



## Review

## Hepatic lipidosis in fattening turkeys: A review

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E-mail: [amrwahab5@mans.edu.eg](mailto:amrwahab5@mans.edu.eg)**Abstract**

The conditions on turkey fattening farms, including management, housing, and feeding, have been constantly improved recently in favor of animal health. Many studies deal scientifically with poultry health. However, specifically concerning liver health, there are still open questions regarding the influence of dietary factors on the metabolism and function of the liver. Consideration of the factors that could influence and alter liver metabolism is therefore of critical relevance. The liver, as a major metabolic organ, is the main site of fat synthesis in turkeys. Under certain conditions, fat can excessively accumulate in the liver and adversely affect the birds' health. The so-called hepatic lipidosis (HL) in fattening turkeys has been known for years. This disease has unacceptable economic and animal welfare impacts, with high animal losses up to 15% within only a few days. To date, little is known about the causes and the metabolic changes in fattening turkeys leading to HL despite the increasing focus on health management and animal welfare. To understand what is different in turkeys compared to other species, it is necessary to discuss the metabolism of the liver in more detail, including HL-associated gross and microscopic lesions. In the current review, aspects of liver structure and lipid metabolism with special regard to lipogenesis are explained to discuss all dietary factors attributing to the development and prevention of HL. As part of the prevention of the HL, dietetics measures can be helpful in the future.

**Keywords:** Turkeys, Hepatic lipidosis, Metabolism, Fatty Liver, Pathogenesis

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**Introduction**

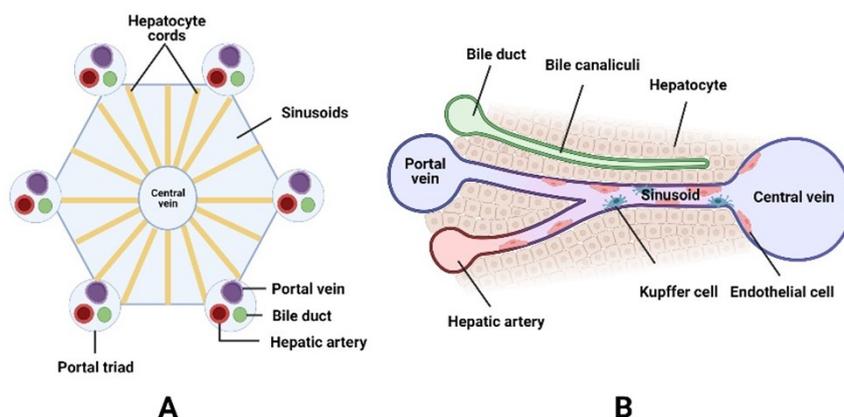
The liver, as a main metabolic organ, plays a major role in digestion and metabolism, regulating different processes such as production, storage, and release of lipids, carbohydrates, and proteins (Denbow, 2000; Zaefarian et al., 2019). The liver is a multipurpose organ, producing different types of proteins, such as blood proteins, enzymes, hormones, clotting, and immune factors. Thus it is considered as an endocrine and exocrine gland (Akers and Denbow, 2013; Hünigen et al., 2016; Wu et al., 2021). To keep a healthy bird, the liver should be kept in a superb condition (Butler, 1976; Aziz, 2008). Therefore, it is essential to better understand metabolic functions and factors that can cause disruptions in the liver to maintain healthy birds (Reavill, 2005; Grunkemeyer, 2010).

The liver is often adversely affected in highly productive animals, particularly in domestic chickens and

turkeys reared under modern farming practices (Her-mier, 1997; Julian, 2005; Claire D'Andre et al., 2013; Crespo and Shivaprasad, 2013). Hepatic lipidosis (HL), also called fatty liver and hepatic steatosis, can affect meat-type turkeys and turkey breeder hens (Gazdzinski et al., 1994; Aziz, 2008; Popp et al., 2014). The current review discusses avian liver anatomy and metabolism in terms of possible importance for disease development. Additionally, metabolic changes, gross and microscopic lesions in the affected livers related to HL will be considered. Afterward, the potential predisposing factors and/or causes that can lead to HL in fattening turkeys are of particular interest.

**Normal liver****Structure**

The liver in birds, the major metabolic gland of the digestive system, is cranially located between the lung



**Figure 1: Liver structure: (A) Functional unit of the liver lobules; (B) Hepatocytes and Kupffer cells (Adapted from Gordillo et al. (2015) and created with BioRender, [www.biorender.com](http://www.biorender.com))**

and heart, and caudally in the gastrointestinal tract (Hünigen et al., 2016). It is divided into two lobes, namely right and left, joined cranially at the midline (Whittow, 1999; Caceci, 2006). The right lobe is larger and separated from the left lobe by a deep fissure. In the domestic fowl and turkey, the left lobe is subdivided into the dorsal and ventral parts (Lucas and Denington, 1956; Denbow, 2000). Lobules are considered the liver's functional units, and each lobe of the liver has about 100,000 lobules (Whittow, 1999; Kmiec, 2001; Maher, 2019). The liver lobule is formed by parenchymal cells (hepatocytes) and non-parenchymal cells (Whittow, 1999; Kmiec, 2001; Caceci, 2006). Hepatocytes take up almost 80% of the total liver size, including many mitochondria, lysosomes, Golgi apparatus, and thus are responsible for many liver functions in poultry (Turk, 1982; Kmiec, 2001; Caceci, 2006).

Generally, non-parenchymal liver cells are located in the sinusoidal wall (Kmiec, 2001; Gunasegaran, 2014). Sinusoids, in general, allow large plasma proteins from the bloodstream to enter the spaces surrounding the hepatocytes (Wisse et al., 1999; Kmiec, 2001). Generally, Kupffer cells or macrophages can phagocytize pathogens, cell debris, and damaged red or white blood cells (Benacerraf, 1964; Kmiec, 2001; Korach-André, 2020). There is a portal triad at each corner of the hexagonal lobule consisting of a branch of the hepatic artery and portal vein as well as the bile duct (Whittow, 1999; Akers and Denbow, 2013). The liver receives oxygenated blood from the hepatic artery and deoxygenated blood from the hepatic portal vein, which contains nutrients, drugs and toxins from the digestive tract of poultry (Whittow, 1999; Caceci, 2006; Akers and Denbow, 2013). In poultry some toxins could be removed via hepatocytes in the liver sinusoids (Zaefarian et al., 2019). Moreover, some metabolites from hepatocytes are secreted into the blood when the blood passes through sinusoid, this blood then passes to the central vein and thereafter into the hepatic vein (Figure 1).

### Function

It is well known that the liver is a unique organ that is involved in different metabolic functions of fat, carbohydrate, protein, vitamins, and minerals (Hermier, 1997; Tanchaonrat et al., 2014). Moreover, liver is the principal storage site of fat-soluble vitamins and some minerals (iron and copper), and is involved also in the activation of vitamin D (Denbow, 2000; Akers and Denbow, 2013). Furthermore, fatty acids released from the liver adipose tissue are oxidized to generate acetyl-CoA and adenosine triphosphate (ATP) that are used as a primary source of energy (Aziz, 2008).

In poultry, some toxic substances from feed and toxins produced in the body are detoxified via the liver, a major detoxification organ (Zaefarian et al., 2019). Some of these toxins include various fat-soluble substances, metabolic end-products (e.g. ammonia, products of blood cell destruction, bile pigments), contaminants (e.g. pesticides, carcinogens), anti-nutrients (e.g. hydrocyanic acid), chemicals (e.g. heavy metals), additives (e.g. antibiotics) and drugs (e.g. various medications) (Akers and Denbow, 2013). The liver turns these toxins into water-soluble waste products, which are then excreted via the kidney in a process named 'detoxification' (oxidation, reduction, hydrolysis, conjugation). Moreover, Kupffer cells in the liver possess antibacterial properties that destroy microorganisms (Akers and Denbow, 2013).

