CHAPTER 28

PATHOGENESIS AND NUTRITIONAL REGULATION OF FATTY LIVER HEMORRHAGE SYNDROME IN LAYING HENS

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INTRODUCTION

Fatty liver hemorrhagic syndrome (FLHS), a disorder of lipid metabolism in laying hens, was first reported by Couch in 1956 as fatty liver syndrome (FLS) (Wolford and Polin 1972). It was later renamed as FLHS by Wolford, because hens with this disease often have varying degrees of hemorrhage in their livers (Wolford and Polin 1974).

The disease occurs in caged hens at the peak of egg production and is characterized by a marked decrease in egg production, sudden death of well-fattened hens, high body weight of dead hens, and severe liver fat deposition on autopsy with certain hemorrhagic features. The disease has become common in many countries, and it has been reported that 74% of the total mortality of caged hens in Queensland, Australia, is due to FLHS (Rozenboim et al. 2016). In addition, FLHS has been reported as the most common non-infectious cause of mortality in northern California, USA (Mete et al. 2013). With the rapid development in the global poultry industry and the expansion of intensive farming, FLHS occurs in chicken farms and professional farmers and has a tendency to increase year by year. The mortality rate is generally less than 5%, sometimes even up to 30%, which brings huge losses to the poultry industry. The disease is mostly disseminated, slow and not as obvious as other infectious diseases, so it has not attracted much attention from the relevant parties. In view of the serious economic losses brought by FLHS to the farming industry, we will elaborate on the disease from the causes and nutritional control, aiming to provide a more effective way for the poultry industry to prevent and control this disease.

Pathogenesis

FLHS is formed by a combination of many factors; nutritional, genetic, environmental, endocrine and toxicological factors are associated with the occurrence of FLHS. Among them, nutritional factors are considered to be the main factors by most researchers.

Nutritional Factors

High-energy, Low-protein Diets

The proportion of energy feeds in the diet is too large and carbohydrates that are not used up by the animals are easily converted to fat in the body. Zhang et al. (2011) found that dietary carbohydrates significantly increased the transcript levels of fat synthesis-related genes SREBP-1c and ChREBP-mRNA and also significantly increased ACC (acetyl CoA carboxylase) and FAS (fatty acid synthase) activity as a way to induce hepatic lipid deposition.

Low protein diets do not provide enough protein to synthesize apolipoproteins, so the fat in the liver cannot be transported out effectively, which leads to FLHS. Low protein in the diet may be one of the causes of fatty liver in laying hens.

High-protein Low-energy Diets

The proportion of protein is too high whereas the energy is too low in high-protein low-energy diets. The high proportion of protein in the diet reduces the proportion of corresponding energy to meet the requirements of laying hens so that some of the amino acids produced after protein decomposition will generate glucose to provide energy, and in this process, amino acid deamination produces large amounts of nitrogen, which synthesizes uric acid in the body's liver, thus increasing the metabolic burden on the liver, inducing or leading to fatty liver.

Energy and Protein Sources

It was found that the incidence of FLHS was significantly higher in laying hens fed with maize as an energy source than that in those fed with wheat as an energy source. It was found that liver fat content was significantly higher in the maize-soybean type diet group than that in the maize-fish meal type diet group, and that triglyceride content in the liver of laying hens provided with the maize/soybean meal was 30 to 50% higher than in those provided with the barley/soybean meal. In addition, the use of oats as a protein source significantly reduced the liver fat content and the degree of hemorrhage in FLHS laying hens (Cross et al. 1987).

Choline, Methionine, B Vitamins, VC, VE and Calcium Deficiency

The output of fat in the liver of laying hens depends on lipoproteins, and lipoproteins cannot be synthesized without choline. VB12, VC and VE can promote the formation of choline from methionine and betaine. Therefore, when these

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substances are deficient, lipoprotein synthesis is blocked and fat cannot be effectively exported from the liver, which in turn leads to massive accumulation of FLHS. The lack or deficiency of calcium in the feed during the peak egg production period makes laying hens to increase the intake as a way to meet the body's demand for calcium, which often leads to excessive energy intake and promotes the development of fatty liver.

