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FAT DIGESTION AND METABOLISM: EFFECT OF DIFFERENT FAT SOURCES AND FAT MOBILISERS IN BROILERS' DIET ON GROWTH PERFORMANCE AND PHYSIOLOGICAL PARAMETERS – A REVIEW

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Abstract

Commercial broilers have a short production cycle and a high requirement for energy (3000 kcal/kg in starter phase and 3200 kcal/kg in finisher phase). Therefore, the need to add energy rich lipids to their diet is inevitable. Digestibility of fat depends on its multiple properties: chain length, the composition of fatty acids, ratio of saturated/unsaturated fatty acids and free fatty acids. The high cost of vegetable oils and less availability due to their consumption in human diet are the main reasons for searching for cheaper alternative fat sources. Animal oils like poultry and fish oil are the by-product of rendering plants and after refining, they are used in poultry diets as an energy source. Due to presence of impurities and free fatty acids, the digestibility of animal fat is lower. There is a limited amount of bile acids and lipase available during early age and when birds are reared on high energy diet (finisher phase). Supplementation of emulsifier or lipase in broilers' diet increase fat utilisation. Emulsifiers increase fat digestibility by increasing active surface area of lipid droplets. Lysolecithin and lysophospholipids are produced from hydrolyses of lecithin and phospholipids by phopholipase A2. The bile acids are mainly composed of cholic acid, hyodeoxycholic acid and chenodeoxycholic acid and have strong emulsification properties. Triacylglyceryl acylase (lipase) is an enzyme involved in catalysis and the hydrolysis of lipids. It can be concluded that use of emulsifier and lipase in broiler diet improves growth performance, nutrient digestibility and intestinal histology in broilers.

Key words: bile acids, broilers, emulsifiers, lipase, oil sources

Feed formulation for poultry is done by keeping in mind to fulfil the optimum nutrient requirement. Modern breeds of poultry perform better than older breeds. To get optimum production, birds require high-quality nutrients i.e., energy, protein, amino acid, calcium, and phosphorus (Applegate and Angel, 2014). Energy providing ingredients account for more than half of the proportion of broiler diet. Grains are considered primary energy ingredients, while fats and oil are also considered good sources of energy. Inclusion of fats and oil has some benefits over grains. Fats and oil contain 2.25 times more energy and a considerable amount of essential fatty acids in comparison to grains. So, the addition of a little amount of fat/oil in the diet had a substantial effect on metabolisable energy (Tan et al., 2016). Birds have an increased energy requirement for which high-density diets are formulated. To meet high energy demands of birds, fats and oils are included in the diet (NRC, 1994).

Feed intake of birds is regulated by energy contents so to monitor feed intake, provision of an adequate amount of energy to the birds is essential (NRC, 1994). In an experiment by De Witt et al. (2009), results showed that by increasing the fat concentration feed intake was reduced. To fulfil the protein and energy requirements of birds other than plant sources, some animal sources are also being used (Donohue and Cunningham, 2009). Except for provision of dietary energy, incorporation of fats and oils in the diet of broiler helps to improve the digestibility of nutrients and shows better performance than those which are formulated without fats and oil comprising similar nutrient profiles (Poorghasemi et al., 2013; Jaapar et al., 2020). Adding fats to the diet reduces dustiness and lowers particle segregation in mash feed, which in turn helps to improve feed intake and lubricate mixing equipment (Tisch, 2006; Latshaw, 2008). Addition of fat helps to promote the palatability of feed (Baião and Lara, 2005). Fatty acids are also considered essential nutrients for optimum growth of poultry. Fats and oil are also good sources of essential fatty acids i.e., linoleic acid, while other fatty acids aid in solubilisation of fat-soluble vitamins (Lesson and Semmers, 2005; Abdulla et al., 2019). Kim et al. (2013) reported that the addition of different fats and oil sources significantly increased the duodenal and ileum length. Diet fortified with fats helps to decrease the passage rate of feed in the gut which allows more enzymatic degradation of nutrients resulting in improvement in digestibility of other nutrients i.e., protein and amino acids. Fats and oils can be used as an energy source during hot weather because of the lower heat of increment (Ayed et al., 2016).

Fats and oils used in the poultry industry are obtained from plant as well as animal sources. Tallow, fish oil and poultry fat are derived from animal sources while oils obtained from plant sources are corn oil, soybean oil and sunflower oil and are being utilised as an energy source in poultry diets (Tabeidian et al., 2005). Fat and oil supplementation become a highly developed practice in the feed sector. But at an early age, the digestive system is not fully developed and chicks are unable to utilise higher fat diet. As secretion of lipase and bile acid is less at 1st week and it tends to increase with age till day 21, this shows that birds cannot properly utilise fats at an early age (Noy and Sklan, 1995).

In poultry, digestion of fats is accomplished by the enzymatic hydrolysis of fats. Fats are broken down into fatty acids. These fatty acids form micelle, which helps to cross the liquid phase of the gut. This process is carried out in the body with the assistance of emulsifiers (Noy and Sklan, 1998). To compensate for the physiological deficiencies, some exogenous sources of enzymes and surfactants are supplemented in broiler diets to improve fat digestibility (Al-Marzooqi and Leeson, 1999; Jaapar et al., 2020). Emulsifiers like lecithin and lysolecithin are being used as supplements for enhancing fat digestibility (Roy et al., 2010; Zhang et al., 2011). Emulsifiers improve growth performance as a result of increased fat digestibility and increased fatty acid absorption. Emulsifiers also increase lipase activity by increasing surface area of fat molecules during the process of fat hydrolysis (Zhang et al., 2011; Tan et al., 2016; Papadopoulos et al., 2018). Dietary supplementation of bile acids and emulsifiers enhanced emulsification, micelle development and fat digestion (Lai et al., 2018 a).

Lipase, also known as triacylglyceryl acylase, is an enzyme that causes catalysis and hydrolysis of fats. The studies on the use of exogenous lipase in broiler diets are limited as compared to phytase, protease or xylanase (Al-Marzooqi and Leeson, 1999). Supplementation of lipase in broiler diet caused better growth performance, oxidative stability, meat quality and shelf life of meat (Nagargoje et al., 2016). If the digestibility of lipids can be improved, feed costs can be lowered. Therefore, it was hypothesized that addition of emulsifier or lipase may increase fat digestibility in broilers and thus have a beneficial effect on broilers' performance when reared on low fat and less total energy of diet. The objective of this review is to examine the effect of different oil sources and fat mobilisers in broilers.

Fat

Definitions

Fats and oils are energy-rich compounds and are esters of fatty acids and glycerol (Baião and Lara, 2005). The terms fat, oil and lipid are often used interchangeably (Figure 1). Lipids are fatty acids and their derivatives (triglycerides) and substances related biosynthetically (lipoproteins) or functionally (cholesterol) to these compounds (Hammond et al., 2003). These lipids can be added to diets as separate raw materials (soybean oil, poultry fat) or as constituents of other raw materials (constituents of grains). However, the term fat can also be considered as that subgroup of lipids that are solid at room temperature (Baião and Lara, 2005), whereas oils are considered as the subgroup of lipid mixtures that are liquid at room temperature (Baião and Lara, 2005). This terminology is not always used properly. For example, palm oil should be referred to as palm fat. Correspondingly, fish oil derived from an animal source. The terms fat and oil are thus commonly based on the origin of the material (animal and vegetable, respectively), rather than on their condition at room temperature.

