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Clinical and Pathological Impact of Fatty Liver Hemorrhagic Syndrome in Caged Hens Farms in Diyala Province

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Abstract

The aim of present study was to show the Fatty liver hemorrhagic syndrome (FLHS) in different areas of Diyala province and its effects on the eggs production. A total of 250 layer hens from different areas of Diyala Province (44) weeks suffering from pale appearance, anorexia, low weight and low egg production without any other signs refers to an outbreak of known infectious diseases were enrolled. Post mortem signs show enlargement of livers in the most cases with fatty appearance. The microscopic study appears the fatty change in hepatic section. The weights of layer hens, blood parameters, lesions score were studied. The results showed higher incidence of case in 11.6% the oldest layer hens in compare with 0.8% the youngest and low weights of affected farms at 31 and 64 wks of age. Birds of Farm 3 had a significantly higher BW than that recommended for Isa brown. No significant increase of all blood parameters measured at 73 wks of age in Farm 1. No significant changes in blood cell profile of hens at different flocks/ages at Farm 1. FLHS cause a significant economic loss by dropping of egg production and mortality increases.

Keywords: Fatty Liver Hemorrhagic Syndrome, Isa brown, Hens, Liver hemorrhage, Hormonal factors

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. FLHS is characterized by excessive fat in the liver and hemorrhage from a ruptured liver. The syndrome occurs in caged laying hens, primarily in birds that are in positive energy balance (Polin and Wolford, 1977). However other factors have also been implicated as potential contributory elements to the occurrence of FLHS (Thomson et al., 2003). 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 (Fig. 1-1 A and B), and in many cases large blood clots in the abdominal cavity (Fig. 1-1 C). Outbreaks occur sporadically in commercial flocks (Squires and Leeson, 1988), and 3-5% of the affected flocks die from the condition. Ugochukwu (1983), Weitzenburger et al. (2005) and Shini et al. (2006) have reported higher mortality (6-20%) due to FLHS. 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.

The etiology of this syndrome is still poorly understood and the occurrence underappreciated. Since the 1990s, the main factors that have been involved in the etiology of the FLHS include:

a) Nutritional factors (e.g. consumption of high energy diets Intake of high-energy diets that allows caged hens to consume energy in excess of the requirements for maintenance and egg production, results in a positive energy balance and increased hepatic fat deposition. The fact that FLHS can be experimentally induced through force-feeding and/or oestrogen administration indicates that the condition might be caused by a surfeit of energy rather than being specific to an

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excess of any nutrient such as fat or carbohydrate . Butler (1975) suggests that excess fat in the liver arises mainly from increased lipogenesis rather than from dietary lipids. Several studies have indicated that high energy maize or wheat diets produce higher incidences of FLHS (Haghighi and Polin, 1982). Branton et al. (1995) observed a high incidence of FLHS in hens that consumed diets containing chelated minerals .

b) Hormonal factors

Oestrogens influence the lipid synthesis which is required for yolk deposition. Polin and Wolford (1977) indicated that the liver hemorrhage score was markedly increased when excess energy intake was combined with exogenous oestrogen treatment. The possibility of a hormonal imbalance has been suggested by the observation of greatly elevated serum calcium and cholesterol in chickens from flocks with FLHS (Harms et al., 1972; Miles and Harms, 1981).

c) Environmental temperatures (i.e. heat and cold stress)

Exposure to cold or heat induces stress and influences lipid metabolism in the fowl (Annison, 1983). The injection of adrenocorticotropic hormone (ACTH) also produces this response (Jaussi et al., 1962). However, most investigators have shown that increased lipogenesis may occur partly due to an excessive intake of carbohydrate during hot weather (Couch, 1956; Pearson and Butler, 1978); while, Jensen et al. (1976) observed more.