Moldy Feed

Moldy feed can produce a lot of harmful substances, especially aflatoxin, which can cause serious damage to the liver of laying hens and lead to disorders of lipid metabolism in liver cells, thus causing fatty liver. It has been reported that the addition of 1.5 mg/kg of aflatoxin to the feed will cause a significant increase in the fat content of the liver of laying hens. In addition, T2 toxin in *Fusarium oxysporum* is also a cause of FLHS.

Drug Use or Poisoning

Some animal drugs can reduce hepatic lipoprotein synthesis by inhibiting hepatic protein synthesis, resulting in dysregulation of lipid metabolism. The antibacterial drug tetracycline is one of the drugs that can cause fatty liver. Tetracycline, in the process of inhibiting bacterial protein synthesis, also affects hepatic lipoprotein synthesis, causing blockage of hepatic fat output and therefore a large accumulation of fat to form FLHS. In addition, heavy metal poisoning, such as mercury, lead and arsenic, can inhibit protein synthesis and cause fatty liver (Schuman et al. 2000).

Genetic Factors

According to an earlier report, genetic analysis of 39 cases of FLHS laying hens showed that 11 laying hens were the offspring of the same rooster, indicating a strong genetic correlation of FLHS (Zhang et al. 2011). Moreover, the liver fat content of laying hens of different breeds or strains under the same feed and environmental conditions was not the same. In addition, inbred strains of single-crowned white Laihang hens (UCD-003) were more susceptible to FLHS, indicating a genetic susceptibility to the occurrence of FLHS (Schuman et al. 2000).

Environmental Factors

Caging

FLHS generally occurs only in caged hens. The intensive caging of hens results in a lack in exercise or insufficient exercise, leading to a dramatic reduction in energy expenditure and a decrease in fat mobilization, which, combined with excessive feeding and increased lipogenesis, can easily cause excessive fat deposition in the body, especially in the liver. As of 2018, cage systems are the dominant method of egg production in all major egg-producing countries/regions except the EU. Several metabolic disorders, including FLHS, are associated with cage systems.

Stress Response

Many stress factors, such as change of feed and feeders, change of flock, shock, injection of vaccine, hunger and lack of water, will cause the body to produce glucocorticoids, which will stimulate gluconeogenesis and promote the formation of fat, so that fat production in the body is increased.

High Temperature

FLHS in laying hens occurs mostly during the peak laying period in summer and less frequently in winter. Because laying hens have low energy requirements under high temperature, excess energy is easily converted into fat and stored in the body. In addition, high temperature can accelerate the synthesis of fatty acids, resulting in a large accumulation of fatty acids in the liver, which enhances the formation of fat. High temperature also reduces the function of the thyroid gland, resulting in reduced secretion of thyroid hormones and weakened lipolysis, which also contributes to the occurrence of FLHS. High temperature itself is a kind of heat stress, which can also contribute to this disease from the heat stress pathway.

