, the same researchers first noted hepatic lipid infiltration after 4 month of feeding hens diets with 3% MO **(11).** The infiltration consistently increased in severity after 5 and 6 month, being statistically significant when compared with controls fed diets with 3% animal-vegetable fat.

A high antioxidative status has been regarded as one of the major factors positively affecting the production performance in the intensive poultry industry **(12)** and **(13).**

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism **(14).** The harmful effects of ROS are balanced by the action of antioxidants, some of which are enzymes present in the body **(15).**

Recent studies have clearly established that dietary lipids and nutrients play important roles in

determining the strength of cellular antioxidative defense mechanisms **(16)** and **(17).** Antioxidants constitute a major cell defense against acute oxygen toxicity and protect membrane components against damage caused by free radicals. Poly unsaturated fatty acids (PUFA), induce an increase in the activities of antioxidant enzymes and a decrease in lipid peroxidation **(18).**

An interaction between n-3 FA and 17b-estradiol on the lipogenic activity of the liver was suggested, thus increasing the hen's susceptibility to hepatic lipidosis. In contrast, **(19),** used hens from an inbred line selected for predisposition to fatty liver hemorrhagic syndrome fed LNA and LCn-3 enriched diets for 4 week. There was a drop in serum triglycerides, lower hepatic dry matter and lipid content when compared with controls although hemorrhage scores were not affected. Reducing liver fat content was not effective in preventing hemorrhage. These workers also suggested that the hepatic oxidative status was not hampered by n-3 FA dietary supplementation although no histopathology was reported.

Flaxseed is known for its unique nutrient profile which provides 534 kcal and contains fat 42%, protein 18%, total dietary fiber 28%. Flaxseed contains 27% of fiber of which two-thirds is insoluble and one-third soluble. Insoluble fiber consists of cellulose, hemicellulose and lignin. Soluble fiber is in the form of mucilaginous material composed of polysaccharides and has proved to reduce cholesterol and regulate blood glucose **(20).** It also contains good amount of α-Linolenic Acid (ALA), omega-3 fatty acid, protein, dietary fiber, lignan. Flaxseed proteins are relatively high in arginine, aspartic acid and glutamic acid whereas lysine, methionine and cysteine are limiting amino acid **(21).**

II. OBJECTIVES

- To investigate the influence of feeding laying hens, diet contains 10% flax seeds, on the incidence of FLHS, from histopathological and liver dysfunction biomarkers prospective.
- To investigate the correlation between the blood oxidative status and the incidence of FLHS.

III. MATERIALS AND METHODS

The experiment was held in the Veterinary Research Institute (VRI), from January to march 2014. The duration of the experiment was eight weeks.

Four, full wire cages were made, each cage was (2X 1.5X 1 meter), and the capacity of each cage was 15 birds. The cages were placed at an open poultry house.

Fifty laying hens (Hisex) breed, 20 week old, obtained from animal production research center (kuku), were utilized in this study. The birds were divided into two groups, 25 birds per group.

Experimental Diets: The diets were formulated to meet the requirements of egg production according to the directions of the national research council**, (22).** Two formulae of diets were prepared, 10% of flaxseeds was inserted into the experimental one.

The supplementary source was subjected to proximate analysis, to determine its content of protein, fat, fiber, N.F.E and energy.

❖ **Management:**

Each group received its diet from day one. Drinking system contained two tanks for each cage, the tanks were cleaned, and the water was changed twice daily. Birds received 24 hour light/day throughout the experiment.

Three ml of blood was collected from twenty bird of each group, the blood was taken using a three ml syringe, and received into EDTA coated vials, and immediately were kept in iced container, the samples were centrifuged at 3000 **rpm** for 20 minutes, and plasma was separated in aliquot and transferred into plane vials. Plasma samples were stored at -20° and -80° C until analysis.

❖ **Fatty Acids Analysis:**

Lipids were extracted in chloroform-methanol (2:1 v/v), according to the method of, **(23).**

Methyl esters of the lipid extract were prepared according to, **(24).**

Table 1: Proximate analysis of flaxseeds

	D.M%	Moisture%	Protein %	Fat %	Fiber%	Ash%	N.F.E%	Energy%
Flaxseeds	96.55	3.45	27.84	35.69	10.78	4.67	17.57	3932.0

Table 2-1: Diets composition

Group Raw Materials%	A	B
Corn %	70	60.0
Wheat hull %	0	4.1
Groundnut cake %	14.3	10
Concentrate %	5	5
Calcium Carbonate%	10	10
Salt (Nacl) %	0.125	0.25
Methionine %	0.34	0.34
Lysine %	0.15	0.15
Mycofix %	0.1	0.1
Flaxseeds seeds%	----	10
Premix*	0.1	0.1

Table 2-2 Nutritional values calculated

Parameter	A	B
ME. Kcal/Kg	2729	2832
C.P%	17.25	18.13
E.E%	5	6.4
C.F%	4.2	4.3
Available phosphorus%	0.52	0.65
Calcium%	3.9	3.9

*Supplied per kilogram of diets: Vitamin A, 5000 IU; Vitamin D , 500 IU; Vitmin E, 5 IU; Vitamin K, 1 IU; Vitamin B , 1.5 mg, Vitamin B , 2.5mg, 1 2 Ca-pantothenate, 2.5mg, niacin acid, 10 mg; pyridoxine,3mg; biotin, 0.1mg; folic acid, 0.25mg; Vitamin B , 0.005mg. Supplied per kilogram 12 b of diets: MnSO. 7H O100mg, FeSO. 7H O, 220mg; ZnSO. 7H O, 150mg; CuSO. 7H O, 20mg; KI, 2mg; Na SeO, 0.4 mg.