Material and Methods

Hens

Layer hens white Hy-Line and Isa brown (250) purchased in different regions in Diyala. The average number of birds per producer per year ranged from less than 10,000 (2 producers); 20,000 to 190,000 (8 producers); and 900,000 birds (1 producer), with the number of sheds ranging from 1 to 16. Only two producers use controlled environment sheds, others have their sheds naturally controlled/ventilated. Eight of the producers use cages housing 5-6 birds/cage, and 4 use cages housing 3 birds /cage. All of producers use cages conforming to the welfare code. Five of producers use Hy-Line brown layers, 2 use Isa brown, 2 use HI-SEX birds and 1 uses both Hy-Line and Isa brown to operate farms. Seven of the producers used farm-mixed feed and only 4 used commercial feed. The mortality rate of flocks ranged from 2% to 11% and the average rate of production for laying cycle ranged from 70% to 89%. None of the producers know the causes of mortalities in their flocks, and only 3 use veterinary laboratories to determine causes of bird mortality; while, all used lighting programs for laying flocks. Only 1 of the producers was aware of FLHS being sporadically observed in their flocks (dead birds).

Blood samples

The blood collected from jugular vein and collected in clean anticoagulant tubes to send to the laboratory to evaluate the hematological parameters for cases.

Histopathology

Necropsy applied for the cases and samples of livers collected to be fix in 10% formaldehyde solution for prepare to routine histopathology.

Statistical analysis

All collected data were analysed using the Chi-square test (x^2) in the SPSS Program (Al-Gharban,

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2017).

Results

Results were showed higher incidence of FLHS in 11.6% the oldest layer hens in compare with 0.8% the youngest. The mortality rates increased with age ($P<0.05$), although there very low differences in mortalities between flocks of similar ages.

The results indicate that for Farm 1 at the 29, 54 and 73 wks of age (end of April) the mortality rate was 2, 4.8 and 11.6%, respectively. At 72 wks, Farm 2 (shed/flock 1) mortality rate (cumulative) was 7.4% of the initial flock, and at 31, 49 and 64 wks of age the mortality rate (cumulative) for Farm 3 (shed 1, flocks 1, 2, and 3) was 0.8, 2.5 and 4.8%, respectively .

As indicated in the methodology only 30-50% of dead birds were necropsied. The results indicate that 42% of birds necropsied from Farm 1 showed clinical signs of FLHS, while for Farm 2 only 28% of dead birds have had FLHS, and for Farm 3, 34% died due to this condition. Interestingly, the results showed that of birds that died in Farm 1, between the ages 37 to 54 wks more than 50% demonstrated FLHS. The average BW of those dead birds was 2008±107 g. The average of BW of birds that died in Farm 2 and 3 was 1821±78 and 1954±92 g, respectively .

Performance parameters

Collected layer hens from Farms 1, 2 and 3 at three sampling points: February, March, and April 2020. For breeder's recommendations at peak of production (32 wks) BW was increased with age. At 32 and 72 wks of age birds of Farm 1 and 2 had a BW comparable with that recommended by the breeder. At 31 and 64 wks of age, birds of Farm 3 had a significantly higher BW than that recommended for Isa brown .

Body weights

Body weight of birds in both feed restricted groups (E2-treated and non-treated group) decreased starting first week post-treatment (Table 1), but this decrease was not significant ($P>0.05$). The decrease was more pronounced ($P<0.01$) on the second week of treatment, and continued to remain at this level (without recovering) even 1 week after the treatments was interrupted .