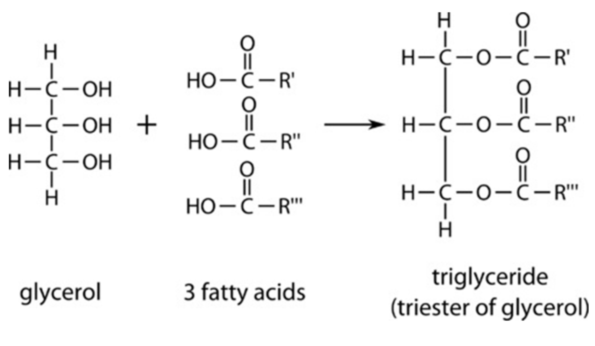

Figure 1. Structure of triglycerides (Baião and Lara, 2005)

Fatty acids can be saturated or unsaturated (Figure 2). A saturated fatty acid has no double bonds between the carbon atoms of the hydrocarbon chain, whereas an unsaturated fatty acid has one or more double bonds between the carbon atoms of the hydrocarbon chain (Raven et al., 2005). Fatty acids also differ in the length of the hydrocarbon chain. They can be divided into short chain fatty acids (SCFA), medium chain fatty acids (MCFA) and long chain fatty acids (LCFA). SCFA are fatty acids with hydrocarbon chains of less than eight carbon atoms. MCFA are those fatty acids with hydrocarbon chains of eight to twelve carbon atoms. LCFA are fatty acids that have more than 12 carbon atoms in their tail (Lairon, 2009).

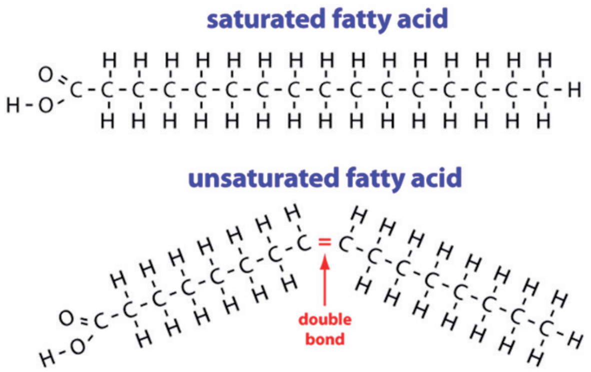

Figure 2. Structures of saturated and unsaturated fatty acids (Lairon, 2009)

Fat digestion

Dietary components such as fats need to go through the digestive tract to be digested. The broiler's digestive tract (Denbow, 2000; Svihus, 2014) starts at the beak and continues with the oesophagus and crop. The oesophagus expands into the proventriculus and is followed by the gizzard. The gizzard empties into the small intestine (duodenum, jejunum and ileum) via a narrow pylorus. Together with the caeca, the ileum ends in a short colon. The colon transfers the digesta into the cloaca where the faecal drops of the broilers are prepared (Nasrin et al., 2012).

The digestion of fat, however, is a complex process that involves several crucial steps that occur throughout the digestive tract (Figure 3). In general, the digestion of fat in broilers is similar to the digestion of fat in other monogastrics (Bauer et al., 2005; Wilde and Chu, 2011; Svihus, 2014). After the feed is ingested, fats are released from the feed matrix and emulsified. Next, the triglycerides of the fat are hydrolysed into monoglycerides and free fatty acids (FFA). These monoglycerides and fatty acids then arrange into mixed micelles that are subsequently absorbed by the enterocytes of the small intestine.

Beak, oesophagus and crop

The digestion of fat starts with the consumption of feed. The purpose of the beak is to pick up the feed particles and direct them to the oesophagus. Before the oesophagus, the beak is extended into a mouth cavity. In this mouth cavity, numerous ducts of the salivary glands release their secretions. The secretions have some limited function by lubrication and humidification of the ingested feed (Samar et al., 2002; Svihus, 2014). The feed is not ground in the mouth but is simply swallowed (Svihus, 2014). The feed is guided towards the oesophagus by the tongue, which is pointy and matches the shape of the beak. The oesophagus connects the mouth to the crop. The crop is a large dilation of the oesophagus. As the storage capacity of the gizzard is limited (5 to 10 g of feed), the main role of the crop is to provide storage and ensure sufficient delivery of feed towards the proventriculus and gizzard (Denbow, 2000). The crop also improves humidification of the feed, but it does not secrete enzymes and no considerable absorption has been reported (Svihus, 2014).

The feed can also pass directly to the proventriculus. In fact, due to the genetic improvements in broilers, the continuous improvements in diet composition and the ad libitum availability of feed, the crop is not used to any significant extent in commercial broilers production. Hence, concerning lipid digestion, no considerable action has taken place during consumption and transfer of the feed to the proventriculus (Svihus et al., 2010).

Proventriculus and gizzard

The proventriculus and ventriculus or gizzard form the stomach of the broiler. Moreover, these organs are commonly referred to as the glandular and muscular stomach of the chicken. The mean retention time in this section is estimated to vary between half an hour and one hour, but it can vary according to the size of the particles in the digesta (Svihus, 2014).

The gastric juice (secreted from proventriculus walls) has a high HCl content which results in a very low pH (2). The pH of gizzard contents from broilers is reported to vary within the range of 1.9 to 4.5 (average 3.5) (Svihus et al., 2002). The presence of a gastric lipase in broilers and poultry, in general, is mentioned in several reviews (Bauer et al., 2005; Blair, 2008). Strangely, no actual data on the presence or activity of gastric or acid type lipase in poultry are available. Nevertheless, due to the shuttling of digesta from the duodenum back to the gizzard and the proventriculus, some lipase action ought to occur in the proventriculus and the gizzard. Moreover, Sklan et al. (1978) have reported enzyme concentrations in the gizzard, including pancreatic lipase concentration, to be approximately 10% of the concentration of enzymes in the duodenum. Therefore, some initial hydrolysis is likely to occur in this part of the digestive tract. In humans and farm animals, it is known that in the gastric phase, primarily triglycerides, are hydrolysed to diglycerides and FFA, whereas the hydrolysis of diglycerides to monoglycerides and FFA is negligible (Lairon, 2009). These diglycerides and fatty acids are thought to participate to some extent in the emulsification process of triglycerides by acting as surface active agents (Lairon, 2009). Absorption of free SCFA in the proventriculus and gizzard of broilers has also been hypothesised, but no actual data are available (Van den Borne et al., 2015).