- **A**=Control group, **B**=10% flaxseeds supplemented group.

❖ **Gas Chromatograph Analysis:**

Fatty acid composition was determined using (Shimadzu-2010) gas chromatograph, fitted with Flame ionization detector (FID). Separation of fatty acids was achieved using DB-WAX column, serial number (us6551263 H), of 0.25um film thickness, 30 meter length and 0.25 mm inner diameter, split less mode.

Fatty acids methyl esters were identified by comparison of retention times with standards, and expressed as percentage of methyl esters.

By the end of the trial, five birds from each group were slaughtered, liver was removed and placed into containers filled with 10% formalin for histopathological analysis.

Liver function tests:

All spectrophotometric analysis was done using spectrophotometer UV/VIS Unicam 8625.

Albumin was determined using commercial kits (Biosystem, Spain), spectrophotometric method described by **(25), (26), (27)** and **(28).**

Table 3: Fatty acids profile of control and experimental group diets

Fatty acid	Control group (A)	Flax seeds supplemented group (B)
(g/100 g total fatty acids)		
SFA	43.53	11.42
MFA	17.68	16.22
PUFA	38.38	72.37
C18:3 (n-3)	1.38	49.10
Σn-3	1.38	49.10
C18:2 (n-6)	36.94	11.67
C20:4 (n-6)	0.21	3.48
Σn-6	37.15	15.15
PUFA/SFA	0.88	6.33
Σn-3/Σn-6	0.04	3.24

SFA= Saturated fatty acids, MFA=Mono unsaturated fatty acids, PUFA=Poly unsaturated fatty acids, C18:3 (n-3) =Linolenic acid (omega-3), C18:2 (n-6) =Linoleic acid (omega-6), C20:4 (n-6) = Arachidonic acid (omega-6).*A=Control group, B=10% flaxseeds supplemented group.*

Graph 1

The concentration of E mg/dl in diets

	vitamin E
A	6
B	7
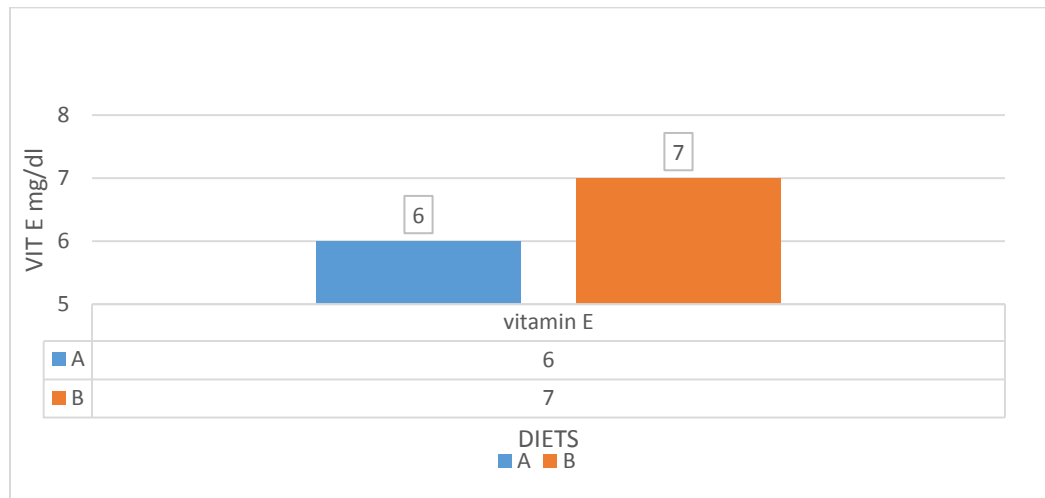
Principle: Albumin in the sample reacts with bromocresol green in acid medium forming a colored complex that can be measured at 630 nm by spectrophotometer.

Total protein was determined using commercial kits (Vitro, Scient), spectrophotometric method described by **(29), (30), (31), (32)** and **(33).**

Principle: Protein in the sample reacts with copper (II) ion in alkaline medium forming a colored complex that can be measured by spectrophotometer at 545 nm.

SGPT, was determined using commercial kits (Biosystem, Spain), spectrophotometric method described by **(34), (27), (26)** and **(28).**

Principle: Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction.

ALP was determined using commercial kits (Biosystem, Spain), spectrophotometric method described by **(35), (36), (37)** and **(38).**

Principle: Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm.

SGOT was determined using commercial kits (Biosystem, Spain), spectrophotometric method described by **(34), (27)** and **(28).**

Principle: Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction.

GGT, was determined using commercial kits (Biosystem, Spain), spectrophotometric method described by **(26)** and **(27).**

Principle: Gamma-glutamyltransferase (GGT) catalyzes the transfer of the g-glutamyl group from g-glutamyl-3-carboxy-4-nitroanilide to glycylglycine, liberating 3-carboxy-4-nitroaniline. The catalytic concentration is determined from the rate of 3-carboxy-4-nitroaniline formation.