Table (1): Body weight of birds in feed restricted groups (E2-treated and non-treated group)

Parameter	Farm 33	Isa brown 1	Farm 2	Farm 12	Hy-Line 1
BW (g)					
32 wks	1985	1985 (1975 at	2117	1872 gm	1980 gm
72 wks	2163	64 wks)	gm	2128 gm	2250 gm

Table (2): Hen Day Production and Mortality rate of enrolled farms

Parameter	Farm 33	Isa brown 1	Farm 2	Farm 12	Hy-Line 1
Hen Day Production		94.3			
(%)	91	75 (79.7 at 64	74%	94.3	94
32 wks	85	wks)		77.4	72
72 wks					

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Mortality					
Cumulative (%)	0.8	1.2	7.4	2.0	0.8
32 wks	4.8	5.8 (4.9 at 64		11.0	4.0
72 wks		wks)			

Blood parameters

All Blood parameters in layer hens affected by FLHS were taken and analyzed at the end of each month (February, March, and April 2020). Data presented here are calculated as an average of 40 birds per shed/age for Farm 1, 18 birds per shed/age for Farm 2, and 27 birds per shed/age for Farm 3 at each sampling point/time. Although, there was a slight increase of all parameters measured at 73 wks of age in Farm 1, this was no significant ($P>0.05$). No significant changes were found in blood cell profile (RBCs, HGB, and HCT) in hens at different flocks/ages at this Farm.

Table (3): Blood parameters in layer hens affected by FLHS

Age (wk)	RBC (x106/L)	HGB (g/L)	HGB (g/L)
21	23.8	132	29.5
25	2.47	134	30.3
29	2.61	138	31.2
46	2.47	132	30.8
50	2.45	135	30.6
54	2.41	126	30.5
65	2.59	132	32.0
69	2.52	139	31.7
73	2.69	146	34.3

Table (4): Blood physiological parameter of hens with FLHS

Age (wk)	Cholesterol (Mmol/L)	Triglyceride (Mmol/L)	GGT (U/L)	Protein (g/L)	Glucose (Ml/L)
12.1	43.7	33.7	12.3	2.5	12.1
14.8	50.7	32.7	11.4	2.7	14.8
15.1	48.7	35.0	11.1	2.3	15.1
12.9	56.7	32.3	19.9	2.4	12.9
14.8	58.3	31.7	23.5	2.8	14.8
15.9	55.0	34.7	19.9	2.5	15.9
12.7	53.7	41.7	20.8	2.5	12.7
14.5	55.3	43.7	23.3	2.6	14.5
15.1	54.0	46.0	24.6	2.3	15.1

Liver weights (g) fat content (%)

Data were recorded during the whole experimental period. Number of birds sacrificed from each treatment at each sampling point was 6. At the end of experimental period all birds were sacrificed and undergone post-mortem examination there were no significant differences between not-treated and oil-treated groups therefore data are pooled and presented together. The dyed layers of livers showed (during experimental period) were pale, swollen and friable with different grades of hemorrhages and haematomas on both surfaces (dorsal and ventral) and/or in the edges of both

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lobes. In advances cases (hemorrhage score 4 or 5) liver tissue was ruptured and large blood coagulations was found inside the abdominal cavity .
Liver

Lesion Scores

Lesion scored as mentioned by (Ginns et al., 2000) by post mortem examination of liver grossly and microscopically.

Organ	Score	Description of lesions
Liver	0	No lesion.
	1	Mild lesion.
	2	Haemorrhages and fatty liver

