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Histopathological, Metabolic and performance Changes in Laying Hens Affected by Fatty Liver Haemorrhagic Syndrome

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Abstract

Background: Fatty liver hemorrhagic syndrome (FLHS) is a metabolic condition occurring worldwide in caged layers and causes significant losses to the egg industry.

Aims: to determine the main pathological, metabolic and performance changes in layers affected by fatty liver haemorrhagic syndrome.

Methods: The present study includes a (300) laying hens at the age fifty-three weeks from six farms suffering from the fatty liver hemorrhagic syndrome. changes in body weight, feed intake, feed conversion rate was determined. blood samples were taken from brachial vein to determine albumin, globulin, total protein, albumin/ globulin, blood urea nitrogen, cholesterol, calcium, glucose, phosphorus. alt, alp, creatinine and creatine kinase. Full postmortem examination was performed and histopathological examination for liver sections stained with hematoxylin and eosin.

Results: Positive significant correlation was reported between body weight gain and feed intake (R= 0.703, P value = 0.000). Positive significant correlation was reported between body weight gain and feed conversion rate (R= 0.850, P value = 0.000). Positive significant correlation was reported between body weight gain and liver lesion score (R= 0.524, P value = 0.000). Significant difference was reported between hens with FLHS and normal hens regarding the values of albumin (P value = 0.000), albumin/ globulin (P value = 0.0018), globulin (P value = 0.009533), total protein (P value = 0.0000). Significant difference was reported between hens with FLHS and normal hens regarding the values of alkaline phosphatase (P value = 0.0000), No significant difference was reported between hens with FLHS and normal hens regarding the values of ALT (P value = 0.57339). Significant difference was reported between hens with FLHS and normal hens regarding the values of creatinine (P value = 0.00003421), creatine kinase (P value = 0.0000). Significant difference was reported between hens with FLHS and normal hens regarding the values of blood urea nitrogen (P value = 0.0015), cholesterol (P value = 0.00000), calcium (P value = 0.0000), phosphorus (P value = 0.000034), glucose (P value = 0.0000). Necropsy findings reveals abdomen filled with large blood spots with hepatomegaly and fat deposition. Liver usually friable and presented with yellow, pale or putty colors. Histopathological necrosis for hepatic cords with moderate dilation and congestion of sinusoidal together with wide hemorrhages.

Conclusions: FLHS induced mainly with consumption of high energy diet causing serious negative effects on liver function as well as most vital metabolic biomarkers in laying hens

Keywords: **Fatty Liver Haemorrhagic Syndrome, Metabolic Biomarkers, Laying Hens**

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Introduction

Fatty liver hemorrhagic syndrome is a metabolic condition that occurs in commercial layers and is frequently the major cause of death in high producing laying flocks**[1]**. FLHS is characterized by excessive fat in the liver and hemorrhage from a ruptured liver**[2]**. The syndrome occurs in caged laying hens, primarily in birds that are in positive energy balance**[3]** . However other factors have also been implicated as potential contributory elements to the occurrence of FLHS **[3]**. The condition is easy to recognize at necropsy with hens having excess abdominal and liver fat, hemorrhages and hematomas of various size in the liver and in many cases large blood clots in the abdominal cavity**[3]**. Outbreaks occur sporadically in commercial flocks, and 3-5% of the affected flocks die from the condition**[4]**.The decrease in egg production and increase in mortality associated with FLHS have implications for the welfare of hens and cause considerable economic losses to egg producers**[5]**. Flocks with FLHS are distinguished by a dramatic increase in mortality notwithstanding high laying percentages**[4]**2.. The mortality is seen mainly in hens which are in full production**[6]**. The mortality is usually 3-5%, but higher mortality rates have been reported. Birds that are found dead can be pale, but usually did not show any other clinical symptoms. In some cases, the mortality can be accompanied by a (sudden) decrease in egg production**[6]**. In live animals it is very difficult to distinguish affected from healthy hens, although some hens do develop pale combs**[7]**.