Determination of oxidative status:

Catalase was determined using commercial kits (Nanjing Jiancheng, China), spectrophotometric method, the instructions of manufacturer was followed.

Principle: Ammonium molybdate can pause H_2O_2 decomposition reaction catalyzed by catalase (CAT) immediately, residual H_2O_2 can react with ammonium molybdate to produce a yellowish complex. It enables calculate CAT activity by measuring OD value at 405 nm.

Superoxide dismutase (SOD) was determined using commercial kits (Nanjing Jiancheng, China), ELISA method, the instructions of manufacturer was followed.

Principle: Superoxide dismutase (SOD) plays an important role in oxidation-antioxidation balance of organisms, this enzyme can remove superoxide anion radicals (O_2^-) to protect cells away from damage.

Malondialdehyde (MDA) was determined using commercial kits (Nanjing Jiancheng, China), spectrophotometric method, the instructions of manufacturer was followed.

Principle: Lipid hydroperoxide decomposition products can condensate with thiobarbituric acid (TBA) to produce red compounds which has absorption peak at 532 nm.

Vitamin E content was estimated by spectrophotometric method, described by **(39).**

Principle: This method involves the conversion of ferric ions to ferrous ions by α-tocopherol and the formation of red colored complex with 2, 2 dipyridyl. Absorbance of chromophore was measured at 520 nm in the spectrophotometer.

The level of vitamin C was estimated by spectrophotometric method described by **(40).**

Principle: Ascorbic acid is oxidized by copper to form dihydroscorbic acid. The product was treated with 2, 4 dinitrophenyl hydrazine to form tris 2, 4 dinitrophenyl hydrazine which undergoes rearrangement to form a product with the absorption maximum at 520 nm in spectrophotometer.

IV. RESULTS

Saturated fatty acids level was significantly ($p < 0.05$), high in control group (A), compared to the treated group (B).

Plasma level of unsaturated fatty acids in the treated group was significantly ($p < 0.05$), high compared to the control group (A).

The plasma concentration of poly unsaturated fatty acids, was significantly high in group (B), the difference was significant at ($p < 0.05$), compared to the control group (A).

The summation of the detected omega-3 fatty acids, was significantly ($p < 0.01$), high in flaxseeds supplemented group (B), compared to group (A).

The control group (A); showed significant ($p < 0.05$) high level of albumin compared to group (B), which was supplemented by 10% flax seeds. By the end of week 8, the difference of plasma albumin concentration between the experimental group and the control one was not significant at ($p < 0.05$).

The 4th week, showed no significant difference between the control group (A) and group (B), while by the end of the last week of the experiment, group (B), recorded significant ($p < 0.02$) high level of plasma total protein compared to the control group (A).

The 4th week recorded significant ($p < 0.05$) high level of ALT and GGT in group (B) compared to the control group (A), while at the last week of the trial, no significant different levels was observed.

There was no significant different concentration of plasma AST, and ALP, between the control group and the experimental one, neither after the 4th week, nor by the end of the 8th one.

Generally, the first month revealed significant ($p<0.01$) high concentration of plasma catalase in group (B), compared to the control group (A), the same result was obtained by the end of the second month.

Group (B), recorded significant ($p<0.04$) high concentration of SOD, at the 4th week, compared to the control group (A), the final week revealed the same result, with significant ($p<0.05$) high level of SOD compared to the control group (A).

The plasma MDA level, was not significantly different between the control group (A), and the treated group, though group (B), recorded slightly low level of plasma MDA, at the fourth week, while There was no significant different concentrations of plasma MDA, between the control (A) group and flax seeds supplemented group (B).

Though the concentration of plasma uric acid in group (B), was higher at week 4, but it was not significantly different at ($p<0.05$) compared to the control group (A), the difference between plasma uric acid concentration between the treated group (B), and the control group (A), remained not significant by the end of week 8.

There was no significant different levels of plasma vitamin C, between flax seeds supplemented group (B), and the control group (A); at the 4th week, the difference of plasma vitamin C level remained not significant, though the slightly higher level of plasma vitamin C level that recorded by the control group (A).

There was no significant different levels of plasma Vitamin E, between the treated group (B), and the control group (A), neither by the end of the fourth week, nor by the end of the eighth week.

Vitamin A, concentration was not significantly different between the control group (A), and the treated one at week 4, but at week 8, it was significantly ($p<0.05$), higher in plasma of flax seeds supplemented group (B), compared to the control group (A).

The liver histopathological findings in the control group, showed large and spherical hepatocytes, nuclei that were centrally located, with a prominent nucleolus and a moderate eosinophilic cytoplasm

Hens fed 10% flax seeds, showed congestion in the central vein, moderate vacculation, two out of the five liver sections, showed mild hemorrhage.