Hormonal Factors

Estrogen, thyroid hormone, cortisol, etc., can affect the development of FLHS by altering lipid metabolism. Most researchers have shown that the estrogen level of FLHS laying hens is significantly higher than that of normal laying hens. On the one hand, estrogen stimulates fat production in the liver of laying hens and estrogen reduces the oxidative capacity of fatty acids in the mitochondria of liver. Choi et al. (2012) showed that exogenous estrogen could induce fat accumulation in liver of male chicks and the average liver weight of estrogen group was significantly higher than that of normal group, and serum TC and TG contents were significantly increased. In addition, serum thyroid hormone levels in FLHS hens are significantly lower than in normal hens. It is generally believed that thyroid hormone reduces body fat deposition by increasing basal metabolic rate and promoting lipolysis. Therefore, the decrease in thyroid hormone levels could also contribute to FLHS (Zhu et al. 2021). Environmental endocrine disrupting chemicals (EDCs) seriously threaten the health of chickens. Bisphenol A (BPA) is classified as an EDC. BPA can act as a selective estrogen receptor modulator (SERM). Exposure to BPA may influence de novo fatty acid synthesis through the increased expression of lipogenic genes, thereby contributing to hepatic steatosis (Marmugi et al. 2012). In addition, BPA could inhibit the insulin signaling and impair the liver insulin sensitivity (Batista et al. 2012). Gao et al. (2021) proved that BPA could aggravate high-energy and low-protein-induced FLHS of laying hens by promoting fatty acid synthesis and inhibiting fatty acid β-oxidation.

Glucocorticoid (GC) excess is another common feature of fatty liver, and clinical studies indicated that the chronically elevated GC level is associated with the occurrence of fatty liver (Targher et al. 2006). But corticosterone (CORT) is the main active form of GC in chickens, and CORT has been regarded as the valid indicator of stress. Researchers found that excessive CORT administration caused liver fatty degeneration, increased abdominal fat, which indicated the classic symptoms of fatty liver bleeding syndrome (Jiang et al. 2008). Further Hu et al. (2017) used exogenous injected CORT and successfully replicated the model of fatty liver hemorrhagic syndrome, which is manifested with mitochondrial dysfunction, lipid peroxidation and inflammation, thereby increasing the susceptibility of liver to more severe damages (Tang et al. 2013; Hu et al. 2017). Glucocorticoid receptor (GR) is the main receptor to accept the signal of corticosterone, and the actions

of GC are primarily mediated by GR (Hollenberg et al. 1985). GR is up-regulated in the liver of FLHS chickens, which indicated that GR is associated with FLHS (Hu et al. 2018; Hu et al. 2020). Corticosterone-induced FLHS could lead to demethylation of N6-methyladenosine (m6A) and post-transcriptional activation of lipogenic genes, which provides new sight in the molecular mechanism of FLHS pathogenesis in the chicken (Feng et al. 2021).

Oxidative Stress Factor

The prolonged accumulation of lipids in the liver results in the formation of lipid peroxides by peroxidation, which generates a large number of free radicals. Free radicals can act on liver membrane and organelle membrane, and also directly oxidize intracellular macromolecules, damaging cell function and integrity. Xing et al. (2020) found that the liver MDA (malondialdehyde) content of laying hens with FLHS was significantly increased and SOD (superoxide dismutase) and GSH-Px (glutathione peroxidase) were significantly decreased, indicating that the occurrence of FLHS was indeed related to oxidative stress. In addition, oxidative stress in the liver can cause endothelial dysfunction and alterations in coagulation and fibrinolysis, leading to hemorrhage in the liver.

Intestinal Flora Factor

The pathogenesis of fatty liver has developed from the previous theory of "double click" to the theory of "multiple strikes" (Tilg and Moschen 2010). The "multiple strikes" hypothesis is that multiple factors act on genetically predisposed individuals together, leading to the occurrence of fatty liver. In "multiple strikes", more and more experiments have shown that the change of the enteric-liver axis is related to the occurrence of fatty liver, and intestinal microbes have been confirmed as a key factor in the enteric-liver circulation, so the intestinal flora has a great relationship with the formation of fatty liver (Federico et al. 2016). Probiotics have been proposed to improve intestinal flora as a way to prevent and treat fatty liver (Guo et al. 2021).

