Review Article

Optimizing Poultry Production: Nutritional Interventions, Management Practices and Diagnostic Approaches for Fatty Liver Disease in Layers

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Abstract |Fatty liver disease (FLD) in layers presents considerable challenges to production, efficiency and animal welfare. This comprehensive review explores the etiology, mechanism of disease development, risk factors, diagnostic methods, and especially the role of nutrition in causing Fatty Liver Disease in layers. FLD is characterized by the excessive accumulation of lipids in hepatocytes. It is a multifactorial disorder influenced by various factors including genetics, nutrition, environment, and management practices. The economic impact of FLD on egg production and quality, as well as the welfare implications for affected birds, necessitates a thorough understanding of its mechanisms and management approaches. Diagnostic techniques such as histopathology, biochemical assays, and imaging modalities are crucial in identifying and monitoring FLD progression. Dietary changes, stress management, and welfare interventions are all included in management techniques because they are meant to minimize stress and maximize liver function. Animal welfare is greatly affected by the living environment of poultry. The influence of cage and cage-free housing systems on overall bird health, disease, stress, and behavior is variable. Preventive measures and early detection are strong strategies to alleviate the possible risk of occurrence of FLD. The current review may help to cover the research gaps regarding FLD in poultry as it provides insights on, different diagnostic tests from published research, the effect of different housing systems on poultry health, pathogenesis, associated risk factors, and good management.

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Introduction

Poultry farming plays an important role in meeting essential protein demands through the production of meat and eggs worldwide. This practice not only addresses nutritional needs but also serves as a significant source of revenue for small-scale farmers (Farooq *et al*., 2022). The poultry industry is the most rapidly expanding agricultural subsector, especially in emerging countries, and it is expected to continue growing in the future as a result of rising incomes, urbanization, and population growth, which will in-

crease the need for meat and eggs (Sriboonyong *et al*., 2022). The poultry industry contributed significantly to the country's economic growth by the production of 2362 tons of chicken meat and 25212 million eggs from a total of 2065 million poultry birds (Economic Survey of Pakistan 2023-24) The poultry sector in Pakistan encounters a range of disease obstacles, comprising infectious diseases like Newcastle disease, fowl typhoid, and salmonella, (Reynolds and Barry. 2022; Mahmood and Sabir 2021) as well as non-infectious diseases such as nutritional deficiencies, nematode infestation, and fatty liver syndrome among poultry (Murwani *et al*., 2022; Sajjad *et al*., 2021). Fats and oils play very important role in digestion process of birds, as they stimulate the release of important digestive substances like enzymes, bile, and mucus, facilitating the breakdown and absorption of nutrients in the digestive tract (Zhang *et al*., 2008). Fatty Liver Syndrome primarily affects commercial caged layers and is characterized as a dietary and metabolic disorder due to less exercise and confinement as compared to free rage birds or birds which are kep in furnished cages with proper place to move and express behavioral characteristics (Trott *et al*., 2014). Additionally, there is an abundance of fat deposited in abdominal cavity. This disease primarily affects commercial layers poultry (caged), which results in decreased egg production, death along with financial losses at large scale (Julian, 2005). Liver rupture causing internal bleeding can be the cause of a laying hen's sudden death (Butler, 1976; Shini *et al*., 2020). When the birds remains alive, it can be quite challenging to detect the indications of this metabolic disorder. When necropsy is done, the liver may be found to be putty-colored and yellowish, with numerous different types of hemorrhages and occasionally big blood clots originating from the liver in the abdominal cavity (Shini *et al*., 2006).

Fatty liver disease can cause a notable drop in egg production and trigger sudden mortalities, leading to substantial financial setbacks in the poultry sector and this condition is characterized by significant fatty degeneration and the buildup of fat within the liver's parenchymal cells (Marchesini *et al*., 2003). FLD has the ability to impair hepatocyte mitochondria, consequently affecting the β-oxidation process of fatty acids. (Wei *et al*., 2008). Furthermore, there exists a strong association between Fatty liver disease and peroxisome proliferator-activated receptors (PPARs), which performs a vital role in regulating β- oxidation. Elevate levels of free fatty acids (FFAs) present in hens' livers during the late egg-laying phase may disrupt biofilm integrity via the generation of free radicals (Sambasiva and Reddy, 2004; Zhou *et al*., 2008). Furthermore, a lot of saturated fatty acids (SFAs) have been shown to be harmful to hepatocytes directly as well as indirectly through causing cellular damage. (Kohjima *et al*., 2015). Moreover, FFAs elevated plasma level can trigger toll-like receptor 4 activation on macrophages as well as adipocytes, leading to the initiation of an inflammatory reaction within the fatty liver. (Yuan, 2001). When Kupffer cells in liver become activated, they have the ability to release significant quantities of cytokines, such as TNF-α, IL-1, IL-6, and IL-8.a (Rutkowski *et al*., 2009). TNF-α stimulates the release of FFAs in tissue which are in peripheries and the synthesis of fatty acids in liver, while Interleukin-1 and Interleukin-6 similarly delay the transport and secretion of triglycerides. (Navasa *et al*., 1998). FFAs disrupt lipid metabolism and glycogen synthesis in primary laying hen hepatocytes by altering the AMPK signaling pathway. This leads to oxidative stress, cell proliferation arrest, and apoptosis (Huang *et al*., 2022). Due to customer concerns about hen welfare, husbandry practices in the egg industry have changed. Consequently, different methods for producing eggs have been implemented over the past 20 to 30 years. Despite this shift, cage production systems remain the dominant method for laying hens in many countries. (Mench *et al*., 2011).