### Body fat

To a large extent, body fat acts as an energy reserve; therefore, it is the most variable among the major body constituents (Griminger, 1986). It has to be noted that the ability of birds to store triglycerides as an energy reserve exceeds that of other classes of vertebrates (Blem, 1976). The fatty acids of these triglycerides are predominantly of the C16 and C18 variety, which are, as a general rule, more saturated than those of mammals (Griminger, 1986). Generally, following feeding, lipids are mostly stored in existing adipocyte vacuoles rather

than in newly formed cells. Even following prolonged starvation, body fat is never completely depleted and is not likely to drop below 4% in order to protect the integrity of tissues and organs (Griminger, 1986).

### Sources and absorption of fat

The diets commonly used for birds contain only a small percentage of fat, and the lipids present in the bird's body are derived mainly from carbohydrates and to a slight degree from proteins (Butler, 1976; Griminger, 1986; Cherian et al., 2002). Dietary lipids are absorbed mainly from the small intestine. The lipases in the small intestine hydrolyze triglycerides to diglycerides, monoglycerides, fatty acids, and glycerol (Butler, 1976; Griminger, 1986). After this partial hydrolysis in the intestinal lumen, they form micelles with bile salts and pass into the mucosal cells where re-synthesis occurs (Butler, 1976). Thereafter, the major portion enters the systemic circulation directly as 'portomicrons' like in poultry rather than via the lymphatic system as 'chylomicrons' like in mammals (Noyan et al., 1964; Bensadoun and Rothfeld, 1972).

### Lipid synthesis

The main site of de novo fatty acid synthesis (lipogenesis) in poultry is the liver, which is very limited in the adipose tissue. Fats synthesized in the liver are derived from three main sources: dietary fat, depot fat, and fat from de novo fatty acid synthesis (from feed carbohydrates) (Griffin et al., 1992; Hermier, 1997; Scanes and Braun, 2013). The activity of the glycolytic system and the dietary carbohydrate content determined in a wide degree the amount of fat synthesized (Butler, 1976). According to Hillard et al. (1980), the decrease of chicken liver lipogenesis is due to the reduction of carbohydrates rather than the increase in fat. In turkey poults (three-week-old), a decrease in lipogenesis in response to an increase in dietary protein was also noted in the liver. In contrast, dietary carbohydrates had the opposite effect (Rosebrough et al., 1982). Moreover, it was indicated that the chicken skeleton was an important site of lipogenesis. However, bones had one-tenth to one-third of the lipogenic activity of the liver (i.e. bone marrow constituted about two-thirds of hepatic activity in chicks) (Nir and Lin, 1982).

The two major enzyme systems involved in fatty acid synthesis are acetyl-CoA carboxylase and the multi-enzyme system fatty acid synthase (Griminger, 1986). Acetyl-CoA carboxylase, which catalyzes the formation of malonyl-CoA, is believed to be the most important rate-limiting enzyme in cytoplasmic saturated fatty acid synthesis (Goodridge, 1973; Butler, 1976). Thus, at this point in the pathway, fatty acid synthesis is regulated by the intake and metabolism of carbohydrates and lipids (Figure 2) through feed-forward and feed-back mechanisms, respectively (Butler, 1976).

The acetyl-CoA may come from oxidative decarboxylation of pyruvate, glycolysis, and the breakdown of ingested or previously synthesized fatty acids and from catabolism of certain amino acids (Griminger,

1986). The non-essential unsaturated fatty acids such as oleic and palmitoleic acids are formed from the corresponding saturated fatty acids by the effect of desaturation enzyme systems (Johnson et al., 1969). Glycerides, phospholipids and cholesterol esters are produced from the CoA derivatives of fatty acids. It is worthy to mention that all fatty acid synthesis is performed in the liver of poultry when considering the susceptibility of the poultry to hepatic steatosis (Goodridge, 1968; O'hea and Leveille, 1969; Leveille et al., 1975; Visscher et al., 2017) and scarce in adipose tissue, whereas in mammals, the latter makes a major contribution. Nir and Lin (1982) stated that estimates of lipogenesis in various tissues in chicks were 45, 23, and 6% of total incorporation for liver, bones, and skin/small intestine, respectively; however, incorporation by muscles, adipose tissue, and kidneys were negligible.

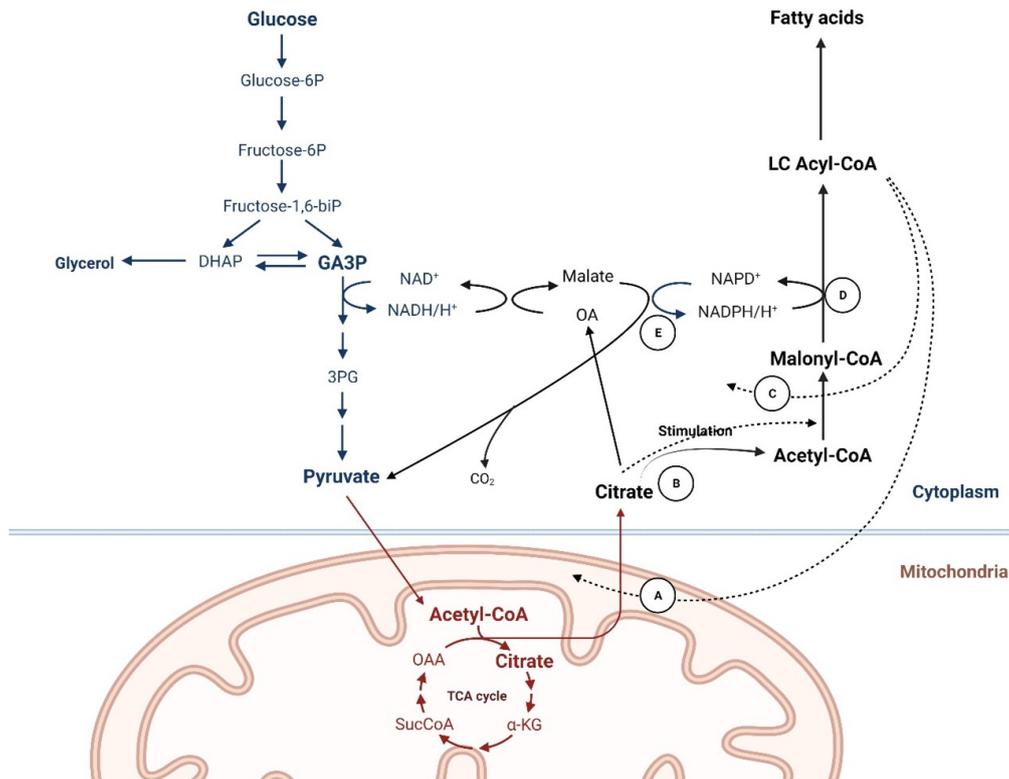
### Dietary fat and body fat

Fats and oils are used in poultry diet formulations to enhance the palatability of diets, the absorption of fat-soluble vitamins, and regulate the passage rate of the digesta in the gastrointestinal tract. They are also the most concentrated sources of energy (Baião and Lara, 2005). Since tissue lipids are derived from lipogenesis and dietary lipids, the nature of the fat consumed will influence the composition of the lipids deposited in various tissues. The triglycerides in each tissue and the compound lipids have a specific fatty acid constitution. Therefore, when lipogenesis is the origin of tissue triglyceride, it corresponds to the composition of specific fatty acids of the tissue in which it is deposited. When significant amounts of fat are consumed, however, the fatty acids of the ingested lipids change the fatty acid constitution of the tissue lipids to varying degrees (Griminger, 1986).

Generally, because of the dynamic nature of adipose tissue, changes occur continuously so that the fatty acid composition of tissue may ultimately return to its constant composition (Griminger, 1986). Although adipose tissue has little capacity for fatty acid synthesis in the fowl, it does have a reasonable capacity for incorporating the acids into glycerides and for generating the glycerol portion. Furthermore, it forms a major storage depot. Lipids are transported to it from the liver as lipoproteins and are released from the protein and hydrolyzed by clearing factor lipase, present in the capillary walls of the adipose tissue. They are then synthesized in adipocytes and stored until they are hydrolyzed and released in response to stress or reduction in the nutritional status (Butler, 1976).