Table 4: Liver function parameters

Weeks	4th week		8th week	
Parameter	Group A	Group B	Group A	Group B
Albumin	2.2846[A]±0.0950	2.0692[B]±0.0950	2.4000[A]±0.1696	2.4133[A]±0.1696
Total protein	3.2000[A]±0.0866	3.1667[A]±0.0866	3.8154[B]±0.1344	4.3308[A]±0.1344
ALT	9.923[B]±0.8135	12.615[A]±0.8135	9.9333[A]±0.5683	9.0667[A]±0.5683
AST	121.93[A]±7.0794	122.21[A]±7.0794	118.13[A]±6.3879	118.88[A]±6.3879
ALP	430.82[A]±22.369	425.76[A]±22.369	418.29[A]±17.786	417.43[A]±17.786
GGT	3.1538[B]±0.1651	3.5615[A]±0.1651	3.0182[A]±0.2275	2.8545[A]±0.2275

A: Control group, B: Fed 10% flax seeds.

Data are means ± standard error. Means in the same row followed by the same letters are not significantly different at ($p < 0.05$).

Table 5: Plasma concentration of MDA, vitamin E and vitamin C

Parameter	Vitamin C (mg/dl)		Vitamin E (mg/dl)		MDA (mmol/L)	
Group	4th week	8th week	4th week	8th week	4th week	8th week
A	$10.600^A \pm 0.65$	$16.000^A \pm 1.116$	$2.9091^B \pm 0.277$	$3.1833^B \pm 0.43$	$10.333^A \pm 1.527$	$10.867^A \pm 1.68$
B	$11.000^A \pm 0.65$	$15.200^A \pm 1.116$	$2.9818^B \pm 0.277$	$4.0000^B \pm 0.430$	$9.333A \pm 1.527$	$10.000^A \pm 1.68$

A: Control group, B: Fed 10% flax seeds.

Data are means ± standard error. Means in the same column followed by the same letters are not significantly different at ($p < 0.05$).

Table 6: Plasma enzymatic antioxidants concentration

Parameter	CAT (U/ml)		SOD (U/ml)	
Group	4th week	8th week	4th week	8th week
A	$41.000^B \pm 6.0431$	$40.667^B \pm 7.8811$	$32.500^B \pm 2.4710$	$33.267^B \pm 2.8718$
B	$70.667^A \pm 6.0431$	$78.333^A \pm 7.8811$	$43.300^A \pm 2.4710$	$45.000^A \pm 2.8718$

A: Control group, B: Fed 10% flax seeds. CAT= Catalase, SOD=Superoxide dismutase.

Data are means ± standard error. Means in the same column followed by the same letters are not significantly different at ($p < 0.05$).
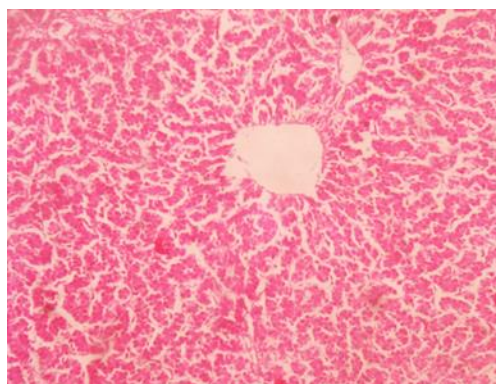
Graph 2

Plasma concentration of Saturated, Unsaturated, Poly unsaturated, Omega-3, and Omega-6 fatty acids (%)

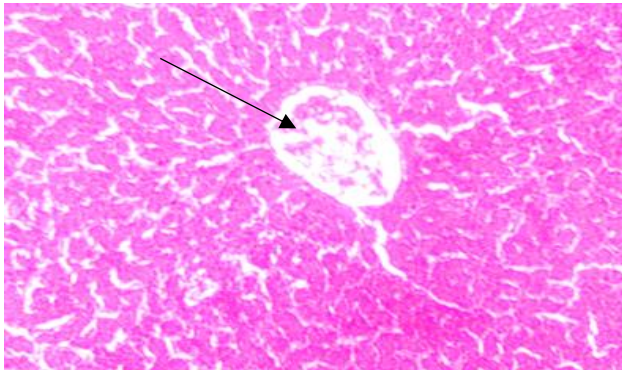|	Saturated	Unsaturated	Poly unsaturated	Omega-3	Omega-6
A	33.01	66.98	42.87	1.69	33.97
B	6.68	93.31	67.4	58.4	9
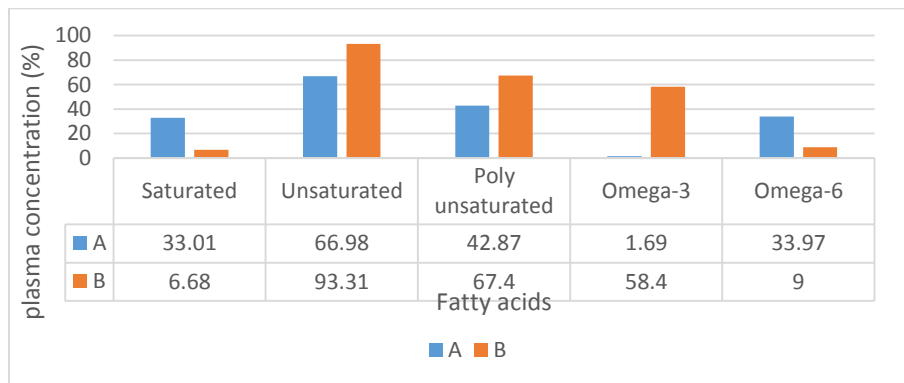
A: Control group, B: Fed 10% flax seeds.