Roughly 85% of layers in the world were housed in traditional cage setups in 2011. (Windhorst *et al*., 2011). According to studies conducted in 2018, the usage of cage systems, mostly conventional cages, was the leading method of egg production in all major egg-producing countries and regions worldwide, The EU-15 being the exception. (Mench and Rodenburg *et al*., 2018). In China, Japan, and the United States, the three countries that produce the most eggs in the world, caged hens provide around 90% of the eggs produced. In the remaining four top countries in terms of poultry egg prduction (Turkey, India, Russia, and Mexico), this figure reaches 100%. (Mench and Rodenburg*et al*., 2018). Research aimed at examining the impact of diet on fatty liver disease in layers found that birds fed higher levels of poultry oil (3% and 4.5%) had increased hemorrhagic liver scores. The study concluded that a high-energy diet is the most typical reason for Fatty liver disease in caged layers. Ultimately, Fatty liver disease is a disorder of lipid

metabolism (Liaqat *et al*., 2023). There are 5 freedom of animal welfare which comprise (1) freedom from thirst and hunger; (2) freedom from discomfort (3) freedom from pain, injury, or disease; (4) freedom to exhibit typical behavior; and (5) freedom from fear and distress (McCausland 2014). To understand animal welfare, three major science-based frameworks have been employed (Fraser, 2003; Hemsworth *et al*., 2015). Among them are: Affective state: animal subjective sensations, biological functions: an animal's capacity to adapt to its surroundings and determine whether its needs are met and natural living: an animal's capacity to live in accordance with its nature and exhibit typical behaviors (Broom, 1986; EFSA, 2005; Fraser, 2003; Hemsworth *et al*., 2015). Lack of exercise in traditional cages can lead to metabolic issues in hens. According to Duncan (2001), paralysis in caged hens around their maximum production is known as "cage layer fatigue" and is caused by spinal cord compression, fracture of the thoracic vertebrae, and weakening of the bones. When comparing traditional cages to methods that provide more opportunities for movement and behavioral expression, non-infectious diseases including osteoporosis and fatty liver are more prevalent. (Weitzenbürger *et al*., 2005; Kaufman-Bart, 2009; Lay *et al*., 2011; Widowski *et al*., 2013). One of the most important metabolic conditions called fatty liver is commonly observed in poultry which are kept in traditional cages (EFSA, 2005; Jiang *et al*., 2014). It commonly results in liver damage or rupture and unexpected mortality. Elevated ambient temperatures, a high degree of stress, and inactivity are the key factors believed to lead to the occurrence of fatty liver (EFSA, 2005). The severe fat buildup in the abdominal cavity and the visceral areas is the first thing that is noticed during a post-mortem examination the liver is swollen, spherical, and extremely delicate. Its color changes from pale-brown to yellow due to fat buildup (Meijering, 1979). A suspected case of fatty liver disease based on postmortem examination was observed at local layer poultry farm at Lodhran, Punjab Pakistan. The Figure 1 shows the pathological signs of fatty Liver disease in layer bird. The labeled Figure 1 shows the liver color changed from normal to pale- yellow and underlying accumulated fat is also indicating fatty liver disease. Figure 2 is showing the enlarged liver size with excessive amount of fat which is suspected indicative of fatty liver disease. Different types of cages have different impact on chicken health, growth and stress level as there are 3 major types of housing system for poul

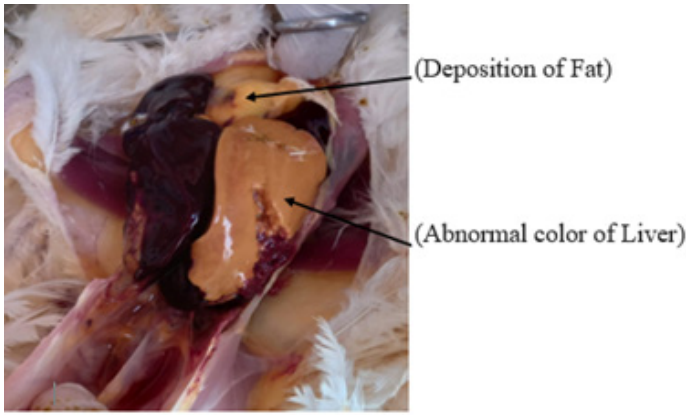

Figure 1: *A suspected case of fatty liver disease. Color of liver changed from normal to pale-yellow. Accumulation of excessive fat is also indicating fatty liver disease (Postmortem examination was observed at local layer poultry farm at Lodhran, Punjab Pakistan).*