### Overview of lipid metabolism

In poultry, lipids are represented mainly by triacylglycerol and are firstly synthesized in hepatocytes and then stored in adipocytes (Nematbakhsh et al., 2021). The lipid metabolism in chickens is different from that of mammals regarding the transportation of dietary lipids to the liver, hepatic lipogenesis, and the unique lipoproteins in the blood. It has been evaluated that the main proportion (70%) of fatty acid synthesis in



**Figure 2: Synthesis of saturated fatty acids from carbohydrate (Butler, 1976).** (A) citrate translocation system, (B) ATP-citrate lyase, (C) acetyl-CoA carboxylase, (D) fatty acid synthetase, (E) malic enzyme (Figure was adapted from “Warburg Effect” and created with BioRender, <https://app.biorender.com/biorender-templates>)

chickens occurs in the hepatocytes through lipogenesis (Figure 2), and only 5% happens in the adipose tissue. However, the diet provided the remaining (25%) of fatty acids (Alvarenga et al., 2011). The consumed dietary fats, mostly triglycerides and neutral fats, are digested in the intestinal tract through emulsification by the bile and then by pancreatic enzymatic digestion. The major products of lipid digestion are fatty acids and monoglycerides, which enter the intestinal epithelial cells where they are used to synthesize triglycerides in turkeys (Aziz, 2008).

Nonetheless, in birds, fatty acids in the diet are drained directly into the portal blood system (instead of the lymphatic system) as very low-density lipoproteins (VLDL), which are termed ‘portomicrons’ because of the poorly developed intestinal lymphatic system (Alvarenga et al., 2011). The portomicrons are structures similar to chylomicrons in mammals. They contain triglycerides (90%) combined with free and phospholipids, esterified cholesterol and lipoprotein in poultry (Bensadoun and Rothfeld, 1972; Griffin and Whitehead, 1985; Hermier, 1997). From the portal blood system and before reaching the rest of the circulation, most of the portomicrons pass through the liver. This unique feature led to accumulation of fat in the liver in laying hens (Cherian et al., 2002). Hermier (1997) found that because of high oestrogen secretion in laying hens producing eggs, the lipogenesis in the liver is high and/or active. Lin et al. (2021) suggested

that estradiol contributes to lipid synthesis in the livers of laying hens.

Liver hepatocytes in chickens can store triglycerides from portomicrons or store the released energy in tissues as fat deposits, metabolize fatty acids to ATP, synthesize phospholipids and lipoproteins (Scott et al., 1982). Therefore, the capacity of hepatic lipogenesis and VLDL secretion is a determining factor of the degree of fattening among avian species (Hermier, 1997). Regarding turkeys, previous studies based on metabolic comparisons with chickens clearly indicated that its specific leanness was associated with a lower capacity of hepatic lipogenesis and VLDL secretion (Kouba et al., 1995). In birds, apart from portomicrons which transport lipids from the gastrointestinal tract to the liver via the portal circulation, VLDL, intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) are the four classes of lipoprotein particles that are synthesized and secreted by the liver in poultry. The density is an important aspect to classify lipoproteins, which varies depending on the proteins they contain and the proportion of lipids (Alvarenga et al., 2011).

As mentioned above, VLDLs are synthesized in the liver and released into the bloodstream, transporting lipids to other tissues. IDLs are the residues of VLDL following their metabolism by other tissues, while LDLs originate from the metabolism of VLDL

and IDL after lipolysis by both lipoprotein lipase and hepatic lipase. HDLs primarily originate in the liver of poultry and presumably also in the intestine (Zaefarian et al., 2019). It has to be underlined that growing turkeys differ from other avian species in their lipid metabolism and hepatic triglyceride synthesis because contrary to mammals and chickens, feeding n-3 polyunsaturated fatty acids (PUFA) does not decrease their hepatic triglyceride synthesis and secretion. In turkeys, n-3 polyunsaturated fatty acids appear to influence HDL metabolism, causing a reduction in muscle growth (Kouba et al., 1995; Mossab et al., 2002). Thus, all efforts towards changing metabolic processes involving lipids may be of fundamental importance to the productive poultry chain in general.

Additionally, in chickens, several hormones such as insulin, and estrogen as well as key enzymes associated with lipid metabolism play an important role in lipid metabolism regulation (Alvarenga et al., 2011) (Figure 1). For example, malate dehydrogenase and fatty acid synthase are the main enzymes involved in lipogenesis (Hermier, 1997). Moreover, adipose tissue development in chickens depends on management conditions, age, sex, species, and fat depots. Diversity in fat depots can affect economic and human health; therefore, it is noteworthy in animal production (Urrutia et al., 2018). In chickens, there is a balance among different sources of lipid deposition, including dietary absorbed fat, lipogenesis and fat catabolism (Liu et al., 2019).

### Metabolism of other nutrients in healthy liver

The liver, together with the pancreas, keeps a steady level of blood glucose. In high blood glucose concentrations, the liver stores glucose in the form of glycogen (glycogenesis) and triglycerides so that the energy can be reused when needed. In cases of immediate glucose needs, the liver can change certain amino acids, fats, and lactic acid to glucose in poultry (Akers and Denbow, 2013), whereas if the level of blood glucose decreases, the liver can breakdown glycogen to glucose (glycogenolysis) and liberates the glucose into the blood.

The liver is also involved in protein metabolism, as protein synthesis in the liver represents about 11% of all protein synthesis in poultry (Denbow, 2000). The dietary proteins are digested into amino acids in the intestine, transported to the liver, and then reach other tissues and organs via the bloodstream. In case of excess amino acids, they will be catabolized by the liver. The uric acid in the liver of poultry is formed by liberated ammonia; thus liver is considered the main organ responsible for uric acid formation (Akers and Denbow, 2013). In addition, hepatocytes in poultry synthesize carbohydrates and fat from certain amino acids and can synthesize various blood clotting proteins and immune proteins (Akers and Denbow, 2013; Zaefarian et al., 2019).

The liver is the metabolic site and storage of vitamin B1, B2, and niacin. Additionally, fat-soluble vitamins are stored in the liver in poultry (Akers and

Denbow, 2013). Generally, the synthesis of 1,25 dihydroxycholecalciferol from vitamin D3 can be done via liver, skin, and kidney (Holick et al., 1989). The metabolism in the liver is therefore extremely high in poultry (Nesheim and Ivy, 1970).

### Damaged liver: Hepatic lipidosis (HL)

Some hepatic disorders characterized by fatty change can affect avian species: e.g. fatty liver hemorrhagic syndrome and fatty liver and kidney syndrome are widely investigated hepatoses affecting chickens (Lee-son et al., 1995a; Hoerr, 1996; Crespo and Shivaprasad, 2003). On the contrary, in turkeys, there is one hepatopathy known as HL typically characterized by locally extensive areas of hepatocellular lipid accumulation, which has been poorly investigated by pathologists.