The aim of current study to determine the main pathological, metabolic and performance changes in layers affected by fatty liver haemorrhagic syndrome.

Material and Methods:

Study Area:

This study was conducted on six flocks of laying hens in the Baqubah city -Diyala province33°45'34.71N; 44°36'23.97E**[8]** .

Ethical Approval: This study was conducted according to the approval of ethical review Committee of College of Veterinary medicine –Diyala University -Iraq, was taken prior to initiation of the work.

Body Weight performance:

The layers were weighed individually and weekly to determine body weight (BW) and body weight gain (BWG). Feed intake (FI) , Feed conversion ratio (FCR) was recorded throughout the study and calculated according to the following formula**[9, 10]**

$$FCR = \frac{\text{Total Feed Consumed}}{Final\ weight - initial\ weight}$$

Clinical Examination of Flocks:

From the clinical examination the main finding was the sudden death of well nutritious hens

Blood sampling

A total number of examined hens was three hundred. The minimum examined hens per flock was five and a maximum of 189 hens per flock were examined. Random samples of birds were selected, and the results from the sample then provided an estimate of the status of the whole flock**[11]**.

Blood samples for hematology and biochemistry were collected from the brachial vein. Haematological parameters were measured using an automated analyzer (CELL-DYN® System 3700CS, Abbott Park, IL 60064). Blood for biochemistry was centrifuged (1500 rpm for 10 min), the plasma stored at -20ºC and subsequently

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analyzed for albumin, globulin, total protein, albumin/ globulin, blood urea nitrogen, cholesterol, calcium, glucose, phosphorus. Alanine amino transferase, alkaline phosphatase, creatinine and creatine kinase using commercial kits and a chemistry system (Vet test chemistry analyzer, IDEXX Laboratories, Inc. USA).

Sampling procedures

After birds were sacrificed by neck dislocation the livers were quickly removed, rinsed with cold sodium chloride solution 0.9 %, and a portion of each liver was used for histopathology

Scoring system for Gross hemorrhagic lesions of liver

Haemorrhage was assessed on both the dorsal and ventral surfaces of the liver and was graded on a scale from 0 to 5**[12]**

Score	Interpretation
score 0	Indicating no haemorrhages
score 1	up to 10 subcapsular petechial or ecchymotic haemorrhages;
score 2	more than 10 subcapsular petechial or ecchymotic haemorrhages
score 3	large haematomas and massive
score 4	
score 5	liver haemorrhage accompanied by rupture of liver capsule

Histopathological Reparation of Liver Samples

All liver samples were fixed in 10% buffered formalin at room temperature. Then 3-4cm of affected liver portion was fixed in paraffin block and thin section (5-6 µm) were sliced and stained with standard hematoxylin and eosin staining procedure. The stained sections were examined under 10x,40x and 100x for evaluation of histopathological changes by an optical microscope**[13]**

Statistical analysis:

Statistical analyses were carried out using SPSS version 17 (SPSS Inc., Chicago, IL, USA) and excel 2016**[11]**. Data were expressed as (mean± SE) **[14]**. Data were analyzed using t -test. P-value less than 0.05 was considered statistically significant**[15]**.

Result

Performance parameters

As shown in table (1), maximum body weight was (2100± 1.32626) in farm no.4, followed by (2000 ±3.16228) in farm no, 6. The minimum weight gain was reported in farm no.5(1750± 0.48795). Positive significant correlation was reported between Body Weight Gain and Feed Intake (R= 0.703, P value = 0.000). Positive significant correlation was reported between body weight gain and feed conversion rate (R= 0.850, P value = 0.000)

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Gross liver lesion score and body weight gain

.As shown in table (2), the selected laying hens from farm no.1, 2 &6 suffering from FLHS, with lesion score 1 for liver. while hens from farm no.3 and 4 suffering from FLHS, with lesion score 2 for liver. While hens from farm 5 have no gross pathological lesion in liver. Positive significant correlation was reported between body weight gain and liver lesion score (R= **0.524**, P value = 0.000).