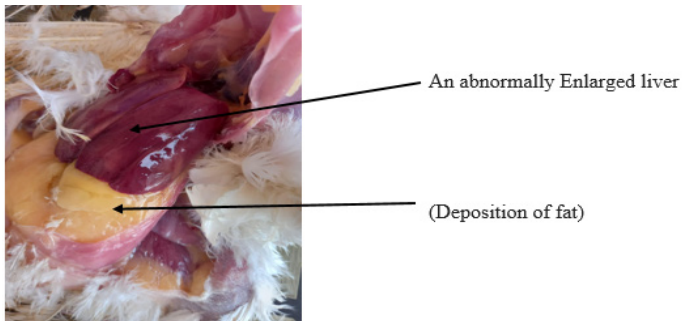

Figure 2: *A suspected case of fatty liver disease. Increased size of liver can be seen. Accumulation of excessive fat is also indicating fatty liver disease (Postmortem examination was observed at local layer poultry farm at Lodhran, Punjab Pakistan).*

try, firstly is the cage free system, secondly, conventional cage system and thirdly, the most important is furnished cage system. Compared to cages, cage-free setups typically provide more opportunities for locomotion and proper movement. The movement level of the bird is usually high due to dispersed resources. Stocking densities need to be low enough to allow for freedom of movement and expression of behavior; otherwise, birds could be restrictive in terms of movement (Leone and Estevez, 2008; Lay *et al*., 2011). Hens kept in cage-free systems may face more fractures during the laying season than hens kept in furnished cage systems during flight or fall on objects like feeders, drinkers etc. (Lay *et al*., 2011; widowski *et al*., 2013). The conventional cage system allows insufficient movement and exercise which can worsen bone fragility and muscle weakness in birds (Webster, 2004; Widowski *et al*., 2013). Hens frequently develop disuse osteoporosis as a result of the severe behavioral limitation they endure in traditional cages,

which further weakens their bones (LayWel, 2006). Out of all the housing systems, conventional caged hens have the lowest bone strength and the highest rate of fractures (Widowski *et al*., 2013). Compared to conventional cages, furnished cages typically provide greater mobility. This system specifically provides more space, perches, nests, and a scratch area. Because of this, hens' behavior is more varied and unrestrained than it would be in conventional cages, and their physical condition is improved. Birds are allowed to engage in some of their most highly driven behaviors, however some issues may be faced while using cage systems which include insufficient delivery of litter in cage, limited movement, less or no ground scratching and wing flapping for very short time due to cage walls (Appleby *et al*., 2002; Lay *et al*., 2011). Another drawback to use cage system when hens are highly motivated to carry out a variety of natural behaviors, such as perching and identification of a nesting site and housing restrictions hinder hens from carrying out these behaviors, they may become frustrated and experience emotional distress, which may manifest as typical back-and-forth pacing behavior (Fraser *et al*., 2013), and Stress may be developed due Hysteria and feather pecking (lay *et al*., 2011).

Mechanism of Fatty Liver Disease (FLD) development

Molecular mechanism such as Insulin resistance is involved in the development of FLD by down grading of insulin receptor substrate 2 (IRS-2), over expression of Sterol regulatory element binding proteins-1 (SREBP-1), upregulation of de novo lipogenesis (DNL), and inhibition of β-oxidation of fatty acids which further promotes hepatic lipid accumulation (Wree *et al*., 2016). Triglycerides are the primary by-product of hepatic de novo lipogenesis, and the liver is the primary location for the synthesis of phospholipids and cholesterol (Piotrowska *et al*., 2011). FLD is developed in layers when there is any disruption in lipid metabolism and layer birds develop this metabolic disease due to a combination of factors including hormones, toxic compounds, nutritional habits, diet, genetics, and environmental conditions (Guo *et al*., 2012). One of the main characteristics of FLHS is insulin resistance, which is necessary for lipotoxicity, oxidative stress, and the inflammatory cascade to be activated (Del *et al*., 2018). An important study showed that FLD is markedly raised the levels of Cidea and Cidec mRNA expression in liver and adipose tissue and these two lipid droplet-associated proteins are crucial in hepatic lipid accumulation and increased steatosis (Peng *et al*., 2019). AMPK signaling pathway is found to play a critical role in the development of FLD in laying hens. The pathway was associated with increased mRNA expression of lipid synthesis-related genes, such as ACC, FAS, GPAT, and cholesterol synthesis-related genes, HMGR, and HNF4α, and decreased expression of fatty acid oxidation-related gene CPT1 (Gao *et al*., 2019). A study using 30-week-old laying hens demonstrated that exogenous estrogen (E2) administration led to elevated liver weights, notable liver modifications, and an increased risk of Fatty Liver Hemorrhagic Disease, particularly when the hens were given an unrestricted diet compared to those with restricted feed intake (Shini and Agim 2014). Zhu *et al*. (2021) suggested a practical model to describe the genetic pathways to understand pathogenesis of Fatty liver disease in chickens. Furthermore, they brought attention to CNTF, SOCS3, and IL6 in the Jak-STAT signaling cascade as possible treatment targets for chicken FLHS. To cause hepatic lipid deposition, Zhang *et al*. (2011) discovered that dietary carbohydrates greatly elevated the transcript levels of the genes SREBP-1c and ChREBP mRNA, which are connected to fat synthesis. Additionally, they significantly increased the activity of FAS (fatty acid synthase) and ACC (acetyl CoA carboxylase). Reduced protein intake prevents the liver from properly moving fat out, which results in F. Low protein diets also do not supply enough protein to make apolipoproteins. One reason why laying hens develop fatty liver could be due to a diet low in protein (Zhang *et al*., 2011). FFAs interferes with AMPK signaling, which impacts oxidative stress, energy metabolism, and cellular functions (Huang *et al*., 2022). Figure 6 demonstrates the interference of free fatty acids with AMPK signaling pathway.