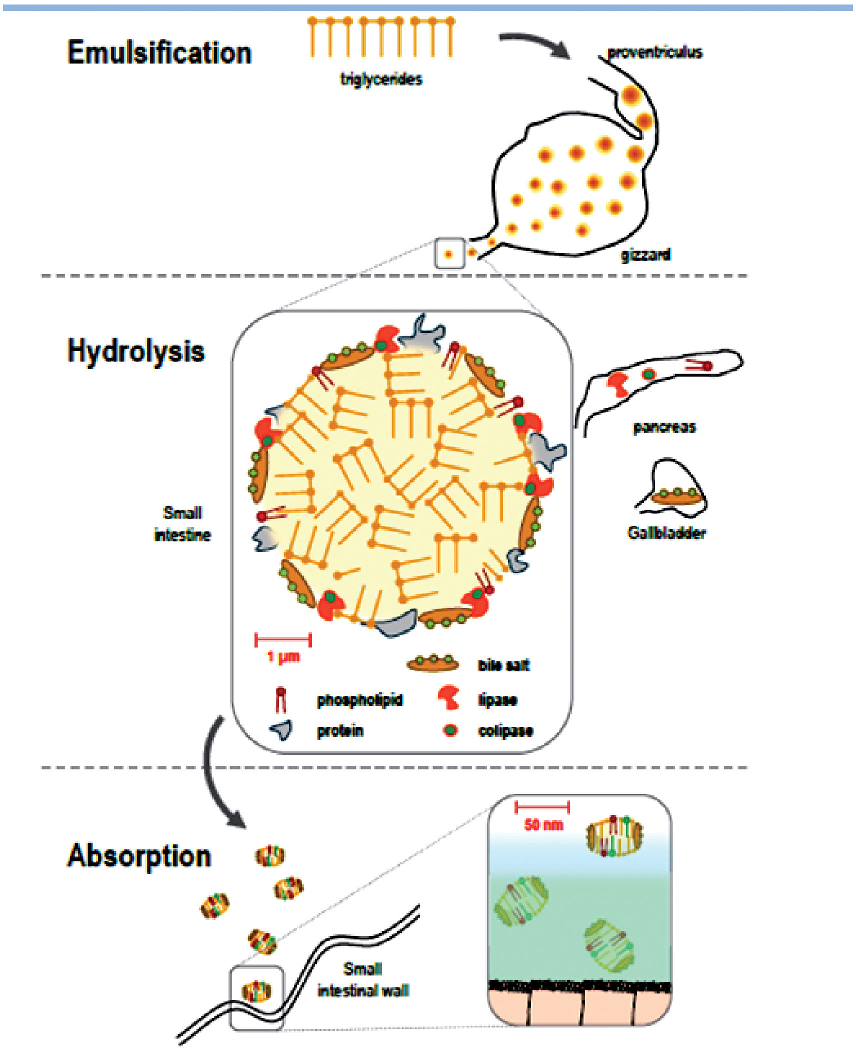

Figure 3. Lipid digestion: emulsification, hydrolysis and absorption (Svihus et al., 2010)

The major contribution of the gizzard and the pylorus to the fat digestion process comes, however, from its grinding action. The gizzard is a powerful muscular organ that is covered on the inside with a hard koilin layer (Denbow, 2000; Svihus, 2011). As a result of these contractions, feed is thoroughly ground and mixed. In terms of fat digestion, this process is of particular importance as first, it releases the triglycerides from the feed matrix and second, it emulsifies the triglycerides. This emulsification is aided by surface active components generated from the feed, such as amphiphilic proteins, but also by bile salts delivered by the reflux of digesta from the duodenum (Bauer et al., 2005; Lairon, 2009).

Small intestinal lumen

Lipids enter the duodenum as emulsion droplets together with the rest of the digesta. These droplets are composed of a core of main triglycerides and fat soluble nutrients (e.g. vitamins, cholesteryl esters) covered with a layer of amphiphilic structures (Bauer et al., 2005). The retention time of the digesta in the small intestine of broilers is between two and four hours (Svihus, 2014). The average retention time increases from duodenum (5 to 10 min) to jejunum (40 to 60 min) to ileum (80 to 120 min) (Svihus, 2014). The entry of the digesta and in particular of these emulsion droplets in the duodenum start the secretion of cholecystokinin, which in turn regulates secretions of bile and pancreatic enzymes by the gallbladder and the pancreas, respectively (Degolier et al., 2013). The bile and pancreatic duct enter the small intestine merely at the distal end of the duodenum (Denbow, 2000). Nevertheless, triglyceride hydrolysis already commences at the start of the duodenum (Svihus, 2014).

Bile formation starts at the hepatocytes of the liver where, in an enzyme driven multistep cascade, bile acids are synthesised from cholesterol and secreted into the gallbladder (Raven et al., 2005). The gallbladder then is connected to the duodenum via the cystico-enteric duct. Both the bile duct and pancreatic duct enter the duodenum with one, common papilla of vater (Denbow, 2000). Bile salts present in chicken bile are taurocholate and taurochenodeoxycholate. In humans and other animal species in addition to bile salts (60 mg/ml), also phosphatidylcholine (3 mg/ml) is secreted into bile (Carulli et al., 2000).

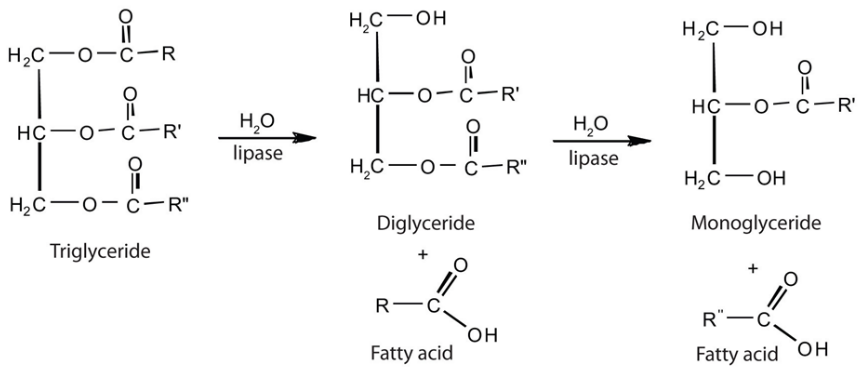

Figure 4. Hydrolysis mediated by pancreatic lipase: hydrolysis of triglycerides to monoglycerides and FFA

The pancreas secretes sodium bicarbonate and a variety of digestive enzymes such as amylases and proteases (Denbow, 2000). With regards to fat digestion the secretions of colipase, lipase, phospholipase A_2 and cholesterol esterase are of particular importance (Bacha et al., 2006; Fendri et al., 2006). Due to the secretion of sodium bicarbonate, the pH in the lumen quickly rises to a level above six (Svihus, 2014). As the pH optimum for lipase in the presence of colipase is reported to be around six to seven, that increase is needed for the efficiency of the hydrolysis process (Karray et al., 2011).

Cholesterol esterase hydrolyses the esters of cholesteryl with fatty acids (Mu and Høy, 2004). Cholesteryl esters are only a minor part of the diet. Cholesterol is an important component in all animal tissues (Lesson and Semmers, 2005). Moreover, only 10 to 15% of dietary cholesterol is present as cholesteryl esters (Lairon, 2009). The vegetable counterpart of cholesterol esters, phytosterol esters are also hydrolysed by cholesterol esterase (Brown et al., 2010). Due to the high inclusion of plant derived materials in the broilers' diet, it can be assumed that their overall contribution would exceed that of dietary cholesterol. Nevertheless, the hydrolysis of cholesteryl esters or phytosterol esters generates respectively free cholesterol and FFA or free phytosterol and FFA (Mu and Høy, 2004; Brown et al., 2010). Phospholipids from bile, predominantly phosphatidylcholine, are hydrolysed by phospholipase A_2. The phospholipase cleaves phospholipids at the sn-2 position to release the respective lysophospholipids and FFA (Joshi et al., 2006).

Enterocytes of the small intestine

The mixed micelles, SCFA and MCFA are then transported towards the enterocytes of the small intestine. Enterocytes are the epithelial cells of the small intestine. Each chicken enterocyte covers approximately 44 µm of the 1 mm long fingerlike protrusions of the small intestine, the so-called villi (Karcher and Applegate, 2008). The apical side of the enterocytes is composed of tiny protrusions, microvilli, which form the brush border. These microvilli are approximately 100 nm in diameter, 1 to 2 µm in length and have approximately 15 to 20 nm of space in-between (Karcher and Applegate, 2008; Lairon, 2009). The enterocytes are covered with the glycocalyx and an unstirred water layer. The glycocalyx is a uniform layer of filamentous material that contains digestive enzymes. This unstirred water layer is a thick viscous layer that covers the enterocytes and surrounds the glycocalyx (Yuan et al., 2007). Tancharoenrat et al. (2014) confirmed that the jejunum is the predominant site of free fatty acid absorption in broiler chickens and that the digestion continues to some extent in the upper ileum. Lipids that are not absorbed by the small intestine enter the colon. In theory, these lipids could be used as a carbon source for the microbiota (e.g. *Escherichia coli*) in the colon and caeca. Fats that are not absorbed by the end of the ileum are therefore expected to be excreted through the faeces (Fujita et al., 2007).