Metabolic parameters

As shown in table (3), significant difference was reported between hens with FLHS and normal hens regarding the values of albumin (P value = 0.000), albumin/ globulin (P value = 0.0018), globulin (P value = 0.009533), total protein (P value = 0.0000).As shown in table (4), significant difference was reported between Hens with FLHS and normal hens regarding the values of alkaline phosphatase (P value = 0.0000),No significant difference was reported between hens with FLHS and normal hens regarding the values of ALT (P value = **0.57339**).As shown in table (5), significant difference was reported between Hens with FLHS and normal hens regarding the values of creatinine (P value = 0.00003421), creatine kinase (P value = 0.0000) . As shown in table (6), significant difference was reported between Hens with FLHS and normal hens regarding the values of blood urea nitrogen (P value = 0.0015), Cholesterol (P value = 0.00000), calcium (P value = 0.0000), phosphorus (P value = 0.000034), Glucose (P value = 0.0000).

Postmortem Examination findings

Necropsy on the birds suffering from FLHS often reveals abdomens filled with large blood spots, arising from the liver. Hepatomegaly with fat deposition. Liver usually presented with different color include yellow, pale or putty colored. Also, the liver was friable with small hematomas in the liver parenchyma. Hemorrhages were found in the margins of the liver lobes. Fatty liver with presence of fat in abdominal cavity around the viscera was reported as shown in figure (1).

Histopathological Findings

As shown in figures (1-5), Histopathological findings include necrotic changes of hepatic cords with moderate dilation and congestion of sinusoidal together with wide hemorrhages. evidence of individualization of hepatocytes results in boron's disrupted of tissues architecture together with clear central vein congestion. marked portal vein dilation with inflammatory edematous exudates as well as portal fibrosis was recorded with sever atrophy of adjacent parenchymal cell zone**.** The main hepatic examination showed moderate perivascular mononuclear cells aggregation associated with slight degeneration changes of adjacent hepatocytes. moderate vacuolation of hepatocytes changes of fat droplets recorded mainly in sub capsular region with few inflammatory cells infiltration also predomination of Kupffer cell with mild cellular infiltration in sinusoids. mononuclear cells infiltration was observed in portal region with obvious vascular congestion. showed moderate vessels congestion with few neutrophils' presence in lumen**.** focal mononuclear cells aggregation in liver tissue with mild perivascular mononuclear cells infiltration. mild sinusoid congestion with mononuclear cells infiltration in some dilated sinusoids together with Kupffer cells hyperplasia. few sections showed enlarged congested portal vein surrounded with carious cell types.

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Table 1:Body Weight Gain, Feed Intake and Feed Conversion Rate for Layers affected by FLHS **at 53 Weeks Of Age**

farms No.	Body Weight Gain (Gram)	Feed Intake (Gram) / 50 Layers	Feed conversion rate
Farm 1	1950 ± 0.2764	5500	2.343
Farm 2	1825 ± 0.56880	5600	2.334
Farm 3	1900± 0.73276	5000	2.355
Farm 4	2100± 1.32626	5800	4.230
Farm 5	1750± 0.48795	6050	3.472
Farm 6	2000 ±3.16228	5500	5.436
R		0.703**	0.850**
P value		0.000	.000

Table *2*: Correlation Between Body Weight and Liver Lesion Score For Laying Hens With FLHS

Farm No.	No of Examined hens	Mean ± SE Hens body Weight	Lesions score
Farm 1	25	1950 ± 0.2764	1
Farm 2	17	1825 ± 0.56880	1
Farm 3	57	1900± 0.73276	2
Farm 4	189	2100± 1.32626	2
Farm 5	7	1750± 0.48795	0
Farm 6	5	2000 ±3.16228	1
R	0.524		
P value	0.000		

Table 3: Liver protein profile for Laying hens with FLHS compared with control at age 53 week