Role of Dietary Intake in Fatty Liver Disease

Nutrition (carbohydrates, fats and proteins) plays very important role as predisposing factor for the fatty liver disease in layers. There have also been reports that genetic, hormonal, and environmental variables contribute to poultry FLHS (Hansen and Walzem, 1993). It is clear that nutrition has a significant role in the onset of fatty liver disease, even though it is caused by a variety of causes, either separately or in combination in poultry because 97% of the affected birds had significant fat deposition or were considered overweight (Trott *et al*., 2014). Certain dietary components may have a role in the advancement of fatty liver disease, according to other studies (Wal-

zem *et al*., 1993; Cherian *et al*., 2002; Yousefi *et al*., 2005) such as maize, when fed throughout the summer as opposed to the winter, a high energy maize feed was observed to cause an increased rate of Fatty Liver Disease than barley feed which is low in energy. poultry fed diets rich in calories, poor in linoleic acid, and low in choline had higher liver weights and hemorrhagic scores (Pearson *et al*., 1981; Yousefi *et al*., 2005). High energy diet is also a predisposing factor It has been observed after different experiments that birds which were effected with fatty liver disease had history of taking high energy adlibitum poultry feed (Polin and Wolford, 1976; Polin and Wolford, 1977; Haghighi and Polin, 1982; Choi *et al*., 2012). The use of oil in poultry feed causes sever liver problems in layers. The important study investigated the impact of poultry oil on liver health and fatty liver syndrome in 120 Lohmann Single Comb layers. Birds fed diets with increasing poultry oil (0%, 1.5%, 3.0%, 4.5%) for 20 weeks showed significant adverse effects on liver health, with the highest oil level causing severe liver damage and elevated plasma lipid profiles. The study concluded that high levels of poultry oil (4.5%) negatively affect liver health, leading to fatty liver syndrome (Liaqat *et al*., 2023). Fats and oils are crucial for bird digestion by promoting the secretion of digestive substances and maintaining gut cell integrity. However, excessive intake can disrupt lipid metabolism, leading to Fatty Liver Syndrome due to excessive liver fat deposition (Zhang *et al*., 2008). High energy diets either from fat or carbs were found to cause FLS, while the group that used fat as an energy source had an average high hemorrhagic score (Rozenboim *et al*., 2016; Zhang *et al*., 2008). Rozenboim *et al*. (2016) reported that layers fed a meal high in energy and poor in protein had higher liver hemorrhage scores. Layer birds' liver had higher levels of fat and dry matter when 4.5% poultry oil was fed. The elevated fat content of the liver was linked to the existence of fatty liver disease in layer birds (Choi *et al*., 2012; Zhang *et al*., 2008). The primary symptoms seen in studies at greater levels of poultry oil are fatty degeneration and hemorrhages, which are crucial indicators of FLS in layer hens (Trott *et al*., 2014). Increased consumption of feed and positive energy can lead to the advancement of Fatty Liver Syndrome in layer birds (Shini *et al*., 2020). Laying hens may naturally be predisposed to Fatty Liver sundrome due to low protein diets with greater ME from fat. (Rozenboim *et al*., 2016). The laying hens fed maize as an energy source had a significantly higher incidence of FLD compared to those fed wheat. The use of oats as a protein source significantly reduced liver fat, the liver fat content and triglyceride levels were 30 to 50% higher in the maize/soybean diet group versus the barley/soybean diet group. The use of oats as a protein source significantly reduced liver fat (Cross *et al*., 1987). According to reports, laying hens' liver fat content will significantly increase when 1.5 mg/kg of aflatoxin is added to their feed. Additionally, FLHS is also brought on by the T2 toxin found in Fusarium oxysporum. Many toxic compounds, including aflatoxin, which can seriously affect laying hens liver and create problems with the metabolism of lipids in liver cells, ultimately resulting in fatty liver, can be produced by moldy feed (Huang *et al*., 2022). Figure 7 demonstrates the onset of metabolic disease (FLD) due to deficiencies of important nutrients in poultry feed.

Diagnostic Tests for Fatty Liver Disease in Layers

Liver function tests reveal the liver's capacity for clearance, metabolism, and synthesis (Jaensch, 2000). Bile acids, plasma albumin concentrations, or clotting factor assays can be used to evaluate synthesizing capacity of liver. It is impossible to evaluate overall liver function with just one test, given the liver's intricate roles in metabolism, excretion, and defense. Therefore, a series of laboratory tests are typically used together to identify liver issues, determine their severity, monitor the disease's progression, and aid in diagnosing the cause and guiding treatment. (Hochleithner *et al*., 2006). The early diagnosis of fatty liver disease in laying hens is determined by many biochemical tests such as serum cholesterol, serum calcium, aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK). The average levels of serum cholesterol, serum calcium, lactate dehydrogenase (LDH), creatine kinase (CK), and aspartate aminotransferase (AST) were found to be 139.4 ± 87.2 (mg/dL), 24.5 ± 5.4 (mg/dL), 1238.3 ± 475.2 (IU/L), 1107.3 ± 422.8 (IU/L), 53.6 ± 23.1 (IU/L) in the Non-FLHS flock, and 210.2 ± 173.2 (mg/dL), 174.3 ± 53.5 (IU/L), 25.2 ± 4.1 (mg/dL), 1104.9 ± 472.9 (IU/L), 1694.9 ± 691.3 (IU/L), in the FLHS flock. With the exception of CK, the activities of serum cholesterol, aspartate aminotransferase (AST), and LDH were considerably greater in the FLHS flock compared to the Non-FLHS flock (So *et al*., 2009). Hepatocyte damage is reflected by serum ALT activity, a very sensitive and moderately selective preclinical and clinical indication of hepa-