Several of the phospho- and lysophospholipids serve as an integral part of membrane structures and have several roles in cell signalling (Lundbaek, 2006). Additionally, lysophospholipids affect membrane formation and proper function (Maingret et al., 2000). In this way, lysophospholipids could have a direct effect on absorption of lipids in the small intestine. In addition, lysophospholipids arrange into mixed micelles which can incorporate, for example, monoglycerides and FFA. By incorporating these lipids into micelles, the transport through the unstirred water layer is improved. Hence, by increasing content of lysophospholipid in the lumen, micelle formation could be increased, improving the transport towards the enterocytes and ultimately improving the absorption of lipids.

Researchers have indicated possible inflammatory or anti-inflammatory properties of several lysophospholipids. Moreover, different molecules (different head groups and/or fatty acid) seem to have different interactions with the immunological system. Mainly lysophosphatidylcholine (LPC) has been linked to inflammatory responses. Patients with atherosclerosis have elevated levels of LPC in their plasma (Lavi et al., 2007). As LPC is a potent chemoattractant for T-cells and monocytes and to induce apoptosis of endothelial and vascular smooth muscle cells (Takahashi et al., 2002), LPC may be directly involved in atherosclerosis. In patients with atherosclerosis, LPC is yielded from the hydrolysis of oxidised phospholipids from oxidised low density lipoprotein (LDL) particles by lipoprotein-associated phospholipase A_2 (Gonçalves et al., 2012). In addition to LPC, also oxidised non-esterified fatty acids (NEFA) are generated by the hydrolysis of these oxidised phospholipids. NEFA is known to stimulate the production of cytokines, such as tumour necrosis factor α and interleukin 6, which increase the inflammatory profile. The inflammatory properties of LPC seem to be highly dependent on the fatty acid composition of LPC (Huang et al., 2008). Recently, lysophosphatidylethanolamine has been shown to express similar inflammatory properties as those found for LPC. These preliminary findings are, however, not yet fully understood and need further investigation. Furthermore, there are no data available on the inflammatory properties of lysophospholipids in broilers. Nevertheless, due to their prominent function in humans, these types of interactions of lysophospholipids with the immune system of the bird could very well be a possible mode of action of lysophospholipids in lysolecithins (Ni et al., 2014).

Fat absorption

Absorption of all lipids through the enterocyte membrane has for a long time been thought to be a passive process that involved a “flip-flop-like” action across the enterocyte membrane (Lairon, 2009). The knowledge of the uptake and transport of lipids has advanced considerably. Nevertheless, not all details of the lipid absorption process are fully understood (Tso et al., 2004). The present understanding is that SCFA and MCFA are absorbed by simple diffusion through the enterocyte membrane. On the other hand, several studies have indicated LCFA to be absorbed through an active, protein-mediated process (Lairon, 2009). Niot et al. (2009) concluded that an efficient LCFA uptake by enterocytes requires both spontaneous and facilitated transfer. Hence, the relative importance of these two mechanisms needs further investigation. The proteins involved in the active absorption process are called fatty acid transfer proteins (FATP). The FATP is a family of proteins of which $FATP_4$ seems the most common and most active protein in the enterocytes (Tso et al., 2004). In addition to FATP, two other transfer protein groups could participate in the active absorption process of LCFA by chicken enterocytes. Platelet glycoprotein 4 (CD36), also known as fatty acyl translocase (FAT), has recently been identified in chickens (Holmes, 2012). Fatty acid binding protein has been identified earlier but was believed to participate primarily in the transfer of fatty acids within the cytosol of the enterocytes (Yuan et al., 2007). Both transfer protein groups are known to play an active role in the absorption of LCFA (Langhans et al., 2011). Similar to LCFA, monoglycerides, phospholipids and lysophospholipids were originally believed to be absorbed by a passive diffusion process (Lairon, 2009). On the other hand, some recent studies have indicated that monoglycerides, phospholipids and lysophospholipids are also, at least partially, absorbed by protein mediated active transport (Glatz, 2014; Gajda and Storch, 2015).

Factors affecting fat digestion

As with the fat digestion process, many factors can affect fat metabolism. The factors that affect fat metabolism can be categorised into diet-related factors and broiler-related factors.

Diet-related factors

Diet-related factors that can affect fat metabolism are either related to the feeding strategy (feeding and feed restriction) or by the macronutrient composition of the diet.

1. Feeding and feed restriction

Limiting the feed intake in broilers by either feed deprivation or by diluting the feed with feed raw materials with low nutritional value such as rice hulls (a.k.a. qualitative feed restriction) result in a reduction of fat deposition (Fouad and El-Senousey, 2014). Hence the fat metabolism is affected, leading to increased utilisation of the dietary fat for energy rather than for storage. In addition to reduced fat deposition, Richards et al. (2003) showed that the hepatic cells of broiler breeders subjected to feed restriction have a significantly lower expression of genes involved in lipogenesis (e.g. SREBP-1 and acetyl-CoA carboxylase) compared to birds fed ad libitum. The findings of Richards et al. (2003) have been confirmed in broilers by Wang et al. (2009) and Yang et al. (2010). The effect of feed restriction on fat metabolism is believed to be induced by alterations in endocrine factors such as thyroid and pancreatic hormones (Fouad and El-Senousey, 2014).

2. Diet composition

The impact of different macronutrients in the diet has been investigated by several researchers (Sanz et al., 2000; Huang et al., 2008; Poureslami et al., 2010 a; Smink et al., 2010; Rosebrough et al., 2011). In a study by Rosebrough et al. (2011) the metabolism of broilers fed a diet with a low protein level (120 g/kg) was compared to those fed a diet with a high protein level (300 g/kg). The authors investigated the lipogenesis in livers excised from those chickens and showed that increasing dietary protein decreases lipogenesis. The latter suggests that the ratio of fat to protein is important. The ratio of protein to metabolisable energy is known to be crucial for bird metabolism (Lesson and Semmers, 2005). Moreover, as a result of excess energy intake, broilers that are fed diets with high fat content but normal protein levels will have an increased fat deposition. Alterations in the essential amino acid content of diets seem to have a similar effect on fat metabolism, as is observed for changes in total protein contents (Fouad and El-Senousey, 2014).

Increasing dietary fat levels, while maintaining the crude protein level will thus increase fat deposition rather than fatty acid oxidation. Additionally, the expression of FABP in enterocytes and hepatocytes is elevated by increasing dietary intake of fats (Gajda and Storch, 2015). The fat type in the diet, especially the impact of unsaturated fatty acids in the diet has received considerable attention (Abdulla et al., 2019). In a study by Sanz et al. (2000) feeding of iso-caloric diets containing either tallow, lard or sunflower oil as a fat source did not affect performance of the birds. These findings were confirmed by Crespo and Esteve-Garcia (2003). They showed that sunflower or linseed oil has a significantly reduced deposition of fat in the abdominal fat pad. Moreover, in a study by Smink et al. (2010) it was shown that diets rich in C18:2 in comparison with diets rich in saturated fatty acids decrease the deposition of fat, especially of monounsaturated fatty acids, which was again attributed to an increased β-oxidation. Along with traditional digestibility parameters, the β-oxidation in broilers fed diets with palm, soybean, linseed or fish oil was evaluated by Poureslami et al. (2010 a) and Abdulla et al. (2019). The β-oxidation of C18:3 was significantly higher for fish oil than any of the other oils. The latter thus confirms that fats rich in unsaturated fatty acids lead to an increased β-oxidation and reduction of fat deposition in broilers. In addition to the macronutrients, a variety of dietary supplements such as L-carnitine, polyphenols and probiotics or larvae meal have been reported to affect the fat metabolism in broilers (Fouad and El-Senousey, 2014; Kareem et al., 2018).