Table (5): Liver lesion scores of hens with FLHS

Farm 33	Isa brown 1	Farm 2	Farm 12	Hy-Line 1
0-1	0-1	1	1-2	1-2

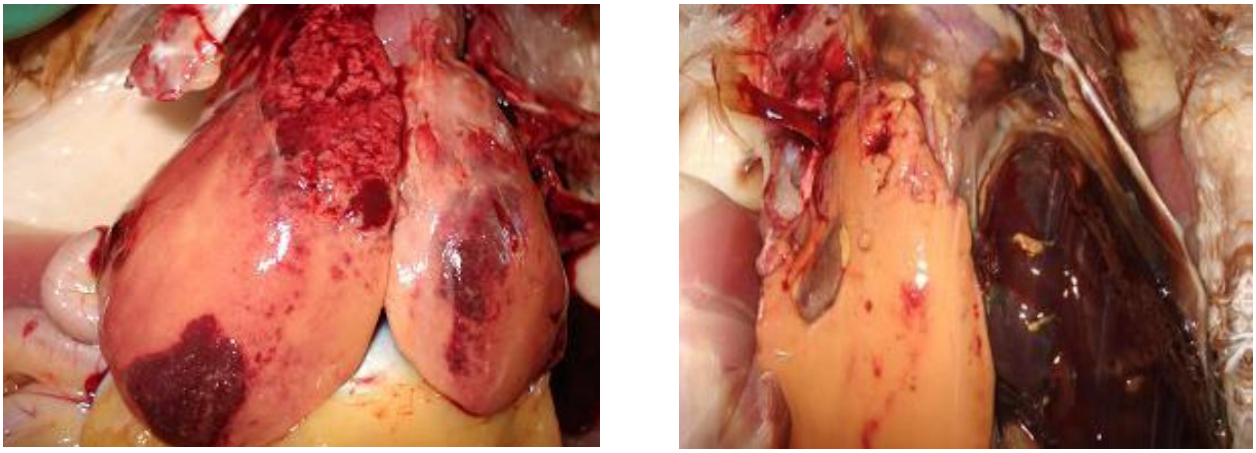

Figure (1): Liver lesion of score 2

Table (6): Birds diagnosed with FLHS, liver weight to BW ratio, fat content and mortality %

No. of birds 2	Birds diagnosed with FLHS (5)	Liver weight to BW ratio (g/100g)	Liver weights (g) fat content (%)	Mortality (%)
32	18.75 6.25	2.07	38.6±5.1 25.2±2.8	0
32	6.25	2.19	39.2±4.8 23.8±2.2	0
16	87.5 12.5	2.91	53.0±6.0 51.4±5.3	18.75
16	68.75 18.75 12.5	2.82	47.4±5.5 43.6±3.8	6.25

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Liver histopathology examination

Oestradiol treatment resulted in an increased infiltration of hepatocytes and liver tissue with fat and fat vacuoles (Fig. 4-10). Histologically, all livers had significant slight and moderate lipid accumulation in livers, however, E2-treated birds demonstrated severe fat deposition and large vacuoles containing fat and distending hepatocytes. In addition to fat deposition, histological sections of E2-treated birds indicated focal inflammatory (heterophilic and/or lymphocytic/ mononuclear) infiltration, hemorrhage and congestion of sinusoids, demonstrating an increased incidence of inflammation and hemorrhage. Massive lipid infiltration, diffuse inflammatory infiltration and congestion were observed especially in the liver parenchyma of birds that macroscopically demonstrated severe lesions of FLHS.

Discussion

In this project a questionnaire followed by an epidemiological survey were used to determine the occurrence of FLHS in caged laying hens in Diyala. The results demonstrate that FLHS is present in caged birds in Diyala. The questionnaire provided important data on hen management practices, and also suggests that most egg producers are not aware of FLHS, but the presence of FLHS was confirmed in the epidemiological study. Post-mortem examination conducted in 3 farms with 7 flocks of different ages indicated that 234 birds (or 36%) of all birds necropsied (597) had FLHS. This indicates that FLHS is the most significant cause of death of laying hens kept in cages. It also confirms our previous observations with a small flock of caged hens at UQ Gatton (Shini et al. 2006) where we found that FLHS was the main cause of death (74% of birds necropsied) in a flock indicating a 6% cumulative mortality rate. The results are also in agreement with previous overseas studies which have shown a high mortality rate (5-20%) due to FLHS in healthy flocks.

Death from FLHS occurs only in extreme cases following massive liver hemorrhage (Squires and Lesson, 1988). Therefore, it is likely that a significant number of hens within a flock are also suffering from subacute and chronic FLHS that may cause a drop in egg production but little increase in mortality (Julian, 2005). These hens may exhibit reproductive dysfunction (Chen et al. 2006), due to chronic liver tissue damage and an impairment of the transport of triglycerides, phospholipids, and cholesterol from the liver to the ovary (Walzem, 1996), resulting in decreased yolk formation and egg production. Our data showed that most deaths occurred in heavier hens over 40 wks of age".