to-pathology (Ozer *et al.*, 2008). The findings imply that measuring enzyme activity in birds that are suggestive of liver damage—specifically, AST, LDH, and GDH glutamate dehydrogenase—is a useful method for identifying FLHS (fatty liver-hemorrhagic syndrome) in a flock of layers (Diaz *et al.*, 1999). Metabolomics is a novel technology that has been utilized in recent years to study and characterize the general alterations in endogenous small molecule metabolites brought on by inside and outside stimuli at a particular time and under a particular set of conditions (Zira *et al.*, 2013; Yu *et al.*, 2020). There are two major analytical platforms of metabolomics which are Mass spectrometry (MS) and nuclear magnetic resonance (NMR) (Patel and Ahmed, 2015). GC-TOF-MS, noted for its high separation efficiency and detection sensitivity, is an effective metabolomics technique for diagnosing and assessing FLHS in laying hens by identifying key biomarkers such as glutamate and oleic acid through ROC curve analysis (Guo *et al.*, 2021). For the purpose of diagnosing FLHS in laying hens, all biochemical analyses were carried out. These analyses included: lipid metabolism indices total triglycerides (TGs), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C); liver function indices, which included aspartate aminotransferase and alanine aminotransferase; liver antioxidant capacity indices, which included glutathione peroxidase (GSH-Px), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px); and mitochondrial function indices, which included hydrogenase (CAT), citric acid synthase (CS), carnitine palmitoyl transferase-I (CPT-1), cytochrome C oxidase (COX), and coupling protein 2 (UCP2) (San *et al.*, 2023). Indicators of fatty liver disease are employed in layers to diagnose certain plasma enzymes at particular concentrations. The important enzymes include Aspartate Aminotransferase (AST), Lactate dehydrogenase (LDH), Glutamate dehydrogenase (GDH) (Diaz *et al.*, 1999). Some Important diagnostic tests from different published articles are given in Table 1. Microscopic image of normal liver (Figure 3) is histologically different from fatty liver in which hepatocytes are distended with fat vacuoles (Figure 4).

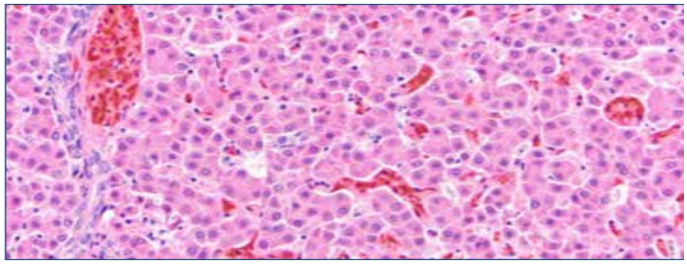

Figure 3: *Microscopic examination of normal poultry liver (Image courtesy Dr. Yuko Sato).*

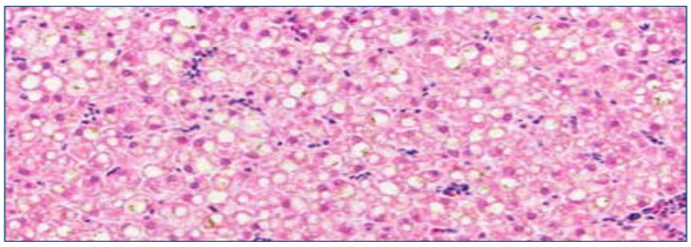

Figure 4: *Microscopic examination of poultry liver effected with FLD (fatty Liver Disease). Hepatocytes are distended with fat vacuoles ((Image courtesy Dr. Yuko Sato).*

Risk factors associated with fatty liver disease in layers

Outbreaks of fatty liver disease in layers are linked to different factors such as environment, genetics, and nutrition. Diets high in calories but low in protein are the main cause of this disease. (Diaz *et al.*, 1999; Choi *et al.*, 2012; Rozenboim *et al.*, 2016). Typically, FLHS is more common in birds in the middle and late stages of egg production as well as those with excess body weight (BW). Furthermore, liver is the primary organ involved in metabolic process of lipids in birds, and a variety of fatty liver diseases are caused by gradual alterations in hepatic lipid metabolism. Laying hens with Fatty Liver disease have increased triglyceride synthesis (TG) and disrupted very low-density lipoprotein (VLDL) production and secretion, which may be caused by an excess of fatty acid supply or hindered oxidation in the liver. (Dong and Tong, 2019; Gao *et al.*, 2019). Based on production-related statistics, aged layers have a higher risk of FLHS, and their body weight is consistently greater than recommended levels (Dong and Tong, 2019). Other studies have also demonstrated that laying hens' fatty liver illness is mostly caused by an excessive accumulation of liver fat, which is brought on by an abnormal build-up of fat caused by an issue in the liver's fat metabolism. Estrogen level effectively caused Fatty Liver disease in hens, with characteristic and systemic effects on liver, coming from a disruption in the metabolism of fat and long-term low-grade inflammation (Dong and Tong, 2019; Shini *et al.*, 2019; Zhuang *et al.*, 2019; Shini *et al.*, 2020). A high body temperature inhibits the thyroid gland's ability to secrete thyroid hormones and weakens lipolysis, both of which are risk factors in the development of fatty liver disease (Huang *et al.*, 2022). Lipid metabolism can be changed by hormones