Molecular Mechanisms

Although some research studies have been conducted on the pathogenesis of FLHS from various perspectives, there are still many challenges in understanding the pathogenesis of the disease, which affects the understanding and prevention of the disease. However, non-alcoholic fatty liver disease (NAFLD) in humans, which has similar pathological changes and clinical diagnosis to FLHS, may provide an insight into the pathogenesis of FLHS (García-Fuentes et al. 2002). According to the pathological histological description, NAFLD ranges from non-alcoholic fatty liver (NAFL), which is pure steatosis with fatty infiltration but no signs of hepatocyte damage, to non-alcoholic steato-hepatitis (NASH), which is pure steatosis with fatty infiltration but no signs of hepatocyte. The former is simple steatosis with fatty infiltration but no symptoms of hepatocellular damage, while the latter has symptoms of inflammation and swelling with or without liver fibrosis (Chalasani et al. 2018).

The seminal view of the pathogenesis of NASH is the "second strike" theory, which states that lipid peroxidation and inflammatory responses are caused by oxidative stress following steatosis (Day and James 1998). Later, researchers have found that there are layers subject to complex and "multiple strikes" in this process, including genetic susceptibility, biological environment, behavioral factors, metabolism, and gut microbiota (Buzzetti et al. 2016; Eslam et al. 2018). Current research studies on NAFLD involve the interaction of multiple cell types in the liver, where lipotoxic intermediates, reactive oxygen species, endotoxins and adipokines can drive the aggregation and signaling of immune cells (including Kupffer cells) and the activation of hepatic stellate cells, which in turn form fibroblasts, produce fibrogenic factors and collagen, and drive the development of cirrhosis through apoptosis (Schuppan et al. 2018). Thus, when the ability of the liver to process primary metabolic energy substrates (carbohydrates and fatty acids) is diminished, chronic oxidative metabolism is observed to enhance the production of reactive oxygen species, creating a pro-oxidant state, and this overall increase in the pro-oxidant/pro-inflammatory state leads to intracellular damage, with hepatocellular injury characterized by endoplasmic reticulum stress, apoptotic signaling pathways, and dysfunctional unfolded protein responses, which subsequently predispose to cirrhosis and hepatocellular carcinoma (Neuschwander-Tetri 2010, Friedman et al. 2018).

It is worth noting that the occurrence of fatty liver hemorrhage syndrome in laying hens is also closely related to insulin resistance. Insulin resistance is a defective metabolic response of target cells (such as muscle cells, hepatocytes and adipocytes) or the whole organism to hormonal influences. Insulin signaling begins with the binding of insulin to the insulin receptor (IR). Then, insulin receptor substrates (Insulin 3 receptor substrates, IRSs) undergo phosphorylation, which subsequently triggers the recruitment of phosphatidylinositol 3-kinase (PI3K) and the activation of protein kinase B (AKT) activation. Systemic insulin resistance means that the ability of insulin to lower blood glucose concentrations to appropriate levels is also hampered by disruption of the Glucose transporter 4 (GLUT4) receptor on the surface of the myocyte membrane, resulting in reduced glucose uptake (Wolford and Polin 1974). Among insulin receptors, Insulin receptor substrate 2 (IRS-2), upon activation, acts as a regulator of SREBP-1 and affects DNL (Luedde et al. 2014). In insulin resistance, IRS-2 is downregulated, SREBP-1 is overexpressed, DNL is upregulated, and β-oxidation of fatty acids is inhibited, thus further promoting hepatic lipid accumulation (Wree et al. 2016). Insulin resistance is a major feature of FLHS and is essential for lipotoxicity, oxidative stress and activation of the inflammatory cascade (Del Campo et al. 2018). Excess FFA in the body causes mitochondrial β oxidation, resulting in mitochondrial dysfunction and oxidative stress, which can inhibit insulin signaling and promote the release of inflammatory cytokines by activating NF-κB (Yang et al. 2019).

In addition, the main product of hepatic de novo lipogenesis is triglycerides and the liver is the main site of cholesterol and phospholipid synthesis (Piotrowska et al. 2011). It has been shown that β-estradiol-17-dipropionate can significantly induce hypercholesterolemia and hypertriglyceridemia. Once exogenous and endogenous lipids are secreted from the liver into the blood, they are transported to the ovaries as a component of lipoproteins. Fatty liver is usual for laying hens and occurs when increased lipogenesis exceeds the ability to synthesize and secrete lipoproteins (Shini et al. 2020).