Moreover, the examination of livers from hens euthanised systematically indicated that more than 50% of hens had focal hemorrhages or haematomas, while 10% of hens euthanised showed focal necrosis and signs of previous subcapsular hemorrhage. Together, the acute and chronic form of the disease suggests that FLHS is a significant source of lost in egg production and confirms our prediction that FLHS is a neglected disease of significant economic importance.

The results of this study also confirm our previous observations that laying hens, in multi-tier cages and in a controlled environment shed, are most at risk of developing FLHS. To our knowledge, we are the first to show the effect of a thermoneutral environmental temperature on the occurrence of FLHS in caged hens. Previous studies that examined the effect of temperature on the occurrence of FLHS were conducted 30 years ago, when controlled environment sheds were not widely used in the industry. In these studies increased mortality due to FLHS was found at temperature extremes. In our study heavier birds in a flock were more likely to have the condition than the lighter birds, particularly in a controlled environment shed. Birds are maintained in a thermoneutral zone and have lower energy requirements. Both factors (lack of activity and controlled environmental

temperature) contribute to increased BW and increased hepatic lipid deposition.

From the first part of the study, it was concluded that FLHS is present in caged flocks in diyala, and the age of the flock and housing conditions influence the incidence of this metabolic disorder.

Induction of FLHS in the laying hens was investigated to study its pathogenesis and establish the role of oestrogen in the production of FLHS, showing that birds with a higher feed and energy intake are more predisposed to the occurrence of FLHS.

Oestrogen-induced hens from feed restricted group also developed FLHS, although with a lower frequency, Body weights and egg production of hens that were restricted to feed was slightly impacted.

In laying hens hepatic lipogenesis is increased dramatically by oestrogen in order to meet the demand for vitellogenesis (Hansen and Walzem, 1993). Although the main products of de novo hepatic lipogenesis are triglycerides, the liver is also the major site of cholesterol and phospholipid synthesis. These lipids along with protein are the main components of lipoproteins. It is well known that, because de novo fatty acid synthesis in birds takes place mainly in the liver (Annison, 1983), adipose tissue growth and subsequent extrahepatic fattening depend on the availability of plasma triglycerides, which are transported as components of lipoproteins (Hermier, 1997). Many factors, e.g. external (nutritional and environmental factors) and internal (hormones and other mediators) may affect lipid metabolism and disturb metabolic, endocrine and immune interactions resulting in hepatic pathology.

Fatty liver occurs in birds when the increase in lipogenesis exceeds the capacity of synthesis and secretion of lipoproteins (Hermier, 1997). Studies in mammals have demonstrated that fat accumulated in the liver and abdominal cavity constitutes an interesting tissue that communicates with other tissues of the body including hepatocytes via adipokines, lipid factors, and lipoprotein particles (Tilg and Moschen, 2008). One of the first organs to be affected when adipose tissue becomes dysfunctional and inflamed is the liver (Attie and Scherer, 2008)

An extremely severe case of a fatty liver will causes an inflammation of the liver cells (steatohepatitis). In chickens, there is a lack of information on the role of a fatty liver in metabolic, endocrine and immune responses.

In this study, elevated leukocyte numbers and fibrinogen levels were highly altered in oestrogen-induced birds and slightly altered in natural cases of FLHS (in birds monitored for 52 wks). As in mammals, in birds the elevation of these parameters demonstrates increased systemic inflammation and tissue repair. Overall, it appears that in addition to the metabolic state of the bird, inflammatory and immune responses might have been involved in the pathogenesis of FLHS. This was also supported from histological data.

Conclusions

FLHS is present in caged layer hens in Diyala and impacts hen health and welfare. Significant economic losses to producers occur because egg production drops and mortality increases. The results demonstrated that FLHS is a major of hen mortality which has the following implications for the industry.

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