Table 1: *Some important diagnostic tests for the diagnosis of fatty liver disease in poultry are given in table 1 with references.*

S.N.	Diagnosis	Diagnostic/Biochemical tests	Reference
1	Lipid Metabolism	Total triglycerides (TGs) Total Cholesterol (TC) High-Density Lipoprotein Cholesterol (HDL-C) Low-density lipoprotein cholesterol (LDL-C)	San *et al.*, 2023
2	Mitochondrial Function	Hydrogenase (CAT) Citric acid synthase (CS) Carnitine palmitoyl transferase-I (CPT-1) Cytochrome C oxidase (COX) Coupling Protein 2 (UCP2)	
3	Liver Function Tests	Aspartate Aminotransferase(AST) Alanine aminotransferase (ALT)	
4	Liver Antioxidant Capacity	Glutathione peroxidase (GSH-Px) Malondialdehyde (MDA) Mlutathione peroxidase (GSH-Px)	
5	Lipid Metabolism, Energy Metabolism, Amino acid metabolism	Serum Mrtabolomic Profiling based on Gas chromatography time-of-flight mass spectrometry (GC-TOF-MS)	Guo *et al.*, 2021
6	Lipid Profile	Total Cholestrol (TC) High Density Lipoprotein (HDL) Low density lipoprotein (LDL) Triglycerides (TG)	Liaqat *et al.*, 2023
7	Blood Serum chemistry	Serum Cholesterol Serum calcium Aspartate aminotransferase Lactate dehaydrogenase Creatinine kinase	So *et al.*, 2009
8	Plasma Enzymes	Aspartate Aminotransferase (AST) Lactate dehydrogenase (LDH) Glutamate dehydrogenase (GDH)	Diaz *et al.*, 1999

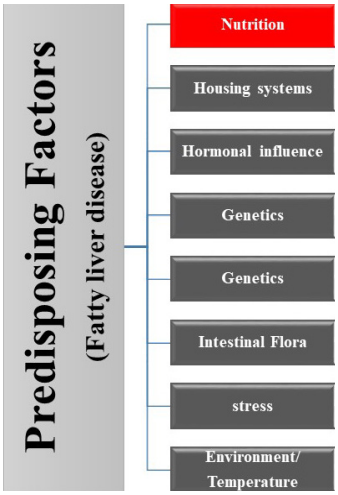

Figure 5: *Demonstrates the most important risk factors of FLD (Fatty Liver Disease) in Poultry. Other factors may also be involved, but the most important factor is Nutrition.*

such as estrogen thyroid hormone, cortisol, and other hormones, which can impact the development of fatty liver disease. The majority of studies have demonstrated that laying hens affected with fatty liver disease have much greater estrogen levels than regular laying chickens. On the other hand, estrogen increases fat synthesis in the laying hens' liver and decreases the ability of fatty acids in the liver's mitochondria to oxidize. Exogenous estrogen can cause male chicks' livers to accumulate fat. The average liver weight of the estrogen group was much larger than that of the normal group, and the serum concentrations of TC and TG were also significantly higher (Choi *et al.* (2012). Hens with fatty liver disease have markedly reduced serum thyroid hormone levels, which may contribute to the condition due to the hormone's role in stimulating lipolysis and boosting metabolic rate to reduce body fat. (Zhu *et al.*, 2021). Figure 5 demonstrates the major risk factors of FLD (fatty liver disease in Poultry). Some genes are also associated with fat development in poultry liver which are ACC (Acetyl-CoA Carboxylase) FAS (Fatty Acid Synthase) PGC-1α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha) PPARγ (Peroxisome Pro-

liferator-Activated Receptor Gamma) SREBP-1c (Sterol Regulatory Element-Binding Protein 1c) and PLIN1 (Perilipin 1) and these genes act differently in different chicken breeds (Kang *et al.*, 2022). 23 target genes are important for FLD and these genes, such as HAO1, ABCD3, and BLMH, are controlled by both DNA methylation and (long non coding RNAs) lncRNAs (Tan *et al.*, 2020). Intestinal microbes have been identified as a crucial component of the enteric-liver circulation, indicating a strong correlation between the intestinal flora and the development of fatty liver (Federico *et al.*, 2016).