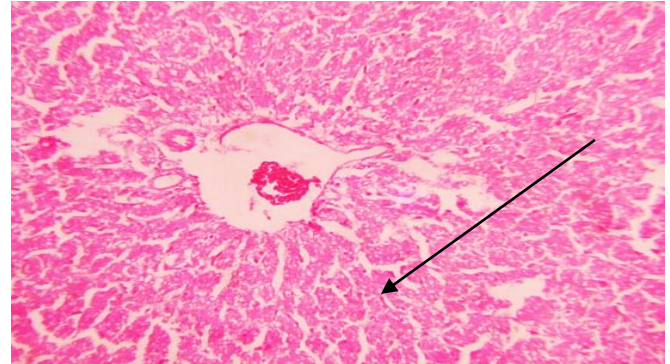**Graph 3**

Control: Normal hepatocytes H & E (10).

Group (B):Congestion of central vein, normal hepatocytes H&E (10)   Group (B): vacuolated hepatocytes H&E (10).

V. DISCUSSION

the effects of dietary supplementation with different fat sources on blood parameters of Japanese quail was investigated by **(41),** their results revealed, there was significant high serum total protein concentration in the group; which was supplemented by flax oil, compared to corn oil and sunflower oil supplemented groups, this result is contrary to the current one, this difference could be attributed to, different forms of flax used in the two experiment, or may be due to the different levels of flax included in the diet, also it could be justified by the different kinds of birds used, or it could be because of difference in feed intake, however, group (B), which received 10% flax seeds, showed slightly high plasma total protein concentration at the final week of the trial, this could be justified be the findings of **(42),** they suggested that, diets enriched in N-3 PUFA, increase protein synthesis through inducing the expression of protein disulfide isomerase PDI-A3.

The high content of omega-3 fatty acids and high content of vitamin E, in flax seeds fed group, could be a synergetic effect resulted in pronounced elevation of plasma total protein level, the effect of vitamin E, on plasma total protein level, was declared by **(43),** that, total serum protein level was increased in broiler supplemented by vitamin E, compared to control group.

On another hand**, (44),** declared that, inclusion of line seeds oil and sunflower oil into Japanese quail diets, enhanced plasma total protein level compared with the control group. This positive improvement in plasma total protein level, may be due to inclusion of these oils on fatty acids which may affect muscle protein synthesis and protein deposition through a prostaglandin dependent mechanism.

the influence of supplementing Japanese quail diets with different fat sources on blood parameters, was investigated by **(41),** their findings showed no significant different serum concentration of albumin between sunflower supplemented group, and corn oil supplemented group, however, the group which received flax oil recorded significant high level of serum albumin compared to the groups mentioned above, the difference between their findings and the current study result, could be attributed to different percentage of flax included in the diet, or even because of the different kinds of birds used in the two experiments.

Alanine transaminase enzyme, was significantly higher in group B, which received 10% flax seeds, compared to group A, but within the wide range of reference values specified for chicken **(45), (46)** and **(47),** while there was no significant different levels between the control group and the experimental one by the end of week 8, these results, are in agreement with what was reported by **(48),** who reported that feeding linseed oil to turkey, significantly elevated serum ALT levels, compared to soya bean oil supplemented group.

Another confirmatory result, was reported by **(49),** that adding different ratio of omega 3/6 fatty acids to laying hens, had no significant effect on plasma AST, and ALT concentration.

The slight high plasma concentration of AST and ALT, is in agreement with the findings of **(50),** who reported that, feeding growing chicks different concentrations of omega-3 fatty acids, resulted in slight elevation of serum AST and ALT concentration, with signs of liver necrosis, This change may be correlated with shifting of the metabolic pathways, which need to be confirmed in future.

Catalase is the first enzyme, to show alteration following induction of oxidative stress, **(51)** and **(52).**

Significant high level of plasma catalase, was observed in the treated groups compared to the control group. The alteration of catalase concentration was fluctuated, this agrees with what was reported by **(53),** when he obtained the same result in a study performed to evaluate the antioxidant enzymes activities of (Cyprinus carpio), fed diet containing moderate level of sunflower seeds meal, this result could be attributed to the intracellular localization of this enzyme which may be responsible for different responses to oxidative stress, because catalase activity found mainly in peroximes **(54).** The values can reflect also an adaption/ acclimation to diet composition **(55).**

Normal level of serum catalase in rats dosed by paracetamol after 30 minute of flaxseeds administration, in comparison with healthy and positive control (received Silymarin 30 minute before administration of paracetamol), was reported by **(56),** these results corroborate our current result, that flaxseeds has potency to improve the oxidative status through elevating the levels of catalase enzyme.

The result of this study revealed, significant high level of plasma SOD concentration in flax seeds supplemented group by the end of the first month and the same result was observed by the end of the second one, this result agrees with what was reported by **(56),** when they conducted a trial to investigate the hepatoprotective activity of omega-3 fatty acids obtained from flax seeds and fish oil, they stated that; administration of previously mentioned oil to rats, dosed by paracetamol repeatedly; achieved normalization of oxidative status, through improvement in levels of antioxidant enzymes and oxidative stress markers.

Polyunsaturated fatty acids (PUFA), can scavenge free radicals, improve the activity of SOD and other antioxidant enzymes, and may exert a preventive antioxidant role against free radicals action **(57),** the fatty acids profile and the percentage of plasma polyunsaturated fatty acids in the treated groups, is agree with the suggestions mentioned above.