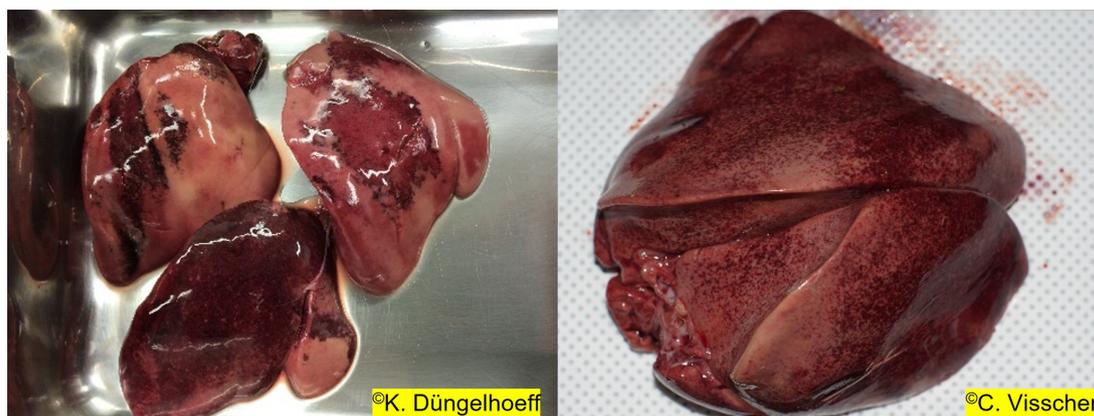
HL is a well-known disease in fattening and turkey breeder flocks (Popp et al., 2014). Usually, in fattening turkey flocks, the condition occurs suddenly at 12 to 16 weeks of age, while female birds aged between 10 and 14 weeks are particularly affected (Gazdzinski et al., 1994). However, HL has been reported only in turkey breeder hens between 12 and 24 weeks of age (Saif and Fadly, 2008). The disease remains for up to ten days, with a high mortality rate of up to 15% (Gazdzinski et al., 1994). However, on three farms with HL outbreaks in turkeys (13-14 wk old), the animal losses were at a common level (under 5% during rearing and fattening period) throughout the fattening period without exceptions reported (Saif and Fadly, 2008; Middendorf et al., 2019b).

### Gross lesions of HL

At disease onset, flocks usually appear hyperactive and nervous. Clinically conspicuous animals show dyspnea and cyanosis and are almost always unable to walk (Gazdzinski et al., 1994; Popp et al., 2014). Similarly, during the days HL occurred, individual animals in the affected turkey flocks (13-14 wk old) showed clinical symptoms such as apathy, dyspnea, and lethargy. The affected animals could not walk, with a highly increased respiratory rate (Middendorf et al., 2019b).

Furthermore, the liver as a major metabolic organ may offer valuable evidence concerning the health conditions of turkeys. The fact that liver lesions were detected at the processing plant makes it clear that the turkeys did not necessarily show any clinical signs of impaired liver function. There were only changes in the liver in most cases, while the rest of the carcass did not show any other lesions. According to Aziz (2008), birds that die of HL are in good body condition with much abdominal fat. The liver is typically enlarged and its surface is mottled due to multiple hemorrhagic foci, and some livers are irregularly shaped with pale areas that represent lipid accumulation (Aziz, 2008; Saif and Fadly, 2008). At necropsy, swollen livers with multiple focal hemorrhages and white or yellowish areas with fat accumulation could be observed (Figure 3) (Galazzi et al., 2007; Saif and Fadly, 2008).

Among other poultry species, fatty liver disease,



**Figure 3: Liver with hepatic lipidosis. Enlarged, mottled liver with large, pale, scattered, well-demarcated foci and dark red areas**

such as fatty liver syndrome and fatty liver hemorrhagic syndrome in laying hens, is well studied and distinguished from HL in turkeys. In the case of fatty liver syndrome in hens, the birds often show no clinical signs and the first sign is a decrease in egg production (Harms et al., 1982). In many cases, these birds die suddenly from liver rupture (Butler, 1976). In contrast, turkeys suffering from HL first attract attention due to their clinical signs (Gazdzinski et al., 1994). However, in some cases, only one out of 24 turkeys without clinical signs during the investigation of HL cases had macroscopically and chemically analyzed fatty liver, and eight out of 49 turkeys with clinical signs had no macroscopically and chemically analysed fatty liver (Middendorf et al., 2019b).

#### Histopathological lesions of HL

There are variations in the histopathological lesions in the affected livers. Former histopathological investigations reported vacuolation, hemorrhagic areas, and necrotic hepatocytes. Meanwhile, the reticular framework was maintained (Gazdzinski et al., 1994; Gallazzi et al., 2007; Saif and Fadly, 2008; Manarolla et al., 2011). Aziz (2008) and Visscher et al. (2017) found that in some cases, the normal architecture of the liver is distorted by marked vacuolation of the cytoplasm of hepatocytes (Figure 4). In turkeys, highly vacuolated hepatocytes dominate the histopathological picture, hemorrhages and necrosis appear to be involved frequently (Butler, 1976; Saif and Fadly, 2008; Trott et al., 2014). In other areas, groups of vacuolated hepatocytes and proliferating bile ductules are separated by hemorrhagic areas. In most cases, the predominant lesions are massive or submassive necrosis of hepatocytes with hyperplasia of bile ductules, and the dead hepatocytes appear as ghost cells (Aziz, 2008), and liver failure is considered the leading cause for mortality.

#### Causes of HL

The causes of HL are uncertain and only few data exist in the literature. Various causes such as nutritional, environmental, management, and viral involvement predisposing factors (infectious and non-infectious) were

discussed (Gazdzinski et al., 1994; Popp et al., 2014). The exact cause of HL is still to be clarified, but genetic components and toxins are possibly involved, too (Gazdzinski et al., 1994; Aziz, 2008). However, it has not yet been possible to etiologically clarify which factors are involved in the phenomenon of HL to occur or, rather, what precisely must be avoided to prevent HL. Nevertheless, it can be considered relatively certain that the animals suffer from a massive change in liver metabolism and consequently die (Visscher et al., 2017).

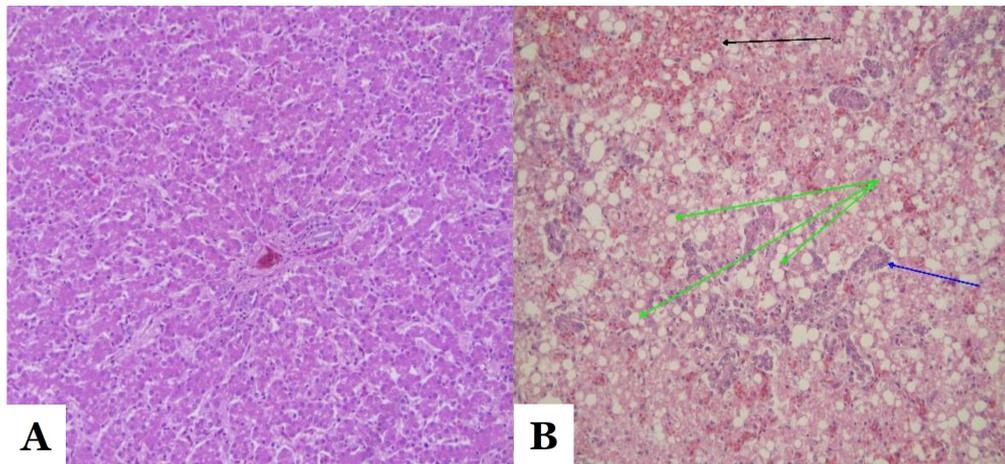
#### Predisposing factors of HL

##### *Dietary intake*

Gazdzinski et al. (1994) described this hepatopathy in turkey breeder hens of 12 to 24 wk of age and speculate about a possible role of nutritional restrictions in the onset of HL. Gazdzinski et al. (1994) and Aziz (2008) pointed out that HL also affects meat-type turkeys whose production is incompatible with diet restrictions. The fasting period and a negative energy balance led to enhanced lipolysis and consequently increased the  $\beta$ -hydroxybutyrate levels in the blood of animals with HL (Aziz, 2008). However, in the majority of cases, the phenomenon HL occurred in flocks with a good feed intake during the fattening period at the time of the fourth and fifth feeding phases, in which animals were fed a complete commercial diet adapted to the energy and nutrients demands, with regard to the age of the birds (Visscher et al., 2017).

##### *Carbohydrates*

A high dietary intake of carbohydrates may be expected to cause fatty liver, and this has been demonstrated experimentally on several occasions by feeding a high energy diet *ad libitum* or by force-feeding (Barton, 1967; Wolford et al., 1971; Ivy and Nesheim, 1973; Wolford and Polin, 1974; Aziz, 2008). In chickens, high-energy and low protein diets induce fatty liver (Zhuang et al., 2019).