Parameter	Hens with FLHS	Control	P value
Albumin (g / dl)	2.08±0.00577	1.2± 0.00577	0.0000
Albumin/ Globulin	0.8±0.0574	1.4±0.0574	0.0018
Globulin (g / dl)	2.51±0.00577	3.1±0.057735	0.009533
Total protein (g / dl)	4.59±0.001154	0.35±0.00577	0.0000

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Table 4: Liver Enzyme profile for Laying hens with FLHS compared with control at age 53 week

Parameter	Hens with FLHS	Control	P value
Alanine Aminotransferase (U /L)	6±0.57735	6.5 ±0.57735	0.57339
Alkaline phosphatase (U /L)	590±0.57735	482.5 ± 0.57735	0.00000

Table 5:levels of Creatinine and Creatine Kinase for Laying hens with FLHS compared with control at age 53 week

Parameter	Hens with FLHS	Control	P value
Creatinine (mg /dl)	0.508±0.00577	1.5 ±0.00577	0.00003421
Creatine Kinase (U /L)	935±0. 57735	220±0. 57735	0.00000

Table 6: Blood parameters for Laying hens with FLHS compared with control at age 53 week

Parameter	Hens with FLHS	Control	P value
Blood urea nitrogen (mg /dl)	14.58± 0.57735	0.1 ± 0.000	0.0015
Cholesterol (mg /dl)	125 ±0.57735	85.14±0.57735	0.00000
Calcium (mg /dl)	32±0.57735	8.5±0.57735	0.0000
Glucose (mg /dl)	226.44± 0.57735	130 ±0.57735	0.0000
Phosphorus (mg /dl)	23±0.57735	6.4 ±0.57735	0.000034

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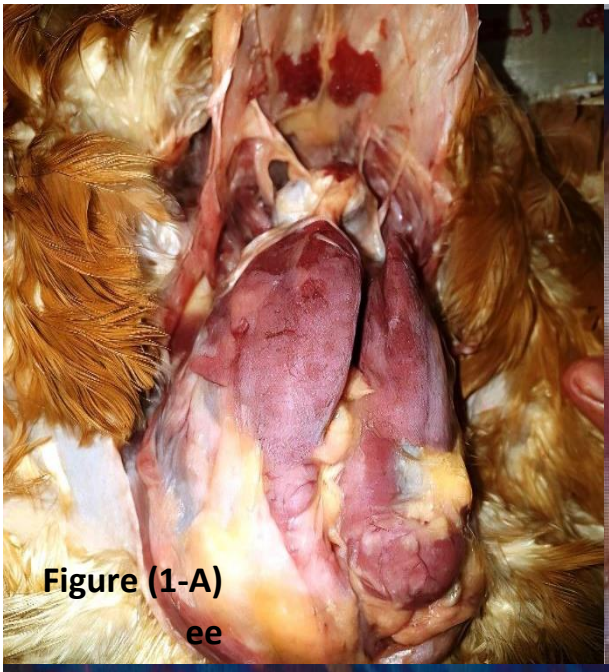