Cidea and Cidec are effector factors inducing cell death. Peng et al. (2019) showed that FLHS significantly increased the

expression levels of Cidea and Cidec mRNA in liver and adipose tissue, and both could be lipid droplet-associated proteins that play an important role in promoting hepatic lipid accumulation and steatosis. In addition, high expression of Cidea and Cidec promoted lipid accumulation in mouse liver, but silencing Cidea and Cidec reduced hepatic lipids. In addition, AMPK pathway-related genes SERBP1a,1c could directly activate Cidea and increase the expression of Cidea mRNA, and Cidea can promote the expression of ACC and FAS, which are related to lipid synthesis.

Gao et al. (2019) study on AMPK signaling pathway of laying hens showed that in liver of FLHS laying hens, the mRNA expression levels of lipid synthesis-related genes ACC, FAS, GPAT and cholesterol synthesis-related genes HMGR and HNF4α were significantly increased, while the mRNA expression levels of fatty acid oxidation-related genes CPT1 were significantly decreased. It can be concluded that the changes in the expression levels of each gene in AMPK signaling pathway are involved in the occurrence of FLHS in laying hens. Therefore, AMPK signaling pathway plays an important role in the formation of FLHS in laying hens.

Diagnostics

Clinical Symptoms

When the chickens are in the peak egg-laying period, they appear depressed, lying down, drowsy and sometimes stand unsteadily. Laying hens with FLHS showed obvious pale and swollen crests and fleshy whiskers, sagging abdomens, and decreased egg production, which could not reach the peak of egg production. Laying hens often die suddenly without obvious symptoms, with high body weights of dead hens (Zhang et al. 2021).

Dissection and Histological Changes

Dead chickens were found to have increased subcutaneous fat. It was found that the entire abdominal cavity was deposited with a large amount of yellowish fat. The mesentery was also covered with more fat. Some chickens also formed thicker fat pad. The liver was grayish yellow and swollen, brittle and fragile, greasy and shiny in appearance. The surface was rich in oil-like droplets and accompanied by varying degrees of bleeder or plaque, and the abdominal cavity was accumulated with light red liquid, sometimes with blood clots next to the liver. When cut with a knife, the knife surface is attached with fatty oil droplets, the cut surface of the liver is raised, the liver lobule is filled with fat, blurred, and the structure disappears. The gall bladder appears enlarged and filled with bile (Tilg and Moschen 2010).

Microscopic observation showed a large number of fat vacuoles and obvious steatosis in the liver tissue. Fat droplets of varying sizes accumulate in the hepatocytes, and the nuclei are often crowded to the side of the cells by the fat droplets. The boundary between cells disappeared. The hepatic cord and sinusoidal structure disappeared, and the liver tissue was completely saturated with fat (Trott et al. 2014).

Nutritional Regulation

Adjustment of Energy-to-protein Ratios

The ratio of energy to protein in the diet of laying hens can be adjusted so that the ratio of protein to energy is 63 to 65:1. Too high or too low energy-to-protein ratios will cause FLHS in laying hens. For laying hens whose body weight exceeds the standard, the objective of preventing FLHS can be achieved by restricting feeding or reducing dietary energy (Wang et al. 2020).

Addition of Choline, Soy Phospholipids, Methionine, Carnitine, Biotin, VB12, Selenium and Calcium

Choline is an important substance in the body of laying hens. It is the precursor for the formation of lecithin. Lecithin is an essential raw material for lipoprotein synthesis, and sufficient choline helps to generate lecithin, which in turn synthesizes lipoproteins to help lipids transportation. Research studies have found that lecithin can significantly up-regulate the expression of the apolipoprotein gene apoB100 in the liver, increase the level of VLDL, improve the capacity of liver fat transport and reduce the occurrence of FLHS. Lecithin can also control appetite and prevent overeating. In addition, supplementation of soy phospholipids in the diet can effectively improve liver function and abnormal metabolism of blood lipids, and prevent the occurrence of FLHS (Yang et al. 2017).