**Figure 4:** Pathohistological picture of livers from healthy turkeys (A) HE-staining, x20) and deceased turkeys with HL. (B) HE staining, x20; black arrow: dilated sinusoids filled with blood; blue arrow: biliary duct proliferates; green arrows: liver cells with fat vacuoles (signet ring cells). Photo by [Visscher et al. \(2017\)](#) © (<http://creativecommons.org/licenses/by/4.0/>)

### **Fatty acids**

Polyunsaturated fatty acids have been known to suppress lipid synthesis and induce lipid oxidation ([Clarke, 2001](#)). Therefore, depletion of PUFA in liver tissue, as observed with the diseased animals, is assumed to be involved in the development of fatty liver and could also be observed in human patients with non-alcoholic fatty liver disease ([Araya et al., 2004](#)). Furthermore, the concentration of specific fatty acids in the liver is a good biomarker for assessing non-alcoholic fatty liver disease in animal models ([Willebrords et al., 2015](#)).

### **Protein**

The insufficiency of dietary protein also contributes to fatty liver diseases ([van Zutphen et al., 2016](#); [Ampong et al., 2020](#); [Chakravarthy et al., 2020](#)). Low protein diets can result in the insufficiency of essential amino acids, such as lysine and methionine. Moreover, dietary effects like low protein diets with low contents of some essential amino acids (lipotropic factors) such as methionine are considered a potential predisposing factor ([Gazdzinski et al., 1994](#); [Aziz, 2008](#); [Saif and Fadly, 2008](#); [Popp et al., 2014](#)). Several studies reported abnormal amino acid profiles in hepatic disease ([Rosen et al., 1977](#)). For example, the low methionine content in liver tissue with a concurrently high level of methionine in blood samples among the animals with HL could suggest an increased mobilization and usage ([Ma et al., 2018](#)). Furthermore, elevated plasma branched-chain amino acids are associated with insulin resistance ([Goffredo et al., 2017](#)), a metabolic disorder predisposing fatty liver disease.

### **Minerals**

Similarities with Wilson's disease, a copper storage disorder whose features include liver fatty change ([Myers and McGavin, 2007](#)), should be considered. [Manarolla et al. \(2011\)](#) stated that copper salts used in turkey breeding in Italy were suggested to prevent aortic rupture and control candidiasis. However, the hypothesis

of abnormal copper accumulation in HL cases can be ruled out in the first weeks.

### **Vitamins**

Vitamin E is an essential micronutrient for humans and animals. Recently, the role of vitamin E and its metabolites in regulating cell signaling and gene transcription has received increased attention ([Azzi, 2018](#)). Moreover, the role of vitamin E in regulating lipid metabolism and abdominal fat deposition has also been investigated ([YanFa et al., 2015](#)). Studies have shown that vitamin E supplementation can prevent human lipid metabolism-related diseases, such as non-alcoholic fatty liver ([Oliveira et al., 2003](#); [Sato et al., 2015](#)) and diabetes ([Yan and Khalil, 2017](#); [Alkholy et al., 2019](#)).

### **Hormones**

In chickens, several hormones, such as estrogen, mostly in female, and insulin as well as key enzymes primarily associated with lipid metabolism, play an essential role in lipid metabolism regulation ([Alvarenga et al., 2011](#)). Thus, it is suggested that HL predominantly affects females, so it seems to be related to gender, leading to gender-related hypotheses, i.e., the role of female hormones like estrogen ([Gazdzinski et al., 1994](#)). Moreover, increased synthesis of fatty acids from glucose (lipogenesis) in the hepatocytes as elevated glucose level in the blood causes increased insulin secretion from the pancreas. Furthermore, high glucose and insulin levels have been found to inhibit fatty acid oxidation and activate lipogenesis via converting excess glucose to fatty acids ([Aziz, 2008](#)).

### **Infection**

The presence of Clostridia could be frequently proved in livers of affected animals without clarifying whether this was a primary or secondary finding ([Sieverding, 2015](#)). [Popp et al. \(2014\)](#) detected picornavirus and parvovirus RNA in liver tissue of animals with HL with PCR techniques. Similarly, [Chin and Woolcock](#)

(1994) stated that picornavirus-like particles suggestive of avian encephalomyelitis virus and low numbers of *Escherichia coli* were identified in the affected livers. These are believed to be incidental because turkey breeder hens in this age range are typically vaccinated against avian encephalomyelitis using live viruses, and it would not be unusual to recover bacteria from livers of seriously sick or dead birds (Saif and Fadly, 2008).

### **Mycotoxins**

Mycotoxins, the secondary metabolites of fungi, can affect animals and affect various organs such as the gastrointestinal tract, liver, and immune system. Toxicity of the mycotoxins depends on the amount of absorption, the number of the metabolites formed, exposure period, and sensitivity of the animal (Murugesan et al., 2015). Cereals also make a significant proportion of animal feed in all parts of the world (Alvarado et al., 2017). Unfortunately, cereals and cereal-based products are prone to aflatoxin contamination.

Aflatoxins, produced mainly by *Aspergillus* fungi, are usually found in feed ingredients used for poultry rations, causing a variety of effects in poultry, including increased liver fat, liver damage, decreased weight gain, poor feed efficiency, etc. (Leeson et al., 1995b; Devegowda and Murthy, 2005). However, when Aflatoxins B1 and Ochratoxin A, produced by fungi mainly belonging to *Aspergillus* and *Penicillium*, co-existed in the poultry feeds, their interaction leads to less additive and more antagonistic effects in biochemical parameters of birds (Pappas et al., 2016). For example, Aflatoxins-B1 and Ochratoxin-A combination can lead to less apparent hepatic and more severe kidney lesions in broiler chickens (Huff and Doerr, 1981). This can be attributed to that Aflatoxins-B1 and Ochratoxin-A show weak cytotoxic effects in liver cell lines when combined than alone, suggesting an antagonistic interaction (Choi et al., 2020).

Until recently, aflatoxin contamination was not a food safety concern in Europe; however, recent climate patterns fluctuations will possibly change this situation (Battilani et al., 2016). In a survey in Serbia, 57.2% of 180 maize samples showed aflatoxin B1 contamination in the concentration range of 1.3 to 88.8  $\mu\text{g}/\text{kg}$ . In another survey in France, 6% of 114 maize field samples and 15% of 81 maize silo samples were found aflatoxin-positive (Bailly et al., 2018). In the United States, the occurrence of aflatoxin in food is generally considered low. Additionally, according to the Corn Harvest Quality Report 2018 to 2019 of the United States Grains Council, of 181 maize samples screened for aflatoxin, 98% have no detectable levels (US Grain Council, 2019). In China, Yang et al. (2019) reported a lower incidence level (0.87%) of aflatoxin B1 in maize.

Deoxynivalenol (DON) is a secondary metabolite produced by *Fusarium* fungi. DON is the most widespread mycotoxin found in grains (Escrivá et al., 2015). A recent survey reported that DON is the most frequent contaminant of feedstuffs in Europe (BIOMIN, 2018). Furthermore, an important list of investigations indicated that this toxic effect could be ob-

served when birds fed a concentration of DON greater than 15 mg/kg (Kubena et al., 1989). This suggests that chickens may be relatively tolerant to this *Fusarium* toxin. However, it has been observed that DON-contaminated feed could affect organ weights, blood biochemical, and immunological parameters (Ghareeb et al., 2013). The most susceptible tissues and organs to DON are those with high protein turnover rates, such as the immune system, the liver, and the small intestine (Feinberg and McLaughlin, 2017). Regards the liver, Ghareeb et al. (2016) suggested that high cholesterol levels in broilers fed DON (10 mg/kg) for 35 days may be due to liver or kidney function damage or stress.