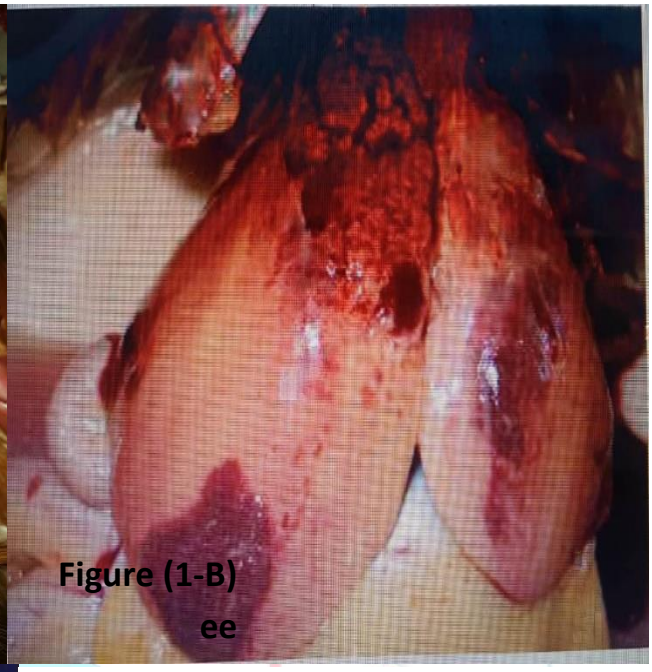

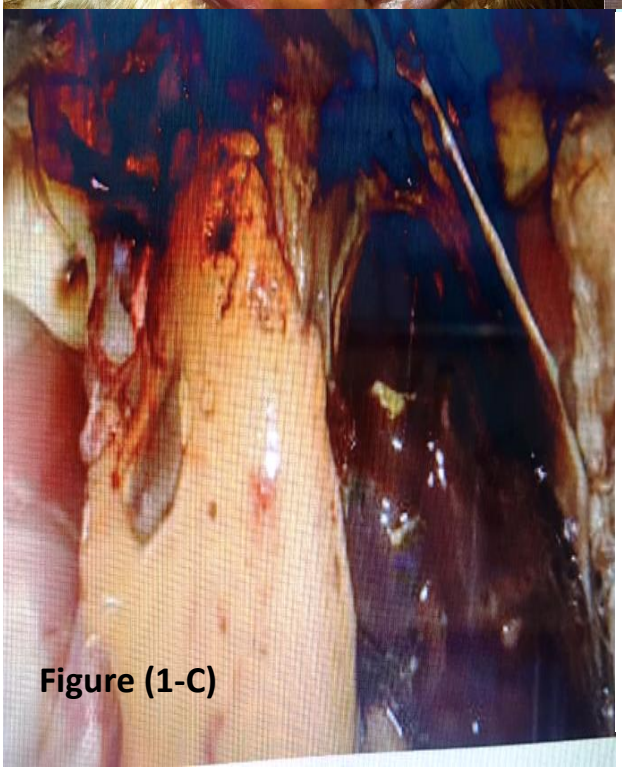

Figure (1): Necropsy on the birds suffering from FLHS reveals abdomens filled with large blood spots, arising from the liver. Figure 1-B, &1-C revealed hepatomegaly with fat deposition. Liver presented with putty colored(Figure1-A).The liver was friable with small hematomas in the liver parenchyma (Figure 1-C). Hemorrhages were found in the margins of the liver lobes as in (Figure 1-A-B). Fatty liver with presence of fat in abdominal cavity around the viscera as in (Figure 1-A).

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Histopathological Examination of Liver Samples :

Histopathological findings were illustrated in figures (1-5)

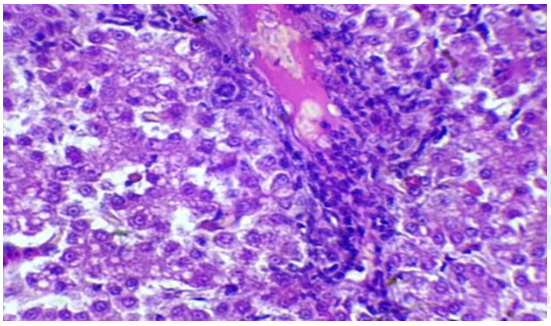

Figure 1: Liver show necrotic finding of hepatic cords with moderate dilation & congestion of sinusoidal together with wide hemorrhages. All evidence of individualization of hepatocytes results in boron's disrupted of tissues architecture together with clear central vein congestion.