The current study, showed no significant different levels of plasma MDA, between the control (A) group and the treated group (B), the data elucidated from a trial conducted by **(58),** to investigate the effect of supplementing diets with different doses of flax oil; on some biological, biochemical and histopathology changes in rats, suffering from nephropathy, had revealed that, the group which was supplemented by glycerol to induce nephropathy (positive control), showed highly significant increase in mean value of lipids peroxidation (MDA), compared to the control negative group, supplementing of 5% and 7% of flax oil, caused highly reduction in (MDA), compared to the control positive group, but at the same time the two doses of flax oil, showed no significant difference in mean of MDA, as compared to the control negative group, these results; agree with our current results.

The biosynthesis of ascorbic acid in mammals and birds takes place in the liver/kidney or both.

In the chickens the synthesis occurs primarily in the kidneys as reported by **(59),** usually, sugars such as glucose, fructose and mannose, serve as precursors for vitamin C synthesis.

There was no significant different levels of plasma vitamin C concentration between the control group (A), and the treated one (B), this may be due to the capability of hens to produce the vitamin de novo at the kidney and liver, and as time passing by the concentration of vitamin C in plasma elevated, regarding individual variation of ascorbic acid synthesis.

Vitamin E, is the primary lipid soluble anti-oxidant, found in food and human blood and tissues, its well-known that vitamin E, inhibits the process of lipid peroxidation in oils and in the biological lipid-protein complexes, such as biological membranes or circulating lipoproteins **(60).**

The level of plasma vitamin E, between the control group (A), and the treated one (B), was not significant at ($p<0.05$), and though the high content of poly unsaturated fatty acids in group (B), diet, the difference of plasma MDA level remained not significant between the two groups, these results could be attributed to the consumption of vitamin E in lipid peroxidation inhibition process.

Hens fed 10% flax seeds, showed congestion in the central vein, moderate hepatocellular vaculation, and two out of the five liver sections, showed mild hemorrhage.

No signs of necrosis was detected, from the signs noticed in group B, we cannot confirm that feeding laying hens 10% flax seeds for two month could be a causative agent of FLHS incidence, especially with no either drop in egg production nor sudden death, the histopathological findings of hepatocellular vaculation, could be attributed to that, liver in birds, is responsible for majority of lipogenesis, and dietary fats in birds is directly transported to the liver by the portal vein. Therefore, birds are expected to normally have a higher hepatic lipid concentration and micro vacuolization **(61).**

The congestion observed in the central vein, is considered a normal outcome of slaughtering the birds.

VI. CONCLUSION

The results obtained from the current study appointed that, feeding laying hens 10% flax seeds for 8 weeks, enhances the oxidative status, without any elevation of plasma **MDA**, though the high content of **PUFA** in the experimental diet. The improvement in oxidative status will be reflected in improvement of laying hen health and production.

Feeding 10% flax seeds to laying hens, did not affect the liver function, represented in liver enzymes, with significant increase of plasma total protein levels.

The current study revealed that, feeding flax seeds to laying hens for two month, did not cause FLHS, though the high content of poly unsaturated fatty acids in the diet. It seems that the good oxidative status which was observed, played a role as liver protector against the incidence of FLHS.

REFERENCES

[1] Butler, E.J. Fatty liver diseases in the domestic fowl – a review. (1976). Avian Pathology; 5:1–14.

[2] Squires, E.J., Leeson, S. Etiology of fatty liver syndrome in laying hens. (1988). British Veterinary Journal; 144: 602–609.

[3] Diaz, G.J., Squires, E.J., Julian, R.J. Effect of selected dietary antioxidants on fatty liver-hemorrhagic syndrome in laying hens. (1994). British Poultry Science; 35: 621–629.

[4] Crespo, R., Shivaprasad, H.L. Developmental, metabolic and other noninfectious disorders. In: Saif, Y.M., Barnes, H.J., Glisson, 364 R.J. Julian. (2005). the Veterinary Journal; 169: 350–369.

[5] Thomson, A.E., Gentry, P.A., Squires, E.J. Comparison of the coagulation profile of fatty liver hemorrhagic syndrome-susceptible hens and normal laying hens. (2003). British Poultry Science; 44: 626–633.

[6] Haghighi-Rad, F., Polin, D. The relationship of plasma oestradiol and progesterone levels to the fatty liver hemorrhagic syndrome in laying hens. Poultry Science. (1981); 60: 2278–2283.

[7] Bhatnagar, M.K., Yamashiro, S., David, L.L. Ultrastructural study of liver fibrosis in turkeys fed diets containing rapeseed meal. (1980). Research in Veterinary Science; 29: 260–265.

[8] Wight, P.A.L., Wells, J.W., Shannon, and D.W.F. Liver hemorrhages induced by rapeseed meal: incidence in adult male and female fowls. (1986). British Poultry Science; 27: 247–252.

[9] Spurlock, M.E., Savage, J.E. Effect of dietary protein and selected antioxidants on fatty liver hemorrhagic syndrome induced in Japanese quail. (1993). Poultry Science; 72: 2095–2105.