### **Stress**

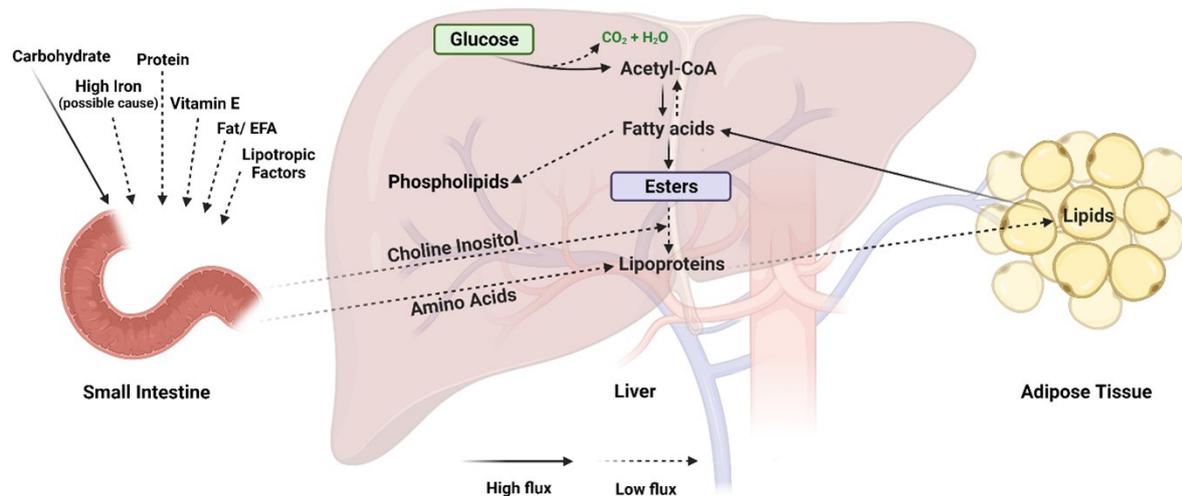
A situation in which stress might occur is through adrenocorticotropin and glucagon, as it probably accelerates the production of cyclic adenosine monophosphate, which inhibits lipoprotein lipase and activates the lipase system in the adipocytes responsible for hydrolyzing lipids before their release (Butler, 1976). In addition, high environmental temperatures and/or changes in lighting programs cause the birds to alter their eating habits, leading to hepatic deposition of lipid and eventually to liver failure (Gazdzinski et al., 1994; Saif and Fadly, 2008).

### **Pathogenesis of HL**

The pathogenesis of fatty liver diseases in chickens is accompanied by an imbalance in lipid homeostases, such as hepatic lipid accumulation, transportation, and metabolism (Zhuang et al., 2019). However, to date, little is known about the pathogenesis of the HL in fattening and turkey breeder flocks. According to Butler (1976), the most important mechanisms by which a fatty liver can be produced involve enhanced lipogenesis, reduced transport of lipids from the liver, reduced deposition in adipose tissue, and decreased oxidation. Additionally, there are several sources and subsequently several pathways of fats leading to fatty liver including peripherally stored adipose tissue that flows into the liver during lipolysis, the uptake of dietary fats, and newly made fatty acids produced by de novo lipogenesis in liver tissue (Donnelly et al., 2005). Several studies suggested various theories and mechanisms (Figure 5), which will be discussed in the following sections.

### **Fat types and fatty acids profile**

In avian species, the amount of fat accumulated in the body depends on the available plasma lipid substrate, which originates from the diet or de novo lipogenesis in the liver (Hermier, 1997). Therefore, the total body fat deposition in poultry is affected by the sources of dietary lipids. Sanz et al. (2000) found that including sunflower oil in the diet of broilers led to a significant reduction in the abdominal fat percentage by inhibiting the activity of fatty acid synthase in the liver and enhancing the activities of carnitine palmitoyl transferase I and L-3-hydroxyacyl-CoA dehydrogenase in the heart



**Figure 5: Possible mechanisms (mostly nutritional) by which hepatic lipidosis can be produced in the liver of affected animals. Modified from Butler (1976) and created with BioRender: [www.bioender.com](http://www.bioender.com)**

compared with the inclusion of fat in the diet. According to the results of Sanz et al. (2000), including sunflower oil in broiler diets enhanced fatty acid oxidation and inhibited fatty acid synthesis, consequently the abdominal fat content was decreased significantly.

From investigations with old breeds, the rate of hepatic lipogenesis is influenced by the intake of fat but in the opposite direction, there being a depression of fatty acid synthesis when fat is added to the diet (Weiss et al., 1967; Pearce, 1968, 1971; Leveille et al., 1975). Similarly, Butler (1976) suggested that excess fat in the liver arises mainly from increased lipogenesis rather than dietary lipid. Also, it was shown that liver lipid levels are not decreased by lowering the dietary fat in laying Japanese quail (Maurice and Jensen, 1978). Moreover, Squires and Leeson (1988) stated that the cause of fatty liver might be due to an excess of energy rather than being specific to an excess of fat in laying hens. This has been related to a reduction in the specific activities of two of the lipogenic enzymes, the citrate cleavage enzyme, the 'malic' enzyme, and may also be due to an increase in the levels of long-chain acyl-CoA derivatives, which are believed to control the pathway by regulating the activity of acetyl-CoA carboxylase and the citrate translocation system.

A reduction in the levels of these long-chain acyl-CoA derivatives may be partly responsible for the increase in hepatic lipogenesis, which occurs when the diet is deficient in the essential fatty acids, linoleic arachidonic (Edwards, 1967; Hopkins and Nesheim, 1967). The comparative analysis of the fatty acid pattern, differences, and shifts in their composition can help to understand the pathogenesis, especially when considering other species (Yamada et al., 2015; Walle

et al., 2016; Wang et al., 2016). Visscher et al. (2017) found that livers of turkeys that died from HL were massively increased in fat levels compared to livers of healthy animals ( $130 \pm 33.2$  vs.  $324 \pm 101$  g/kg DM, respectively). Also, in all fatty livers, different fatty acids concentrations were significantly increased compared to control (palmitic acid: 104 g/kg DM, +345%; palmitoleic acid: 18.0 g/kg DM, +570%; oleic acid: 115 g/kg DM, +437%). Middendorf et al. (2019a) observed a shift from unsaturated to saturated fatty acids in the diseased turkeys, i.e., most of the fatty acids were highest in affected turkeys with HL, but the sum of n-3 and n-6 as well as the sum of PUFA were lowest. This partly can provide an explanation for the severe liver damage of the affected animals, and highlight the importance to consider the type of fatty acids used in the feed.

The fatty acid pattern in liver tissue of diseased animals showed a greatest share of palmitic and oleic acids. Both fatty acids and a general increase in unsaturated fatty acids are involved in cell apoptosis and death (Wang et al., 2016). Therefore, they could partly explain the severe clinical course of HL. High carbohydrate diets, especially in de-novo lipogenesis, had high palmitic acid contents (Chong et al., 2008; Walle et al., 2016). This is common in diets for fattening turkeys, particularly in the last fattening periods, where most diets consist of cereals. It is well stated that palmitic acid is considered toxic for the liver (Yamada et al., 2015; Mota et al., 2016). Yamada et al. (2015) found that in comparison to humans, palmitic acid content was numerically slightly higher in healthy turkeys ( $8.26 \pm 2.57$  g/kg liver tissue in turkeys vs.  $5.45 \pm 0.67$  g/kg liver tissue in humans).

However, the palmitic acid content in simple steatosis and steatohepatitis cases is significantly higher in humans than in turkeys.