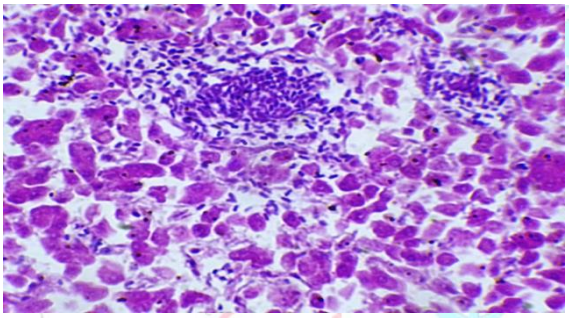

Figure 1: Section showed marked portal vein dilation with inflammatory edematous exudates as well as portal fibrosis was recorded with sever atrophy of adjacent parenchymal cell zone**.**

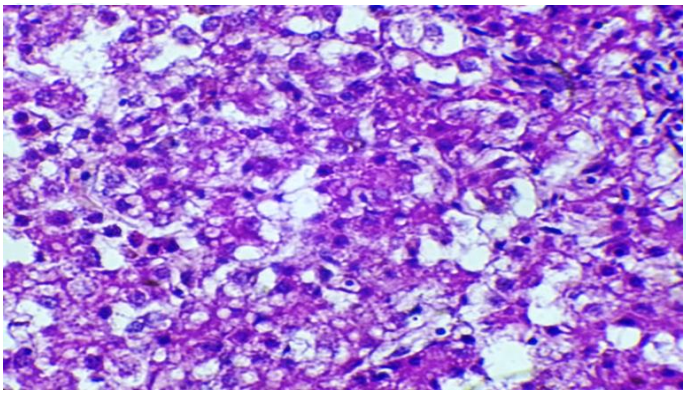

Figure 3: The main hepatic examination showed moderate perivascular mononuclear cells aggregation associated with slight degeneration changes of adjacent hepatocytes .moderate vacuolation of hepatocytes changes of fat droplets recorded mainly in sub capsular region with few inflammatory cells infiltration also predomination of Kupffer cell with mild cellular infiltration in sinusoids.

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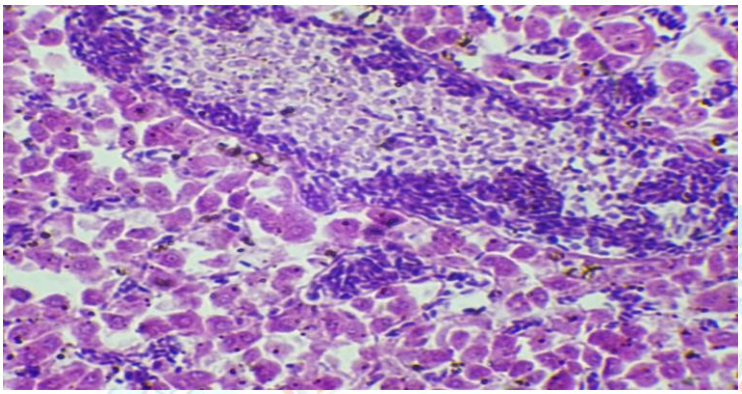

Figure 4: mononuclear cells infiltration was observed in portal region with obvious vascular congestion. showed moderate vessels congestion with few neutrophils' presence in lumen.

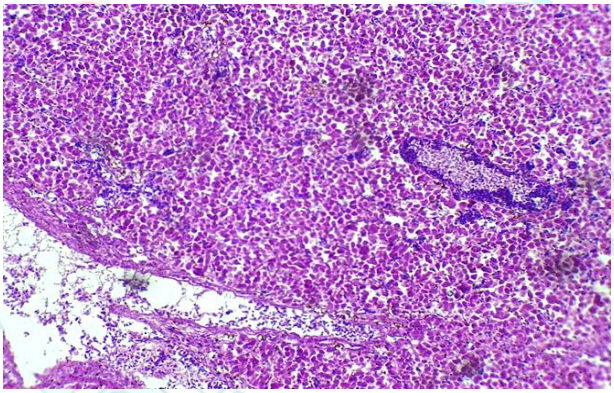

Figure 5: focal mononuclear cells aggregation in liver tissue with mild perivascular mononuclear cells infiltration. mild sinusoid congestion with mononuclear cells infiltration in some dilated sinusoids together with Kupffer cells hyperplasia . few sections showed enlarged congested portal vein surrounded with carious cell types.