Management and Control of Fatty Liver Disease

Taurine can control fat metabolism in the liver, decrease fat buildup in the liver, prevent oxidative stress, and improve mitochondrial dysfunction hence protecting laying hens from FLHS (San *et al.*, 2023). Taurine, one of the limited free amino acids, earned

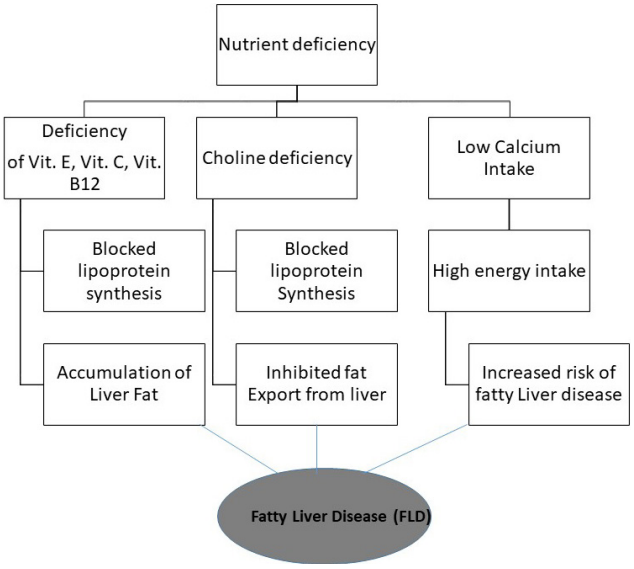

Figure 6: *Demonstrates the interference of free fatty acids with AMPK signaling pathway (Huang et al., 2022).*

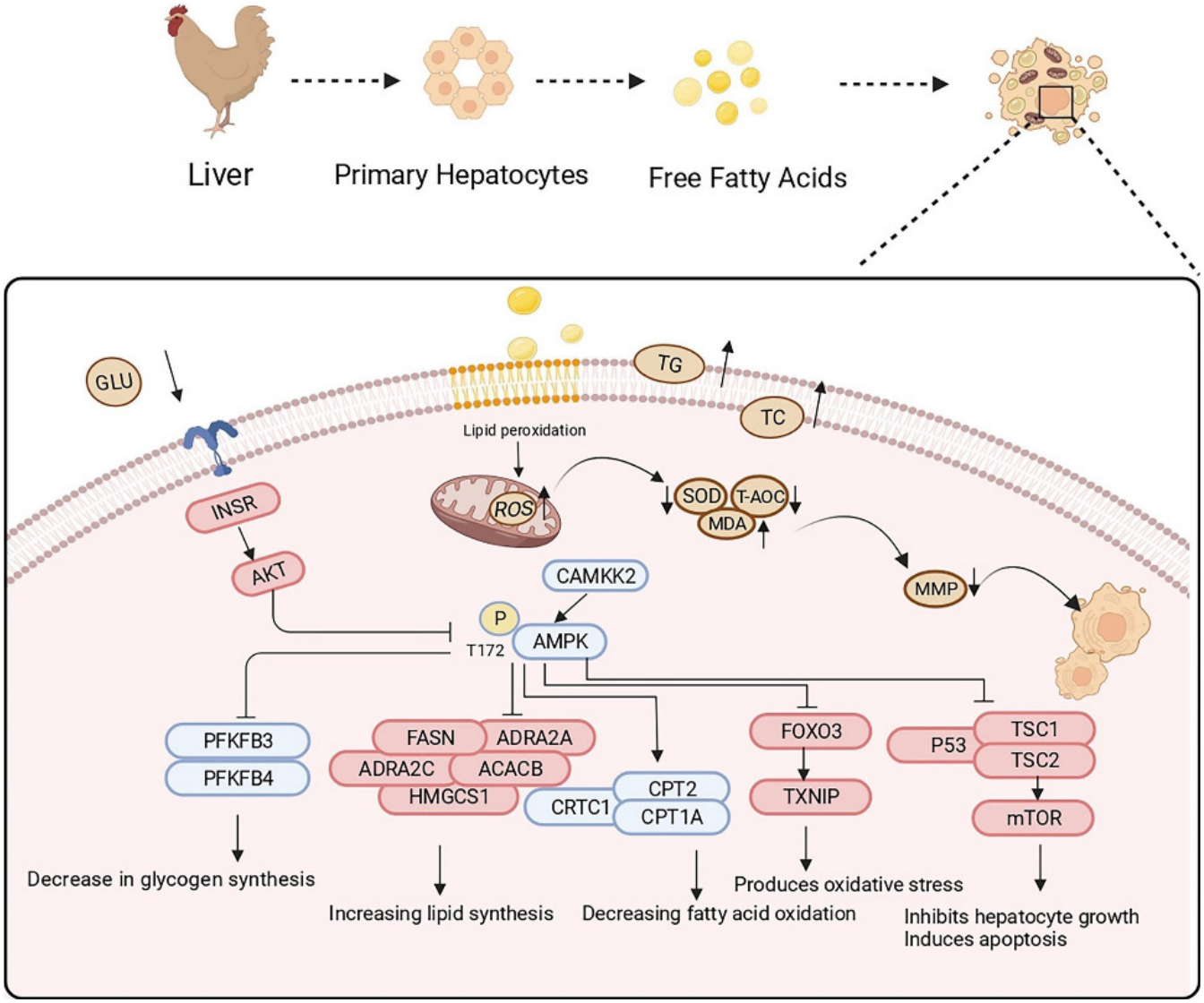

Figure 7: *Demonstrates the onset of metabolic disease (FLD) due to deficiencies of important nutrients in poultry feed. Due to continuous ingestion of deficient feed, poultry birds are prone to this disease. (Huang et al., 2022)*