Abdominal fat deposition in broilers is not dependent only on the source of oils. For example, the composition of oil (fatty acid isomers) is one of the major factors affecting body fat deposition in broilers, as stated by [Simon et al. \(2000\)](#). Their data showed that including conjugated linoleic acid in chicken diets reduced the skin fat (major fat depots in poultry) compared with diets with no linoleic acid. Similarly, [Badinga et al. \(2003\)](#) noted that dietary conjugated linoleic acid decreased fat deposition in the chicken livers compared to those fed conjugated linoleic acid and corn oil (a rich source of linoleic acid). Additionally, [Zhang et al. \(2007\)](#) found that conjugated linoleic acid decreases total body fat deposition in broilers via inhibiting the action of lipoprotein lipase. In addition, [Zhou \(2008\)](#) proposed that conjugated linoleic acid reduced abdominal fat deposition in Yellow-feather broiler chickens, a local Chinese strain, by downregulating peroxisome proliferator-activated receptor- $\gamma$  mRNA expression in abdominal adipose tissue. Thus, generally in avian species, it can be concluded that conjugated linoleic acid reduces total body fat deposition directly via different ways, such as by reducing lipoprotein lipase activity and downregulating peroxisome proliferator-activated receptor  $\gamma$  mRNA expression, or indirectly by elevating the activity of carnitine palmitoyl transferase I.

### Protein content

Protein is an essential nutrient of poultry diets. [Yalçin et al. \(2010\)](#) found that the total carcass fat deposition in chickens increased when fed diets containing 19.2%, 16.6%, and 15.5% protein (low protein) compared with those fed diets containing 22.9%, 19.9%, and 18.2% protein following the standard recommended by National Research Council ([NRC, 1994](#)) in the starter, grower and finisher phases, respectively. Therefore, we believed that dietary protein content could play a role in fatty liver pathogenesis. Thus, dietary effects like low protein diets should be considered ([Gazdzinski et al., 1994](#); [Popp et al., 2014](#)). Removing lipids from the liver in the form of lipoproteins depends on the availability of the protein moiety and the phospholipid components essential for the complete assembly of the lipoproteins. [Choi et al. \(2005\)](#) also showed a significant reduction in fatty acid synthase mRNA expression in the liver of broiler chickens fed increasing dietary protein content compared with the control.

Furthermore, [Rosebrough et al. \(2008, 2011\)](#) found that the mRNA expressions of hepatic malic enzyme, acetyl coenzyme carboxylase, and fatty acid synthase were suppressed when feeding broilers a diet containing a high protein level when comparing low protein and high protein diets. The fatty acid synthase is an essential enzyme in the de-novo lipogenesis pathway in the chicken livers ([Fouad and El-Senousey, 2014](#)). Therefore, body fat deposition is affected directly by dietary

protein content ([Fouad and El-Senousey, 2014](#)). Overall, we think that it is better to cover the protein requirements of birds with low fat deposition and a high quality meat.

### Amino acid pattern

Particularly methionine as a precursor for carnitine plays an important role in VLDL secretion and is therefore involved as a lipotropic factor in removing fat from the liver ([Gruffat et al., 1996](#); [Zhang et al., 2017](#); [Peng et al., 2018](#)). Consequently, an insufficient supply or an increased need for methionine could reduce fat from the liver ([Hazel, 2009](#)). Several studies showed a significant increase in abdominal fat after feeding broilers a methionine-deficient diet ([Yao et al., 2006](#); [Zhan et al., 2006](#); [Opoola et al., 2012](#)) and a reduction in liver weight due to methionine supplementation ([Jariyahatthakij et al., 2018](#)). Moreover, the fat-lowering effect of dietary L-methionine might be associated with changes in lipogenesis and/or lipolysis. [Takahashi and Akiba \(1995\)](#) found that the body fat content was regulated by the inclusion of dietary L-methionine via reducing the lipogenesis (fatty acid synthase activity) and increasing lipolysis (hormone-sensitive lipase activity).

Furthermore, methionine and lysine are the precursors of carnitine. Carnitine is involved in the transportation of long-chain fatty acids from the cytoplasm into the mitochondria, in which fatty acids can be catabolized via  $\beta$ -oxidation ([Longo et al., 2016](#)). In addition, methionine and cysteine are required for apolipoproteins ([Saif and Fadly, 2008](#)). Furthermore, feeding chickens diet supplemented with high lysine content reduced abdominal fat deposition. Therefore, lysine addition to poultry diets could reduce the carcass fatness via lipogenesis inhibition ([Grisoni et al., 1991](#)).

Arginine is considered one of the essential amino acids implicated in decreased carcass fat deposition ([Fouad et al., 2012](#)). [Crespo and Shivaprasad \(2003\)](#) indicated that feeding broiler chickens diets with high levels of arginine than the recommendations of the National Research Council could reduce carcass abdominal fat ([NRC, 1994](#)). In avian species, therefore, dietary L-arginine supplementation inhibits hepatic fatty acid synthase mRNA expression and improves carnitine palmitoyl transferase I and L-3-hydroxyacyl-CoA dehydrogenase mRNA expression, which causes a reduction in the size of abdominal adipose cells and consequently decreased the abdominal fat content ([Wu et al., 2011](#); [Fouad et al., 2013](#)). Histidine is the only amino acid whose amount in liver tissue was higher among HL animals than non-affected ones ([Middendorf et al., 2019b](#)). [Lee et al. \(2005\)](#) reported increased histidine concentrations in plasma and organs of diabetic mice and postulated an improvement in antioxidant activity and insulin restoration via histidine supplementation. Considering the elevated ammonia levels in the blood of animals with HL and the high concentration of glutamine together with the function of histidine as

a possible inhibitor of mitochondrial glutamine transporter (Rama Rao et al., 2010), histidine may play an important mediatory role in reducing oxidative stress in animals with HL.

Remus and Firman (1991) reported an increase in liver glutamic acid simultaneously with increased muscle catabolism. However, this could not be proved in the study by Middendorf et al. (2019a). On the one hand, glutamine is important for immune cell function and inflammation response (Wu et al., 2018) and can be elevated during catabolic stress (Wilmore and Shabert, 1998). On the other hand, several studies reported increased glutamine levels in the plasma of ketoacidosis and hyperglycemic patients (Biolo et al., 2008).

Furthermore, Middendorf et al. (2019a) observed that the aromatic amino acids in blood samples in animals with HL were about four times higher than in the non-affected group, whereby tyrosine constituted the major level, and tryptophan was highest among non-affected animals. The clinical signs, including coordination difficulties, apathy, and high levels of aromatic amino acids, and decreased ratio of branched chain amino acids/aromatic amino acids in blood samples of HL affected animals, could suggest the involvement of neurological disorders HL.

## Minerals and vitamins

### *Manganese content*

Manganese plays a vital role in carbohydrate and lipid metabolism; therefore, it is a necessary trace mineral in poultry nutrition (Klimis-Tavantzis et al., 1983). Moreover, it has been noted that manganese's dietary inclusion could reduce the body fat content in broiler chickens effectively (Fouad and El-Senousey, 2014). Also, it was found that manganese may reduce the carcass abdominal fat significantly by downregulating the activity of lipoprotein lipase (Lu et al., 2006). In avian species, the liver is the site for the synthesis of fatty acids. Those fatty acids derived from the diet are transported to the adipose tissues via LDL or portomicrons before storage in the adipose tissue. So, lipoprotein lipase hydrolyses the triglycerides of portomicrons and LDL to produce free fatty acids and glycerols (Hermier, 1997). Therefore, lipoprotein lipase is an important enzyme for the uptake of fatty acids via adipose tissues.

### *Iron content*

There are very few to no reference values for turkeys regarding the iron content in liver tissue. Therefore, it is rather difficult to judge if high levels of iron are pathological. High levels of iron in the liver can occur for various reasons. During an infection defense, the circulating iron is stored in the liver to make it less available to pathogens (Lowenstine and Petrak, 1980; Klasing, 1998). There are also reports of high hepatic iron contents during chronic ingestion of high amounts of dietary iron (Dierenfeld et al., 1994; Gerlach et al., 1998) due to the less strictly regulated intestinal absorption of iron by birds (Ward et al., 1991). Richards et al. (1987) examined the iron content in livers of turkey poults after one, up to four days of starvation

and refeeding. After only one day of starvation, the iron contents in livers had almost doubled. In future studies, the hypothesis of starvation as a possible reason for HL should be verified.