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Discussion

Fatty liver haemorrhagic syndrome (FLHS) is defined as " a non-infectious (metabolic) disease of laying hens characterized by excessive accumulation of fat in the liver and abdominal cavity, liver rupture and haemorrhage, and sudden death"**[12]**

Current study reported positive significant correlation between body weight gain and feed intake (R= 0.703, P value = 0.000) which come in accordance with **[16]**. Current study reported Positive significant correlation between body weight gain and feed conversion rate (R= 0.850, P value = 0.000) which come in accordance with **[16]**. The result of current study come in accordance with opinion that changes in body weight accounts for about 50–70% of the variation in feed intake **[16]** .Thus, "variation in body weight and variation in feed intake are inextricably linked; hens which consume more feed on average tend to gain weight and heavier hens will consume more feed" .Thus, "flocks whose BW are managed around the breed standard or at a recognized optimal weight for age tend to achieve significant improvements in production performance, with improved persistency of egg production" **[17]** .Current study reported positive significant correlation between body weight gain and liver lesion score in FLHS (R= **0.524**, P value = 0.000) which come in accordance with **[16]**.

Current study revealed significant difference between hens with FLHS and normal hens regarding the values of albumin, albumin / globulin , globulin , total protein which come in line with that reported by **[18]**.

In current study, significant difference was reported between hens with FLHS and normal hens regarding the values of alkaline phosphatase (P value = 0.0000),which come in accordance with that reported by **[19, 20]**on the other hand **[21]**, stated that "Elevations are most common with liver disease even though the level of activity in this organ is low as well as elevation of alkaline phosphatase level takes place at laying period**[21]** .On the other hand **[22, 23]** stated that no significant difference was reported between hens with FLHS and normal hens regarding the values of alkaline phosphatase. While **[24]** stated that "In chickens, elevated Alkaline phosphatase activity has been predominantly related to increased osteoblastic activity and used as a marker for evaluating skeletal health and bone disease, such as skeletal growth, nutritional secondary hyperparathyroidism, rickets, fracture repair, and osteomyelitis". According to **[21]**, Alkaline phosphatase activity increase in FLHS and other conditions such as "Hyperparathyroidism-induced stimulation of osteoplastic activity and Enteritis" . The activity of this isoenzyme is labile and difficult to measure **[25]**.

In current study ,no significant difference was reported between hens with FLHS and normal hens regarding the values of ALT (P

value = **0.57339**) which come in concordance with that reported by**[21]**

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who stated that" ALT activity occurs in many different tissues. Specific diagnostic value of these enzymes in birds is poor. In many cases, patients with severe liver damage have had normal ALT activities, reflecting a low level of enzyme activity in liver cells from certain species". ALT activities often increase due to damage in many different tissues. On the other hand **[25]** stated that "In some avian species, normal ALT activities are below the sensitivity of many analyzers".

In current study, significant difference was reported between hens with FLHS and normal hens regarding the values of creatinine (P value = 0.00003421), creatine kinase (P value = 0.0000). which disagree with that reported by **[26]**who stated that the value of creatinine kinase was lower in FLHS affected layers compared with non-FLHS layers. Creatinine kinase functions in skeletal muscle, heart muscle and brain tissue. In muscle, this enzyme makes ATP available for contraction by the phosphorylation of ADP from creatinine phosphate**[21]**. Elevations in activities are mostly seen because of muscle cell damage. This enzyme has value in distinguishing muscle from liver cell damage**[27]**. However, the clinician should consider that muscle and liver cell damage can occur simultaneously from the same or different pathologic processes. Increase in activity of creatine kinase in laying hens has been linked to muscle cell necrosis**[27]**

In current study, significant difference was reported between hens with FLHS and normal hens regarding the values of blood urea nitrogen which come in contrary with that reported by **[26]**who stated that " no difference in BUN between FLHS and non-FLHS laying hens ,