Carnitine plays an important role in regulating lipid metabolism. It can help long-chain fatty acids to enter the mitochondria and promote the oxidation of fatty acids. In addition, the daily average egg production rate of the layers provided with biotin was significantly increased, and the liver fat rate and abdominal fat rate of the layers were significantly reduced. It may be that biotin improves the performance of laying eggs, so that the fat in the liver of laying hens can be continuously transported to the ovaries, reducing the deposition of liver fat.

Selenium has a protective effect on vascular endothelium. Adding a certain amount of selenium to the diet can significantly reduce liver bleeding in laying hens. When the hens are in the peak egg production period, enough calcium should be added to the feed to meet the body' needs of the laying hens and ensure normal egg production.

Change of Energy and Protein Sources

In the supply of layer diets, replacing corn with wheat and barley of equal energy, and replacing soybean meal with fish meal and yeast meal can reduce the occurrence of FLHS to a certain extent. Most researchers believe that when corn is used as an energy source for laying hens, the liver fat content and hemorrhage are much higher than that for laying hens which use wheat or barley as energy sources; when fish meal, yeast powder, etc. are used as protein sources, the occurrence of FLSH in laying hens is significantly lower than that of laying hens which use soybeans as a source of protein (Cross et al. 1987).

Addition of Vegetable Oil

Vegetable oil contains a lot of unsaturated fatty acids (PUFA), and PUFA has the effect of regulating lipid metabolism. Studies have shown that n-3 PUFA can significantly reduce liver fat (Peng et al. 2019). In addition, adding sunflower oil and linseed oil to the diet can significantly reduce the fat content of the liver of laying hens and inhibit the occurrence of FLHS. Davis et al. (2016) also found that adding flaxseed to the diet can inhibit the development of fatty liver to a certain extent and reduce steatosis.

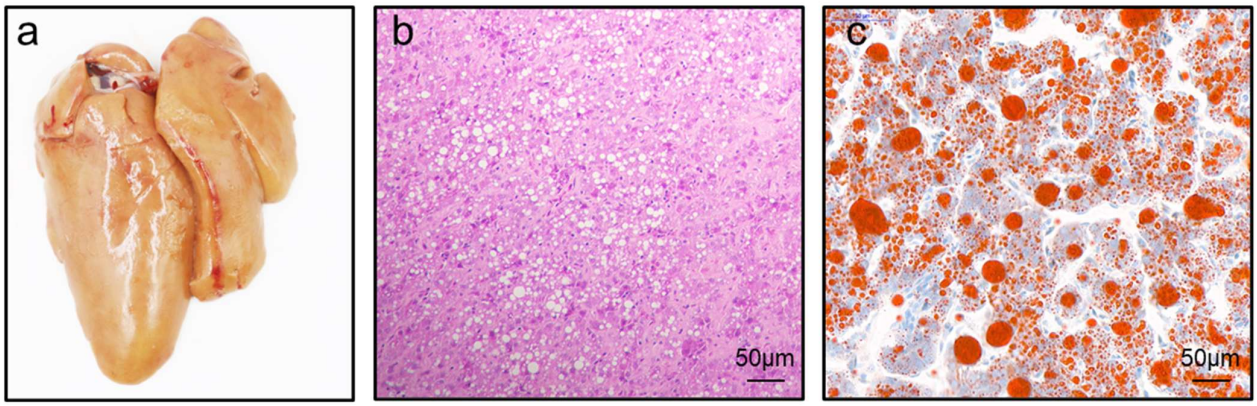

Fig. 1: Observation of liver tissue and sections of fatty liver in laying hens. (a) Morphological observation of liver tissue of fatty liver in laying hens. (b) Representative images of HE staining of fatty liver in laying hens. (c) Representative images of oil red O staining of fatty liver in laying hens