Broiler-related factors

An important broiler-related factor that can affect fat metabolism is the age of the broiler. Besides the age of the broiler, gender and strain of the broilers can affect fat metabolism.

1. Age

The synthesis of FABP has been reported to be low in young broilers and increases with broilers' age, especially after three weeks of age. In the previously mentioned study by Poureslami et al. (2010 b) the accumulation of C18:2 and C18:3 in body tissue increased, whilst β-oxidation of fatty acids decreased with age. The accumulation of fat tissue is shown to be age dependent and increases with broilers' age. Therefore, it seems that with increasing broilers' age dietary fats are less easily used as an energy source through β-oxidation, but rather deposited as fat in the adipocytes (Tancharoenrat et al., 2013).

2. Gender

It is well known that female broilers tend to deposit more fat than male broilers (Rondelli et al., 2003; Fouad and El-Senousey, 2014). Nevertheless, in the previously mentioned study by Poureslami et al. (2010 b) no significant effect of the broilers' gender on β-oxidation was found and it was concluded that the gender of the broilers had only a marginal effect on the fat metabolism.

Fats in broiler diets

The amounts and the types of fat or oil added to the diet are highly dependent on the price of feed raw materials. For example, if the price of soybean oil is low and the price of corn is high, part of the corn in the diet will be replaced by soybean oil. Lesson and Semmers (2005) have suggested a minimum inclusion level of 1% fat in broilers' diets. Typical inclusion levels for fats and oils are around 2 to 5%. In some diets, inclusion level of fat or oil may reach up to 8% or more. Some researchers have shown the potential of vacuum coating for increasing fat content post pelleting (Lamichhane et al., 2015). This technique is not common in commercial broiler feed mills. Additionally, inclusion of fat and oil in the diet is typically lower in starter diets and is often gradually increased in the grower and finisher diets (Lesson and Semmers, 2005).

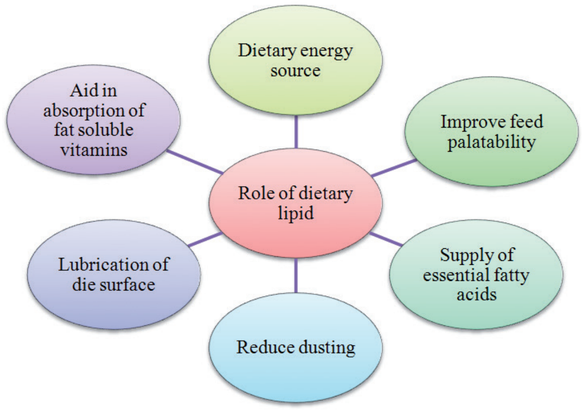

Figure 5. Role of fat in broilers

Not all fats that enter the broilers' digestive system are coming from fats added to the diet. Some percentage of oil is also present in soybeans or grains such as wheat and corn. The lipid contents of soybeans, wheat and corn are around 18–20, 2 and 3.5–4%, respectively (Hammond et al., 2003; Lesson and Semmers, 2005; Wrigley, 2010). Therefore, a diet with 50% corn will already supply approximately 2% of fat to the diet. A variety of dietary fat sources is available for adding to broiler diets. Traditionally, tallow and poultry fat as animal fat sources and soybean oil, canola oil and palm oil as vegetable fat sources have been principal fat sources in broiler diets. As the formulation of feed is cost-driven, so selection of dietary fat sources is important. Therefore, a variety of alternative fat sources such as refined poultry fat and fish oil, soap stocks have entered the feed market (Baião and Lara, 2005; Lesson and Semmers, 2005).

Animal fats are obtained by the rendering of fresh slaughter by-products. The raw materials are chopped in a crusher, followed by heating the material directly or indirectly with steam. By heating, the fat is liquefied and freed from its matrix. The slurry of fats and solids is then separated by drainage. The solid phase is further processed to form poultry by-product meal. The fat phase can also be further purified by sieving, centrifugation and sedimentation (Anderson, 2006). Typical animal fats used in broiler diets include tallow, poultry fat, pig lard and fish oil (Baião and Lara, 2005; Lesson and Semmers, 2005).

Motives for adding fat to broilers' diets

Fats and oils have the highest energy density among all macronutrients and therefore, primarily have been used in broiler feed industry as a supplemental dietary energy source. In literature, physiological fuel values of fats, proteins and carbohydrates are reported as 9, 4 and 4 kcal/g, respectively (Donato and Hegsted, 1985). Moreover, supplementation of fats to broiler diets increases the metabolisable energy of the total diet by increasing the utilisation of other dietary components. This so-called "extra-caloric" effect of the fat originates from the reduced passage rate through the gastrointestinal tract which enhances absorption of all nutrients of diet (NRC, 1994; Swennen et al., 2004; Baião and Lara, 2005). Besides their energetic contribution, addition of fats to broiler diets also improves the absorption of fat-soluble vitamins and essential fatty acids (Villaverde et al., 2004; Baião and Lara, 2005). Dietary fats are a major carrier of fat-soluble vitamins (Baião and Lara, 2005). The added fats enhance the production rates in pellet mills, primarily because of their lubricating effects. In this way, the dustiness of mash feeds is reduced and the quality and durability of pelleted feeds can be improved (Thomas et al., 1998).

A variety of animal and plant fats and oils are used (as energy source) in the formulation of poultry diets (Sanz et al., 2000). Energy value of different fats and oils used in poultry diet is given in Table 1. Digestibility of fats has a great concern about the chemical structure of fats incorporated in the broiler diets. Rapeseed oil that contains less than 2% erucic acid is termed as canola oil (Leeson and Summers, 2001). Apparent metabolisable energy (AME) of diet is determined by the contents of its saturated and unsaturated fatty acids. Birds will obtain less AME, if diet is formulated by using saturated fat source i.e., tallow. Oils are derived from plant sources and contain a significant amount of unsaturated fatty acids. Fats are obtained from animal sources and include a considerable amount of saturated fatty acid (Baião and Lara, 2005).

Addition of sunflower oil (6%) in broilers' diet had improved FCR (1.54 vs 1.68) than those fed palm oil (6%) (Khatun et al., 2018) due to increased unsaturated fatty acid content in sunflower as compared to palm oil. Similarly, sunflower oil inclusion at 2% in broilers' diet resulted in lower feed consumption (3467 vs 3919 and 4150 g) and improved FCR (1.66 vs 1.94 and 1.91) than those fed soybean and fish oil (Liu et al., 2017). Zaefarian et al. (2015) showed that birds fed on soy oil achieved better weight gain (WG) (2125 vs 2027 g) and FCR (1.45 vs 1.49) in comparison to the birds fed on diets fortified by tallow as a dietary energy source. This is because soy oil shows higher fat retention than those containing tallow. Higher weight gain (1512 vs 1390 g) and feed intake (FI) (2809 vs 2616 g) were recorded in birds fed palm oil in their diet than lard oil (Zhong et al., 2014). Use of soybean oil in broiler diet caused greater weight gain and better FCR compared to those fed poultry fat and tallow (Zhang et al., 2011). Addition of poultry fat (PF) and canola oil (CO) at 3% in broilers' diet caused higher weight gain (PF: 2000 g, CO: 1965 g) and better FCR (PF: 1.70, CO: 1.74) (Shahryar et al., 2011). Hosseini-Mansoub and Bahrami (2011) used four levels (0, 1, 2 and 4%) of fish oil in broilers' diet and showed that body weight gain was greater in birds fed diet having 2% fish oil (2248 vs 2101 g).