In current study, significant difference was reported between hens with FLHS and normal hens regarding the values of Cholesterol which come in line with **[28, 29]**. Liver lipid metabolism is involved in many physiological processes such as energy supply, inflammation, and cellular signaling, which plays a key role in fatty liver disease **[30]** . Massive lipid hoarding is an outstanding characteristic of FLHS. Cholesterol is a major lipid that is a precursor of all the steroid hormones and bile acids as well as a component of the plasma membrane of cells. It is obtained from the animal protein sources in the diet as well as being synthesized by the liver. Elevated and decreased cholesterol concentrations may occur from a number of physiologic influences and different diseases. As stated by **[21]**"Very high cholesterol concentrations usually accompany lipemia, especially in fatty liver degeneration. Elevations can occur because of hypothyroidism, liver disease, bile duct obstruction, starvation or high fat diets".

In current study, significant difference was reported between Hens with FLHS and normal hens regarding

the values of glucose which come in agreement with that reported by **[19, 31]** reported that "FLHS hens had a higher

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blood glucose than control hens which indicate a serious damage for beta cells in pancreas ”

This study reported significant difference was reported between hens with FLHS and normal hens regarding the values of calcium which come in agreement with that reported by**[32, 33]** who stated that “ Serum calcium levels in hens with FLHS are elevated, suggesting interference with the formation of active Vitamin D (1, 25 (OH2) D3) which is vital in the egg shell formation process” . Current result come in contrary with that reported by **[24]**who reported no significant difference between hens with induced fatty liver and control group .

Current work reported significant difference between hens with FLHS and normal hens regarding the values of phosphorus which come in accordance with that reported by **[34]** who stated that “plasma inorganic phosphorus is related to dietary phosphorus in hens with an elevated plasma inorganic phosphorus level associated with fatty liver syndrome”. Current result come in contrary with that reported by **[24]**who reported no significant difference between hens with induced fatty liver and control group .

In the present study The necropsy findings for the birds suffering from FLHS often reveals abdomens filled with large blood spots, arising from the liver ,hepatomegaly with fat deposition which come in agreement with **[2, 12, 13]**. Liver usually presented with different color include yellow, pale or putty colored which come in agreement with **[2]**. Also, the liver was friable with small hematomas in the liver parenchyma which come in line with that reported by**[7, 13, 35]** . Hemorrhages were found in the margins of the liver lobes which come in accordance with **[36]** and these haemorrhages “may extend to the surface, or can form hematomas under the liver capsule”. Fatty liver cases usually associated with presence of fat in abdominal cavity around the viscera which is the common finding as stated by **[7, 36]**

In current study, histopathological findings include necrotic changes of hepatic cords with moderate dilation and congestion of sinusoidal together with wide hemorrhages. evidence of individualization of hepatocytes results in boron's disrupted of tissues architecture together with clear central vein congestion which come in accordance with that reported by **[12, 13]**.Also current study reported marked portal vein dilation with inflammatory edematous exudates as well as portal fibrosis was recorded with sever atrophy of adjacent parenchymal cell zone which agree with that reported by **[13].** In current study**,** there was moderate perivascular mononuclear cells aggregation associated with slight degeneration changes of adjacent hepatocytes with moderate

vacuolation of hepatocytes due to fat droplets mainly in sub capsular region with few inflammatory cells infiltration with predomination of

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Kupffer cell with mild cellular infiltration in sinusoids which come in agreement with **[12, 13]**. Mononuclear cells infiltration was observed in portal region with obvious vascular congestion. Focal mononuclear cells aggregation in liver tissue with mild perivascular mononuclear cells infiltration and mild sinusoid congestion with mononuclear cells infiltration in some dilated sinusoids together with Kupffer cells hyperplasia which come in agreement with that reported by **[2]** .

Conclusions: FLHS induced mainly with consumption of high energy diet causing serious negative effects on liver function as well as most vital metabolic biomarkers in laying hens

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