Prevention of Feed Mildew

Moldy feed contains a lot of harmful substances, among which mycotoxins can cause lipid metabolism disorders in the liver by damaging liver cells, leading to FLHS in laying hens. Therefore, during the feeding process, attention should be paid to moisture prevention and feed mildew, and dry and fresh diets for laying hens should be provided.

Addition of Antioxidants

The fat accumulated in the liver for a long time will undergo a peroxidation reaction to produce a large number of free radicals, which can cause damage to liver cells through oxidative stress. Therefore, adding antioxidants such as VC, VE, betaine, pentoxifylline (PTX), selenium, and dihydropyridine to the diet can effectively reduce the oxidative stress of liver cells, thereby reducing the occurrence of fatty liver (Diaz et al. 1994).

Addition of Probiotics, Metformin, Chitosan, Eucommia, Resveratrol, Flaxseed, etc.

Many studies have shown that intestinal microbes play an important role in the formation of fatty liver, and the different stages of fatty liver and fibrosis are closely related to the intestinal microflora (Yang et al. 2017; Zhu et al. 2021). Therefore, probiotics can be added to the diet to improve the intestinal flora and prevent the occurrence of FLHS. In addition, metformin can reduce lipid deposition in the liver. It can increase the activity of liver ATGL (lipase) and activate the AMPK signaling pathway. The activation of the AMPK pathway inhibits the expression of fat synthesis-related genes ACC and FAS (Sun et al. 2021).

Early studies have found that adding chitosan to feed can significantly reduce the triglyceride content of the liver of mice, as well as the fatty degeneration of the liver. Adding 10-20g/L of eucommia leaf decoction to drinking water can significantly reduce the fat content of the liver of laying hens. Xing et al. (2020) found that resveratrol could significantly reduce fatty liver lesions in laying hens, so resveratrol can be tried to control FLHS in laying hens. Schumann et al. (2003) showed that consumption of flaxseed can reduce the content of TC and TG in serum and liver lipid rate, reduce the mortality of laying hens, and have a better therapeutic effect on FLHS of laying hens.

Betaine a methyl donor, as an important component of the methionine cycle, could also effectively prevent FLHS of chickens. Betaine could be used as a methyl donor to relieve FLHS induced by corticosterone or high-energy and low-protein diet, effectively reduce abdominal fat and inhibit liver steatosis, reduce fat synthesis, and promote the β oxidation of fatty acids in liver (Leng et al. 2016; Omer et al. 2020).

In addition, daily management in the feeding process can be strengthened to ensure a peaceful and clean environment in the chicken house. Disinfect regularly and maintain good ventilation to avoid the accumulation of toxic gases in the house. Try to grasp the feeding density to ensure that each chicken has enough space for exercise. When it is hot in summer, pay attention to the cooling treatment in the house to reduce the occurrence of heat stress. At the same time, avoid choosing susceptible breeds when selecting breeds, check the flock regularly and cull those chickens that exceed 15-20% of their normal body mass, and check the diet from time to time to prevent chickens from accidentally eating moldy and toxic feed, which can increase the risk of FLHS. These are also key measures to reduce the risk of FLHS in chickens (Gao et al. 2021; Meng et al. 2021).

In the following Fig. 2, the authors summarize the nutritional regulation for the prevention and control of FLHS, with a view to providing reference for the prevention and control in production practice.