its name from being initially discovered and isolated from cattle bile. Taurine demonstrates numerous physiological and pharmacological impacts, such as antioxidant, stabilizing cell membranes, detoxification, regulating osmotic pressure, and contributing to the development of the brain and retina (Roysommuti and Wyss, 2014). According to Cross *et al.* (1987), the degree of damage to liver caused by hemorrhages and liver fat content in fatty liver disease in laying hens were considerably decreased when oats were used as a protein source. It has been observed that addition of components such alfalfa, wheat bran, or fermentation leftovers greatly lowers liver cholesterol levels (Maurice *et al.*, 1978). Energy intake is therefore crucial, but it doesn't seem to be the only nutritional component influencing the buildup of lipids in birds. Several dietary components have been shown to have implications for liver lipid levels that cannot be explained in the context of dietary energy content. For example, it was found that the occurrence of subclinical fatty liver disease and liver lipid levels decreased in that order with isocaloric diets based on maize, wheat, or barley (Perason *et al*, 1978). Thus, a positive energy balance seems to be a contributing component to FLHS, and it has been found that environmental and nutritional factors that impact this also affect the buildup of liver fat. Thus, consumption of lower-energy diets can diminish the fat content in the liver. Exercise also plays a role in energy balance; chickens kept in cages were found to have higher liver lipid contents than hens kept in floor pens. It has also been demonstrated that hens kept in cages can have lower liver fat levels when pushed to exercise (Whitehead, 1979). Energy balance can also be impacted by environmental temperature; birds housed at higher temperatures have been shown to have higher liver lipid levels (Perason and Butler, 1978). Studies have indicated that the fatty liver syndrome is most likely to occur in summer season (Ivy and Nesheim, 1973). Lipid metabolism's homeostasis is destroyed by excessive triglyceride buildup in the liver and fatty acid oxidation inhibition (Miao *et al.*, 2021). By functionally preventing the buildup of extra lipid in the liver, osteocalcin (OCN) lowers the possibility of FLD (Miao *et al.*, 2021; Lin *et al.*, 2020). Osteocalcin protects chickens from fatty liver disease (FLHS) by activating the JNK and AMPK signaling pathways, down-regulating PPARα signaling to mitigate liver damage from various factors, and stimulating pancreatic β-cells to enhance insulin synthesis and reduce insulin resistance (Tu *et al.*, 2023). Probiotic are also a good source to mitigate the FLD in poultry (Gao *et al.*, 2021).

Conclusion and Recommendations

Fatty liver disease represents a complex and multifactorial disorder in layer poultry, with substantial implications for production efficiency and bird welfare. Understanding the etiology, pathogenesis, risk factors, diagnostic methods, and management strategies associated with FLD is crucial for mitigating its negative impact on the poultry industry. Implementing preventive measures, early detection, and targeted interventions are essential for optimizing production performance and promoting the welfare of layer poultry affected by FLD. Future research should be focused on innovative approaches, including modulation of the gut-liver axis and precision nutrition, holds promise for improving outcomes and addressing the challenges posed by FLD in layer poultry production. Accurate diagnosis of FLD in layer poultry is crucial for timely intervention and management. Diagnostic methods encompass a combination of clinical assessment, biochemical assays, imaging techniques, and histopathological examination. Clinical signs such as decreased egg production, lethargy, and hepatomegaly provide initial clues, while biochemical assays measure serum biomarkers indicative of liver dysfunction. Imaging modalities such as ultrasonography and computed tomography facilitate non-invasive evaluation of liver morphology and lipid content. Histopathological examination of liver biopsy specimens remains the gold standard for confirming FLD diagnosis and assessing histological changes associated with disease severity. There may be additional risk factors responsible for fatty liver disease in poultry that have not been discussed in published research. The different type of housing system have different impact on poultry production and occurrence of diseases but comparatively, furnished cage system have positive impact in terms of higher production, desirable welfare and less chances of disease occurrence. This review offers a thorough understanding of the gaps in the diagnostic literature as well as other management-related concerns pertaining to fatty liver disease in poultry, which could be beneficial in reducing the issue within the poultry industry.

Future Directions

Emerging research on the gut-liver axis and the role of gut microbiota in FLD pathogenesis offers prom-

ising avenues for novel therapeutic interventions. Modulating gut microbiota composition through probiotics, prebiotics, or dietary additives may help prevent FLD development and improve liver health in layer poultry. Furthermore, advances in genetic selection for FLD resistance and precision nutrition strategies tailored to individual bird requirements hold potential for enhancing production efficiency and welfare outcomes. Continued research efforts aimed at elucidating the underlying mechanisms of FLD and evaluating novel interventions are essential for addressing this significant challenge in layer poultry production.

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Novelty Statement

This review aims to fill critical research gaps in the understanding of fatty liver disease (FLD) in poultry by providing a comprehensive synthesis of current knowledge. It offers insights into various diagnostic tests, examines the impact of different housing systems on poultry health, and elucidates the pathogenesis and associated risk factors of FLD. Additionally, the review highlights effective management strategies, serving as a vital resource for researchers and poultry practitioners dedicated to improving animal welfare and health outcomes.

Authors Contributions

Bilal Javid: Idea and conceptualization, write up of original article.
Hafiz Muhammad Bilal Akhtar: Collection of relevant data, Visualization and arrangement of data.
Tahira Kamal: Proof reading of review paper.
Muhammad Naeem Riaz: Technical support and guidelines.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

Ethics Approval

Not applicable

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