[10] Hargis, P. S., Van Elswyk, M. E. and Hargis. B. M. Dietary modification of yolk lipid with menhaden oil. (1991). Poultry. Sci; 70: 874–883.

[11] Van Elswyk, M. E., B. M. Hargis, J. D. Williams and P. S. Hargis. Dietary menhaden oil contributes to hepatic lipidosis in laying hens. (1994). Poultry. Sci; 73:653-662.

[12] Lin H., Decuypere E., Buyse J. Acute heat stress induces oxidative stress in broiler chickens. (2006). Comp. Biochem. Physiol. A Mol. Integr. Physiol; 144: 11–17.

[13] Mujahid A., Akiba Y., Toyo mizu. M. Acute heat stress induces oxidative stress and decreases adaptation in young White Leghorn cockerels by down regulation of avian uncoupling protein. (2007). Poultry Sci; 86: 364–371.

[14] Durackova Z. Some current insights into oxidative stress. (2010). Physiol. Res; 59: 459–469.

[15] Halliwell B. Uric acid: an example of antioxidant evaluation. (1996). In: Cadenas E, Packer L. editors. Handbook of antioxidants. New York: Marcel Dekker; p 243–56.

[16] Chow, C.K. Nutritional influence on cellular antioxidant defense systems. (1979). Am. J. Clin. Nutr; 32(5):1066-1081.

[17] Avula, C.P., Fernandes, G. Modulation of antioxidant enzymes and apoptosis in mice by dietary lipids and treadmill exercise. (1999). J. Clin. Immunol; 19(1):35-44.

[18] Yang, X.J., He, X., He, L.X., Liu, Y.X., Yang, Y., Guo, Y.M. The effect of PUFA on antioxidation parameter of broiler chickens. (2008). Chin. J. Anim. Nutr; 20(3):299-304 (in Chinese).

[19] Schumann, B. E., Squires, E. J. and Leeson, S. Effect of dietary flaxseed, flax oil and n-3 fatty acid supplementation on hepatic plasma characteristics relevant to fatty liver hemorrhagic syndrome in laying hens. (2000). Br. Poultry. Sci; 41: 465–472.

[20] Tarpila A, Wennberg T, Tarpila S. Flaxseed as a functional food. (2005). Current Topics in Nutraceutical Res; 3: 167.

[21] Jain Rohini, Grover Kiran, Singla Neerja. Oilseeds for better health. (2015). J Nut Res; 3(1): 50-53.

[22] National Research Council (NRC), (1994). Nutrient requirements of poultry, Ninth revised edition.

[23] Folch, J., Lees, M and Sloane-Stanely, G. H. A simple method for the isolation and purification of total lipids from animal tissues. (1957). J. Biol. Chem; 226:497–507.

[24] Wang, Y., Sunwoo, H., Cherian, G., and Sim, J.S. Fatty acid determination in chicken egg yolk: a comparison of different methods. (2000). Poultry Science; 79: 1168-1171.

[25] Doumas BT, Watson WA and Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. (1971). clinical chemistry acta; 31:87-96.

[26] Young DS. (2000). Effects of drugs on clinical laboratory tests, 5th edition. AACC Press.

[27] Tietz. (2005), Textbook of clinical chemistry and molecular diagnosis, 4th edition. Burtis CA, Ashwood ER, Burns DE. WB Saunders Co.

[28] Friedman. (2001), effects of disease on clinical laboratory tests, 4th edition. AACC Press.

[29] Doumas, BT, Bayse, DO, Carter RJ. Candidate reference method for determination of total protein in serum.1. Development and validation, Ⅱ. Tests for transferability. (1981). Clinical chemistry; 27:1642-1654.

[30] Brobeck, JR. (1973). Physiological basis of medical practice 9th ed. Baltimore, MD: Wilkins and Wilkins; 4-7.

[31] Hiller, A, Plazin, J, and Van Slyke, DD. (1948):J. Biol.Chem. 176-1401.

[32] Young, DS (1990). Effects of drugs on clinical laboratory tests. Third edition. 1990: 3: 6-12.

[33] Koller, A. (1984). Total serum protein in: Kaplan, LA, Pesco, AJ, eds. Clin Chem, theory, analysis, and correlation. St. Louis: Mosby Company 1316-1319.

[34] Gella FJ, Olivella T, Cruz Pastor M, Arenas J, Moreno R, Durban R and Gomez JA. A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. (1985). clinical chemistry acta; 153: 241-247.

[35] Rosalki SB, Foo AY, Burlina A. Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. (1993).   clinical chemistry; 39:648-652.

[36] Tietz. (1994), Textbook of clinical chemistry, 2nd edition, Burtis CA, Ashwood ER, WB Saunders Co.

[37] Friedman and Young. (1997), Effects of disease on clinical laboratory tests, 3th edition. AACC Press Co.

[38] Young DS. (1997), effects of drugs on clinical laboratory tests, 3th edition. AACC Press.

[39] Palan PR, Mikhail BS, Basin J, Romney SL. Plasma Levels of antioxidant beta-carotene and tocopherol in uterine cervix. (1973).   Nutr Cancer; 15: 13-20.

[40] Omaye S.T., Turnbull J.D., Sauberlich H.E. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. (1979). Meth. Enzymol; 62: 3.