Conclusion

Investigation of the formation mechanism of fatty liver in laying hens can provide a fundamental solution to the problem. Most researchers believe that nutritional factors are the direct cause of FLHS in laying hens. The lack or imbalance in nutrients makes the lipid metabolism of laying hens unbalanced. The production of fat in the liver is more than the output, which continuously accumulates to form fatty liver. Other factors such as genetics and environment have accelerated or weakened the formation of FLHS to a certain extent. In recent years, many domestic and foreign scholars have found that AMPK signaling pathway is closely related to the occurrence of fatty liver. In the laying hens with FLHS, the gene expression in AMPK signaling pathway has changed. For example, carbohydrates can significantly increase the expression of fat synthesis-related genes. The expression of ACC and FAS can induce lipid deposition, so many factors may affect the AMPK signal

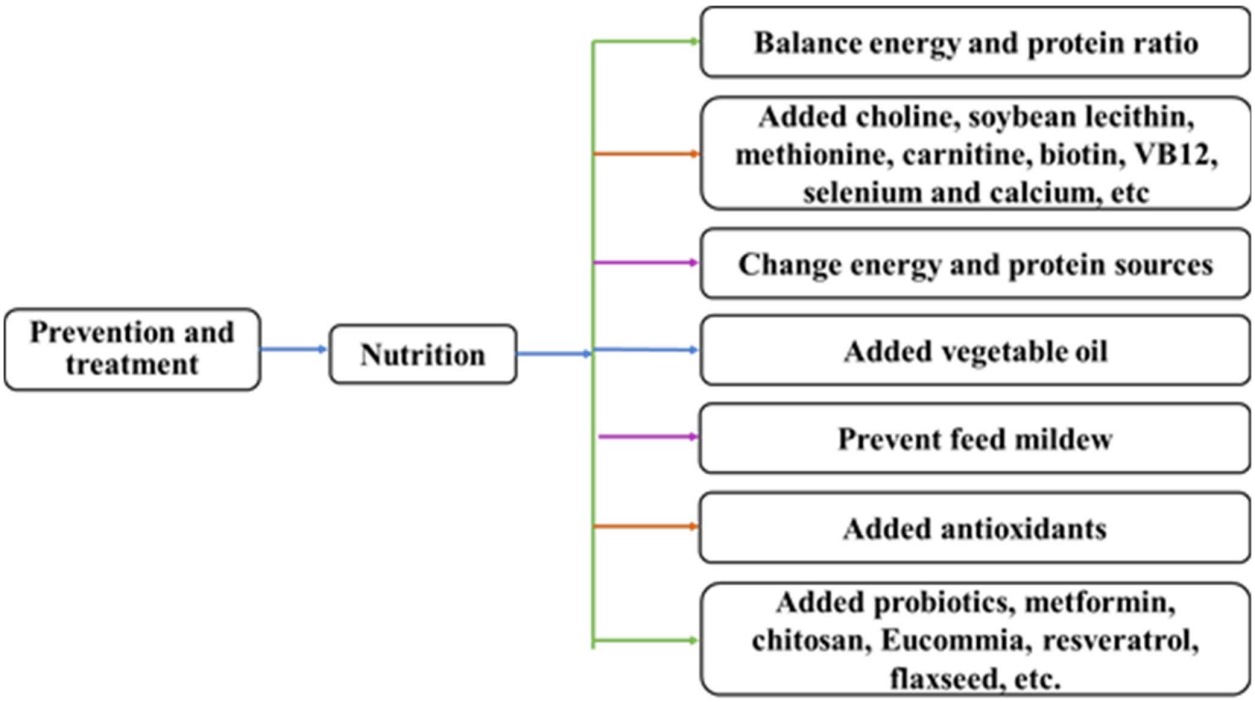

Fig. 2: Nutritional regulations to prevent and treat FLHS

pathway to cause the occurrence of FLHS. To prevent and treat FLHS, we can start with the AMPK signal pathway, and inhibit the occurrence of the disease from the molecular mechanism by activating the AMPK signal pathway. In addition, the relationship between AMPK signaling pathway and the occurrence of fatty liver still needs to be further studied.

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