Addition of palm oil sludge in broilers' diet at 5% had improved BW (1.67 vs 1.44 and 1.27 kg), FI (4.14 vs 3.88 and 3.87 kg) and FCR (2.48 vs 2.71 and 3.06) compared to those fed palm oil sludge at 0 and 2.5% (Dada, 2003). Fascina et al. (2009) reported that birds fed 75:25 ratio of soybean and tallow oil had improved weight gain (875 vs 810 g) and FCR (1.4 vs 1.48) during starter phase. In contrast, effect of poultry fat and soybean oil in broilers' diet had no difference on FI, WG and FCR (Polycarpo et al., 2014). Addition of soybean oil and blend of different vegetable oils had no effect on FI, WG and FCR (Abudabos, 2014). Different oil sources (beef tallow and canola oil) in broilers had no effect on growth performance (Meng et al., 2004).

Table 1. Energy value of different fats and oil for poultry (BTPS, 2017)

Fat and oils	Gross energy (Kcal/kg)	Metabolisable energy (Kcal/kg)	Net energy (Kcal/kg)
Canola oil	9399	8784	7906
Sunflower oil	9332	8840	7250
Corn oil	9350	8773	7896
Soybean oil	9333	8790	7911
Poultry fat	9282	8681	7812
Lard fat	9369	8080	7272
Beef tallow	9408	7401	6660
Palm oil	9400	8817	7934
Coconut oil	9229	7924	7132
Fish oil	9298	8600	7150

1. Vegetable oils

A long-range of vegetable oil has been used as an energy source, but availability is limited due to the utilisation of fats and oils in human consumption and the only refusal by human industry is the presence of impurities in oil (Lesson and Semmers, 2005). Soybean, sunflower and corn oil are being utilised as a dietary energy source in poultry diets. Vegetable oil is a good source of polyunsaturated fatty acids (PUFAs). PUFAs are those fatty acids that contain more than one double bond. These polyunsaturated fatty acids are highly digestible by chickens but more prone to oxidation. Vegetable oil contains essential fatty acids i.e., arachidonic linoleic and linolenic acid that is considered necessary for the optimum growth of broiler chicken (Balevi and Coskun, 2000). Canola oil is being used as an energy source, but the only limitation of the addition of canola oil in the feed is the presence of erucic acid. The occurrence of erucic acid makes the bird unable to utilise energy from the diet (Leeson and Summers, 2001).

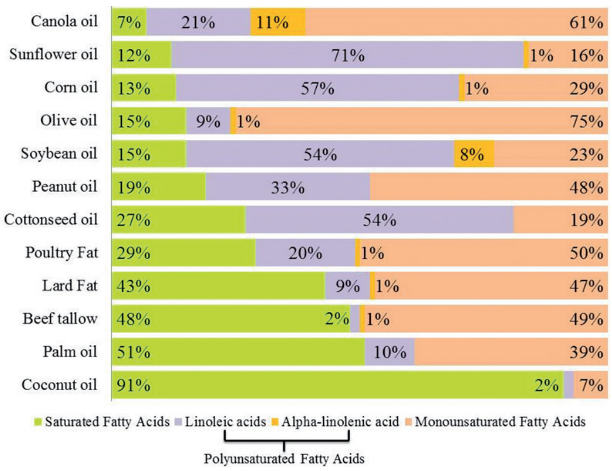

Figure 6. Fatty acid composition of different fats and oils (Kaur et al., 2014)

Long et al. (2018) reported that weight gain (2448 vs 2305 g) and FCR (1.55 vs 1.66) were improved by feeding combination of soybean with coconut, coconut, palm, linseed and corn oil than along soybean oil. Kang and Kim (2016) showed that 5% replacement of rice bran oil with soybean oil caused lower FI (2769 vs 2889 g) and better FCR than 0% replacement. Nobakht et al. (2011) used sunflower oil, canola oil, soybean oil and a combination and showed that inclusion of oil in diet had improved FCR (1.83 vs 2.14). Soybean oil at 4% in low energy diet caused higher body weight (2472 vs 3407 g) and better FCR (1.8 vs 1.88) than those fed beef tallow (Monfaredi et al., 2011). Zhong et al. (2014) showed that broiler birds fed on a mixture of linseed and palm oil (60:40) had higher WG than palm and linseed oil alone (1646 vs 1512 and 1386 g). Poorghasemi et al. (2013) observed that birds fed with tallow and canola oil had higher WG and better FCR. This increase in WG is due to the actual ME of each dietary fat source and higher retention time of feed in the gut which is an advantage of the addition of fats/oil.

2. Animal fats

A range of animal fat sources is utilised in poultry diets to fulfil the energy requirement of birds i.e., tallow, lard and poultry fat. Shoaib et al. (2021 b) observed that poultry fat caused lower feed intake (3134 vs 3304 and 3278 g), higher weight gain (1832 vs 1789 and 1741 g) and improved FCR (1.72 vs 1.84 and 1.88) than fish and palm oil. Shahryar et al. (2011) revealed that addition of canola oil and poultry fat at 3% in broilers' diet resulted in higher weight gain (PF: 2000 g, CO: 1965 g) and better FCR (PF: 1.70, CO: 1.74). De Witt et al. (2009) reported that use of different oils (fish oil, high oleic sunflower oil, lard, sunflower) at 3 and 6% of both levels had no effect on growth performance in broiler birds. Firman et al. (2008) experimented evaluating the difference between seven different fats and oil sources (tallow, lard, poultry fat, yellow grease, soy oil, palm oil and vegetable and animal blend). Results showed addition of rendered fat produces almost equivalent results in comparison to plant-sourced oils. Ghazalah et al. (2008) reported that increasing poultry fat level in broilers' diet from 0 to 5%, resulted in higher body weight (1812 vs 1720 g), improved FCR (2.19 vs 2.39) and performance index (82.7 vs 73.5). Birds fed diet having 7.5% tallow had higher WG (1323 vs 176 g) in broilers fed diet having low energy than NRC recommendation levels (Sadeghi and Tabiedian, 2005). Skrivan et al. (2000) showed that feeding 0.5% lard oil in broilers' diet had improved WG (1973 vs 1799 g) compared to those on 0.5% rapeseed oil.

Effect of fat sources on nutrient utilisation in broilers

Shoaib et al. (2021 b) observed that poultry fat had higher digestibilities of EE (69 vs 64 and 63%) and CP (65 vs 61%) than fish and palm oil. Birds fed diet supplemented with palm oil had higher phosphorus digestibility than those fed linseed and soybean oil (Abdulla et al., 2016). Soybean oil in broilers' diet resulted in greater nitrogen digestibility (57.96 vs 55.94%) in broiler birds (Polycarpo et al., 2014). In contrast, different oil sources had no effect on nutrient digestibility (Meng et al., 2004; Abdulla et al., 2016). This might be due to lower oil levels or variations in data recorded. Firman and Remus (1994) experimented for examining the effect of the addition of corn oil on the digestibility of amino acids of bone meal and feather meal. Corn oil was added at 5% and 10% inclusion rate and the result showed that birds fed with the inclusion of corn oil showed a 5–6% increase in amino acid digestibility. This increase in digestibility is related to an increase in the retention time of feed in the gut leading to more exposure to digestive enzyme. Pesti et al. (2002) studied the utilisation of poultry fat, yellow grease, white grease, palm oil and vegetable oil. It was noted that the metabolisable energy content of fat sources was significantly different from each other. Lowest ME was obtained from supplementation of poultry fat. Less utilisation of ME is due to the structural differences between each fat and oil source. Soy oil provides ME with the highest value as it includes more polyunsaturated fatty acids, which are more digestible.