In a recent study, Visscher et al. (2017) revealed a change in iron metabolism and a high iron level in the livers of affected turkeys (Figure 5). It has not been clarified why these high iron contents in the liver occur in birds suffering from HL. High hepatic iron contents are also reported in human patients with fatty liver disease and have been associated with fibrosis and progression of liver damage (Bacon et al., 1994; George et al., 1998). High iron contents can generate oxidative stress, which leads to cytotoxicity and inflammation (Kruidenier and Verspaget, 2002; Ahmed et al., 2012). The acute hepatocellular injury itself may result in increased iron uptake from the intestine (Batey and Johnston, 1993). High iron contents can also modify the fatty acid pattern in membranes and organs by lipid peroxidation (Bacon et al., 1986; Myers et al., 1991). In addition to examining liver tissue, determining ferritin and transferrin levels is also of interest for the diagnosis of iron overload (Piperno, 1998). Both parameters are acute phase proteins, which change in concentrations during infection, inflammation, or stress (Xie et al., 2002; Murata et al., 2004; Rath et al., 2009).

Ferritin is the iron storage protein, and transferrin binds iron to transport it through the body (Hallquist and Klasing, 1994; Arosio et al., 2009). Besides their involvement in iron homeostasis, ferritin and transferrin are also acute phase proteins and are used as markers for inflammation, infections, and stress, among other things (Murata et al., 2004; O'Reilly and Eckersall, 2014). Increased serum ferritin levels are also associated with insulin resistance in human patients, a common phenomenon in fatty liver diseases (Wrede et al., 2006). However, Middendorf et al. (2019b) found that the measured ferritin values were not significantly different between normal and HL infected birds and did not match the high iron contents in liver tissue of turkeys with HL. On the other hand, acute phase proteins do not increase during starvation or liver damage due to a general impaired liver protein synthesis (Reeds et al., 1994; Gruys et al., 2005).

Transferrin is an iron transport protein and acts in mammals as a negative acute phase protein (Murata et al., 2004). Transferrin is a positive acute phase protein in broilers and increases during inflammation and infection (Tohjo et al., 1995; Xie et al., 2002). In a recent study, transferrin levels in blood samples were significantly the highest in the normal birds and lowest in the turkeys infected with HL (Middendorf et al., 2019b). Regarding the high values in the control group, which consisted of slaughtered animals, it must be taken into account that the animals were transported and probably stressed. This can also potentially lead to an increase in transferrin. Additionally, serum transferrin levels were used as markers for the nutritional status and reflection of visceral protein stores in patients (Fletcher et al., 1987). Consequently, the

low transferrin levels among animals with HL could suggest a previous starvation period or liver damage. The low acute phase protein levels, together with the high iron contents, could indicate a previous malnutrition/starvation period and/or severe liver damage (Middendorf et al., 2019b).

Hepcidin is the primary regulator of iron homeostasis in vertebrates (Drakesmith and Prentice, 2012; Michels et al., 2015). The liver plays a significant role in regulating iron homeostasis because it is the primary producer of hepcidin and the main iron depot in the body (Drakesmith and Prentice, 2012; Pietrangelo, 2016). The synthesis is normally induced by systemic iron levels and inflammatory stimuli (Michels et al., 2015). The liver responds to inflammatory signals originating extra-hepatically by increasing the hepcidin level. Infections or stimuli likely induce liver hepcidin expression, reduce serum iron and increase iron accumulation in reticuloendothelial cells (Drakesmith and Prentice, 2012). Concerning human medicine, hepcidin levels were significantly higher in obese children with NAFLD than in those without NAFLD (Demircioğlu et al., 2014). Therefore, changes in the hepcidin regulation would be possible but should be the focus of future investigations.

#### *Vitamin E content*

Dietary vitamin E has also been shown to prevent diet-induced lipid accumulation in the liver of guinea pigs (Podszun et al., 2014). Moreover, dietary vitamin E supplementation decreased abdominal fat in broiler chickens (Li et al., 2009; Zaboli, 2013). The regulatory roles of vitamin E on lipid metabolism may involve inhibiting adipose cell conversion by regulating signaling pathways and modulating gene transcription. A previous study showed that vitamin E inhibited the adipose conversion of 3T3-L1 cells at micromolar levels (Kawada et al., 1990). Further studies indicated that vitamin E induced adiponectin expression in rat adipose tissues and 3T3-L1 cells via a peroxisome proliferator-activated receptor  $\gamma$ -dependent mechanism (Landrier et al., 2009). Adiponectin is important in lipid metabolism because it promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation (Fu et al., 2005).

Vitamin E can also affect fat deposition by regulating the expression of genes involved in lipid metabolism, such as those involved in lipogenesis, lipolysis and transport. In broilers, 200 mg/kg dietary vitamin E supplementation increases peroxisome proliferator-activated receptors- $\beta$  and heart fatty acid-binding protein genes expression in the pectoralis (Li et al., 2009). However, there have been few studies on the role of vitamin E in body fat. Moreover, the regulatory mechanisms of vitamin E on lipid metabolism are not completely understood.

#### **Probiotics and prebiotics**

Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) define probiotics as 'live microorganisms which when

administered in adequate amounts confer a health benefit on the host (Bajagai et al., 2016). Some studies have focused on the importance of probiotics as a kind of regulator of lipid metabolism in chickens (Saleh et al., 2014).

Unlike probiotics, prebiotics are not microorganisms (Alloui et al., 2013). According to FAO and WHO (2007), a prebiotic is a selectively nondigestible ingredient that allows specific changes in the composition and activity in the gastrointestinal microbiota and benefits the host (FAO, 2007). Up to our best knowledge, no studies have been investigated on the effect of prebiotics on lipid metabolism in turkeys. However, in humans, prebiotics and synbiotics have had a significant impact on decreasing the degree of hepatic steatosis (hepatic fat infiltration) in patients with non-alcoholic steatohepatitis (Ferolla et al., 2016; Bomhof et al., 2019). In this scenario, Bomhof and co-workers observed that fructooligosaccharides supplementation decreased steatosis (Bomhof et al., 2019).

#### **Conclusions**

The manifold analyses in the current review provided insights into how a change in feed composition could potentially maintain animal health in a preventive manner. Provoking factors could not be consistently identified but are certainly not exclusive to the context of diets may suggest a preceding period of stress (metabolic, e.g., induced by fluctuations in feed intake as a possibility). However, the disease condition seems to be a multifactorial problem in which HL plays a central role and to which different stressors, including viruses, contribute. Monitoring at the slaughterhouse helps determine husbandry deficiencies and implement a benchmarking system for all turkey farms. Standardization of the evaluation, including photographic means and experts, is of major importance. More studies are needed to determine the leading causes and pathogenesis of the condition and to investigate whether it has any genetic components. Also, future research should primarily focus on exploring the possibility of exposure to a toxin, especially microbiological tests to find pathogenic agents.

Prevention of HL in turkeys with higher levels of vitamin E in the complete feed is a successful strategy practiced in the field today. The successful use of high-dose vitamin E administration has been reported several times (Gazdzinski et al., 1994; Popp et al., 2014), indicated a metabolic etiology. Administering vitamin E to affected flocks (25 IU/hen) via water for 7 d reduced mortality (Gazdzinski et al., 1994). In flocks that have been affected by an occurrence of HL in previous flocks, this approach is sometimes used in practice, which could be of preventive interest. However, systematic evidence that higher vitamin E levels have an effect is still lacking.

HL is prevented by having adequate methionine (0.2%) and methionine plus cysteine (0.4%) in the ration (NRC, 1994). In addition, the disease was prevented by supplementing the standard feed with 1 kg 60% choline, 1 kg methionine, and 20 g vitamin

B12/ton (Gazdzinski et al., 1994). In some areas, successful prevention of HL disease was apparent following a protocol in which the initial avian encephalomyelitis vaccine was given via water, followed by a booster vaccination in the wing-web instead of administering two wing-web inoculations (Saif and Fadly, 2008).

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