[41] Hazim J. Al-Daraji, Ali S. Al-Hassani, H.A. Al-Mashadani, W.K. Al-Hayani and H.A. Mirza. Effect of Dietary Supplementation with Sources of Omega-3 and Omega-6 Fatty Acids on Certain Blood Characteristics of Laying Quail. (2010). International Journal of Poultry Science; 9 (7): 689-694.

[42] Abeer A Ahmed1, Kayode A Balogun1, Natalia V Bykova2, 3 and Sukhinder K Cheema. Novel regulatory roles of omega-3 fatty acids in metabolic pathways: a proteomics approach. (2014). Nutrition & Metabolism; 11:6.

[43] Shashi Kant, Nazim Ali, Gulab Chandra, Riyaz Ahmad Siddique. Effect of shatavari and vitamin E on hemato-biochemical profile ofbroilers during the winter season. (2014). Veterinary World, EISSN: 2231-0916.

[44] El Yamany, Hewida M.H. El Allawy, Laila D. Abd El-Samee and A.A. EL-Ghamry. Evaluation of Using Different Levels and Sources of Oil in Growing Japanese quail Diets. (2008). American-Eurasian J. Agric. & Environ. Sci., 3 (4): 577-582.

[45] Ross J.G., Christie G., Halliday W.G. Hematological and blood chemistry „comparison values" for clinical pathology in poultry. (1978). Vet. Rec; 102:29-31.

[46] Bowes V.A., Julian R.J., Stirtzinger T. Comparison of serum biochemical profiles of male broilers with female broilers and white leghorn chickens. (1989).   Can. J. Vet. Res; 53: 7-11.

[47] Meluzzi A., Primiceri G., Giordani R., Fabris G. Determination of blood constituents reference values in broilers. (1992). Poultry Sci; 71: 337-345.

[48] Czech. A and K. Ognik. The effect of using soyabean or linseed oil with RRR-d-α-tocopherol or dl-α-tocopherol acetate on hematological parameters and rearing performance of young turkey hens. (2014). Journal of Animal and Feed Sciences; 23: 262–268.

[49] Nadia L. Radwan, M. H. Abd El-Samad and Sherin A. Nada. Effects of different dietary ratios of linoleic acid to a- linolenic acid on productive performance, immunity of laying hens and egg yolk fatty acids composition. (2012). Egypt. Poultry. Sci; 32(I):163-188.

[50] Roy R., S. Singh and S. Pujari. Dietary Role of Omega - 3 Polyunsaturated Fatty Acid (PUFA): A Study with Growing Chicks, Gallus domesticus. (2008). International Journal of Poultry Science; 7 (4): 360-367.

[51] Stara, A., Machova, J. and Velisek, J. Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp (Cyprinus carpio L.). (2012). Environmental Toxicology and Pharmacology; 33(2):334-343.

[52] Jin, Y. X., Zhang, X. X., Shu, L. J., Chen, L. F., Sun, L. W., Qian, H. F., Liu, W. P. and Fu, Z. W. Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (Danio rerio). (2010). Chemosphere; 78:846-852.

[53] İlknur Meriç. Evaluation of sunflower seed meal in feeds for carp: Antinutritional effects on antioxidant defense system. (2013). Journal of Food, Agriculture & Environment; 11 (2): 1128-1132.

[54] Trenzado, C, A. Antioxidant enzymes and lipid peroxidation in sturgeon Acipenser naccarii and trout Oncorhynchus mykiss. A comparative study. (2006). Aquaculture; 254:758-767.

[55] Bastrop, R., Spangenberg, R. and Jurss, K. Biochemical adaptationof juvenile carp (Cyprinus carpio L.) to food deprivation. (1991). Comparative Biochemistry and Physiology; 98A:143-149.

[56] Tejaswi Chavan, Suresh khadke, Shubhangi Harke, Abhijit Ghadge, Manjiri Karandikar, Vtjaya Pandit, Prabhakar Ranjekar, Omkar Kuikarni and Aniket Kuvalekar. Hepatoprotective effect of poly unsaturated fatty acids against repeated subacute Acetaminophen dosing in rats. (2013). International Journal of Pharma and Bio Sciences; 4(2): 286 – 295.

[57] Liu L.C. Effect of unsaturated fatty acid and monensin on rumen and blood fatty acid synthesis in sheep. (2009). Ha Erbing, Northeast Agricultural University.

[58] Hanaa. H. El-Sayed, Abd El-Moneim. H. Darwish, Ensaf. M.Ysein. Ghada. D.zehairy. Biochemical and Biological Study on The Effect of Flaxseed on Rats Suffer from Nephropathy. (2014). IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402, p- ISSN: 2319-2399. Volume 8, Issue 1 Ver. III (Jan. 2014), PP 59-66.

[59] Roy, R.N. and B. Guho. Species differences in regard to Biosynthesis of ascorbic acid. (1958). Nature; 182: 319-320.

[60] Fellenberg, M.A. and H. Speisky. Antioxidants: their effect on broiler oxidative stress and its meat oxidative stability. (2006). World Poultry Science Journal; 62:53-70.

[61] Trott K. A., F. Giannitti, G. Rimoldi, A. Hill, L. Woods, B. Barr, M. Anderson, and A. Mete. Fatty Liver Hemorrhagic Syndrome in the Backyard Chicken: A Retrospective Histopathologic Case Series. (2013). Pathology Online journal.