Effect of fat sources on carcass response and meat quality in broilers

Shoaib et al. (2021 b) observed that canola oil had higher water holding capacity (63.31%) than fish

(53.31%) and palm oil (54.93%). Addition of poultry fat and canola oil in broilers' diet caused lower gizzard weight and abdominal fat, while carcass weight was not affected compared to the control group (without oil) (Shahryar et al., 2011). Birds fed a dietary blend of sunflower, rapeseed and tallow oil had higher carcass yield than those fed only animal fat (Mohammed and Horniaková, 2012). Different oil sources in broiler diet had no effect on carcass response (Neto et al., 2011; Dorra et al., 2014; Polycarpo et al., 2014). Different oil sources (soybean oil and beef tallow) and levels of oil (2 and 4%) had no effect on carcass response (Monfaredi et al., 2011). Use of sunflower oil and palm oil in broiler diet had no effect on meat quality parameters (Khatun et al., 2018). Meat quality parameters were similar by the use of recovered oil in the diets (Dorra et al., 2014). Replacement of rendered poultry fat and soybean oil with red palm oil had no effect on sensory evaluation (Nyquist et al., 2013).

Effect of fat sources on blood haematology and serum biochemistry in broilers

Use of palm oil (6%) caused higher serum cholesterol (3.26 vs 2.49), triglycerides (0.68 vs 0.42) and LDL (1.51 vs 0.71) content than sunflower oil in broilers' diet (Khatun et al., 2018). Some alternative ingredients, such as earthworms, algae and azola can be considered as source of oil enriched with omega-3 and omega-6 PUFA (Alshelmani et al., 2021). Long et al. (2018) studied the effect of replacement of soybean oil with two different combinations of multiple oils. Combination I contained 25% soy, 10% coconut, 15% peanut, 20% palm, 15% linseed and 15% corn oil. Combination II included 50% from combination one and 50% extruded corn. Results showed a decline in total cholesterol in the birds fed on first combination (2.8 vs 3.12 mmol/l). Canola oil in broiler diet had lower serum triglycerides and LDL cholesterol than sunflower oil (Ghasemi et al., 2016). Birds that received contaminated water with crude oil at 10.5 ml had lower plasma protein, HBC, mean corpuscular haemoglobin concentration (MCHC) and PCV than those that received clean water (Akporhuarho et al., 2015). Dietary red palm oil in broiler diet had lower cholesterol than rendered animal fat and soybean oil (Nyquist et al., 2013). Hosseini-Mansoub and Bahrami (2011) used four levels (0, 1, 2 and 4%) of fish oil in broilers' diet and reported that inclusion of 2% fish meal increased blood glucose (110 to 123 mg/dl) and decreased protein (3.85 to 3.65 mg/dl), albumin (2.1 to 1.9 mg/dl) and globulin level (1.72 to 1.65 mg/dl). These changes are due to enrichment of diet with omega-3 fatty acids from fish oil. Addition of higher level (4%) of soybean oil and beef tallow caused higher cholesterol (89.8 and 98.5 vs 84.9), higher HDL (54.8 and 40.4 vs 37.7) and lower LDL content (28.1 vs 39.4) of blood than control group (Monfaredi et al., 2011). Soybean and lard oil in broiler diet had no effect on serum cholesterol, triglycerides and LDL content (Burlikowska et al., 2010).

Effect of emulsifier on production performance in broilers

Fats are not soluble in water (Upadhaya et al., 2017), while in the gut of birds there is an aqueous environment. For that reason absorption takes place through the process of emulsification (Gu and Li, 2003). Emulsifiers are known to be a potential source for improving fat utilisation, by increasing fat digestibility and AME of fats (Allahyari-Bake and Jahanian, 2017; Jaapar et al., 2020). Emulsifiers are also defined to lower the surface tension of water which helps in the formation of emulsion droplets (Melegy et al., 2010). Natural emulsifiers are nutritional emulsifiers like lecithin and lysolecithin (Ravindran et al., 2016). Lecithin obtained by the processing of soy oil is hydrolysed to produce lysolecithin. Hydrolysis of phospholipids with phospholipase A_2 leads to the formation of lysophospholipids which has better oil in water emulsification properties (Joshi et al., 2006). The functionality of emulsifiers is true for almost every type of fat/oil. Supplementation of emulsifiers on lard fortified diets increases up to 3% digestibility of fats (Zosangpuii et al., 2015), in soybean oil based diets there was 3.5% increase in digestibility of fats (Hosseini et al., 2018). Following points should be considered before choosing an emulsifier for poultry.

1. Hydrophilic-lipophilic balance

While selecting emulsifier for supplementing in poultry diet, it is considered that the hydrophilic-lipophilic balance (HLB) value of emulsifier should be higher (Table 2). As birds drink almost double the amount of water in comparison to feed, the amount of water is much higher in the intestine. So, in this case, emulsifier having high HLB is required (Siyal et al., 2017). Supplementation of emulsifiers having a high value of HLB will perform better and supplementing such emulsifiers will lead to increased fat digestibility and this resulted in a higher apparent metabolisable energy on nitrogen corrected basis (AMEn) value of the diet (Rovers and Excentials, 2014).

2. Critical micellar concentration (CMC)

The size of the micelles is reported to vary between 4 and 60 nm. The formation of bile salt micelles depends on the concentration of bile salts in solution. A minimum concentration exists, which is necessary to form a micellar solution, the so-called critical micellar concentration (Bauer et al., 2005). The CMC values of bile salts depend on the solution in which they are. The CMC of taurocholate is situated between 4 and 20 mM and that of taurochenodeoxycholate between 6 and 9 mM (Maldonado-Valderrama et al., 2011).

3. Phospholipids and lysophospholipids as emulsifiers

Phospholipids (glycerophospholipids) and lysophospholipids are key components of all biological membranes, but also have a variety of other functions such as cellular messaging and enzyme activating. Phospholipids are composed of a sn-1,2-diacylglycerol with a phosphate residue in position sn-3, which in turn is bound to an amino-alcohol (choline or ethanolamine), amino acid (serine), carbohydrate (inositol) or other (e.g. hydrogen) functional moiety. Lysophospholipids are derivatives of phospholipids. They are composed of either a sn-1-monoacylglycerol or a sn-2-monoacylglycerol with a phosphate residue in position sn-3, which is linked in turn to the respective functional moiety (Figure 7). Phospholipids and lysophospholipids are polar lipids of different classes. Therefore, they will behave and interact differently (Allahyari-Bake and Jahanian, 2017). In an aqueous environment, phospholipids tend to form double layers or liposomes, regardless of the functional moiety attached to the phosphate residue. Lysophospholipids on the other hand will tend to form micelles (Lairon, 2009).

Table 2. Selection of emulsifier on hydrophilic-lipophilic balance

Ratio (%)		HLB value	Emulsifying role in water	Function
hydrophilic (water loving)	lipophilic (fat loving)			
0	100	0	High lipophilic emulsifier	Advantage for water in oil emulsion
10	90	2		
20	80	4	↑ ↑ ↑	
30	70	6		
40	60	8		
50	50	10	↓ ↓ ↓	
60	40	12		
70	30	14		
80	20	16	High hydrophilic emulsifier	Advantage for oil in water emulsion
90	10	18		
100	0	20		

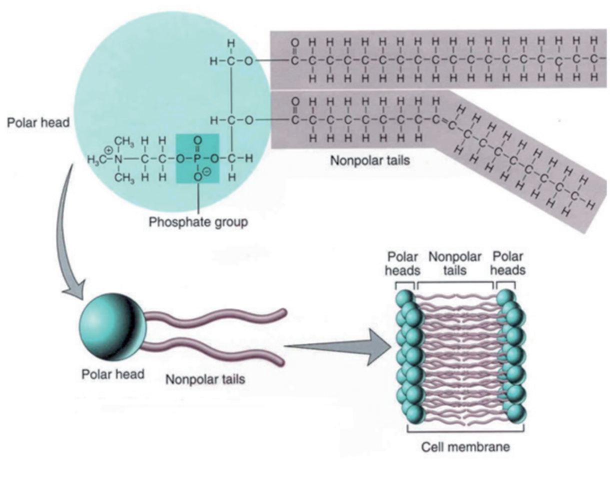

Figure 7. Chemical structure of phospholipid

4. Lecithin and lysolecithin as emulsifiers

Lysophospholipids are supplemented to broiler diets under the form of lysolecithin. Lysolecithin is generated by enzymatic conversion of lecithin (Figure 8). Lecithin itself is obtained during the water degumming step of crude vegetable oil processing. The obtained lysolecithin can be further purified to obtain fractions rich in specific (lyso) phospholipids on treatment with phospholipase (Peña et al., 2014). Lecithin has been used to refer to PC alone or to a group of lipids containing phosphorus. From a commercial point of view, lecithin (E322) is a mixture of polar and nonpolar lipids with a minimum of 60% acetone insoluble matter.

Addition of 0.1% emulsifier in broilers' diet had increased weight gain and FCR during the period of 0 to 19 days (An et al., 2020). Kamran et al. (2020) used different levels (0, 0.025, 0.035, 0.045%) of polyglycerol polyricinoleate (PGPR) in broilers' diet on three oil sources (poultry fat, soy oil and oxidized soy oil) and concluded that use of PGPR in soy oil based diet had improved FCR than control diet in broilers. Use of 0.05% lysophospholipid in broilers' diet had improved feed intake and FCR (Chen et al., 2019). Addition of Orffa energizer 2 at 0.035% in rice bran based diet had improved body weight gain and FCR (Kulkarni et al., 2019).

Siyal et al. (2017) used two levels (0.05 and 0.1%) of soy lecithin in diet containing palm oil as energy source. Birds fed on soy lecithin achieved significantly better average WG and FCR in comparison to control group. Addition of emulsifier (0.1%) in broilers' diet had improved FCR as compared to 0 and 0.05% inclusion of emulsifier, while feed intake and body weight was not affected (Zosangpuii et al., 2015; Zhao and Kim, 2017). Zampiga et al. (2016) reported that lysolecithin in broiler diet significantly improved FCR. Addition of emulsifier (0.05%) with rice bran oil in broilers' diet had higher weight gain (2281 vs 2201 g) than control group (Tan et al., 2016).

Kaczmarek et al. (2015) used two levels (SE = standard energy, LE = low energy) of apparent metabolisable energy to evaluate the response of emulsifier by using a blend of rapeseed oil and tallow. Reduced AMEn (LE) of supplemented diet reduced WG (2005 vs 2041 g). However, emulsifier supplementation on low energy diet improved WG (2060 vs 1950 g), particularly. Birds fed diet having 0.1% multi-enzyme + 0.05% emulsifier in low density diet had greater feed intake than high density diet (Cho et al., 2012). Broilers reared on diet having soybean oil with emulsifier had higher weight gain and better FCR (Neto et al., 2011). Addition of emulsifier at 1% of total oil (palm oil) caused higher body weight gain and better FCR than control group (without emulsifier) (Roy et al., 2010). Supplementation of ox bile at 0.5% in broilers' diet had increased weight gain (1624 vs 1379 g) and improved FCR (2 vs 2.39) compared to control group (Alzawqari et al., 2011). There was no interaction between emulsifier and different oil sources (poultry fat, soybean oil and tallow) on growth performance in broilers (Zhang et al., 2011). Melegy et al. (2010) prepared negative control diet of low nutrient density and a positive control diet of optimum nutrient density. While, negative control diets were supplemented by 250 and 500 g/ton of emulsifier, respectively. Supplementation of emulsifier on negative control diets helps birds to achieve almost equal WG and FCR in comparison to birds fed on positive control diet (WG: 2262, 2289 and 2252 g, FCR: 2.0, 1.98 and 2.0). Zaefarian et al. (2015) reported that supplementation of emulsifier improved feed intake (3049 vs 2966 g) and WG (2103 vs 2049 g). Khonyoung et al. (2015) reported that emulsifier in broilers had improved feed efficiency up to 21 days (Table 3).

Effect of emulsifier on carcass response and meat quality in broilers

Emulsifier (0.035%) in broilers fed diet containing different oil levels (1, 2 and 3%) caused greater heart weight (8.51 vs 7.01 g) than control group (Abbas et al., 2016). Kamran et al. (2020) used different levels (0, 0.025, 0.035, 0.045%) of PGPR in broilers' diet reared on three oil sources (poultry fat, soy oil and oxidized soy oil) and reported that lightness, redness, yellowness and pH were not influenced by different dietary treatments. Broilers' diet supplemented with multi-enzyme and emulsifier along with low density diet had no effect on breast yield with high density diet (Cho et al., 2012). Use of emulsifier in broiler diet had no effect on meat quality and sensory evaluation parameters (Zhao and Kim, 2017). Zampiga et al. (2016) reported that lysolecithin in broilers' diet had no positive effect on breast and thigh yield. Use of emulsifier (0.1%) had no effect on meat quality parameters in broilers (Zosangpuii et al., 2015).

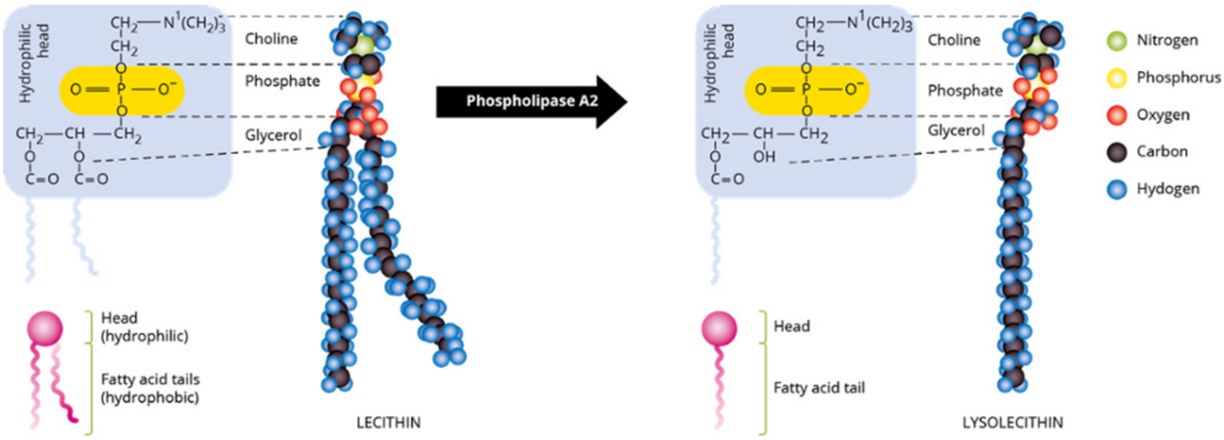

Figure 8. Conversion of lecithin to lyso-lecithin

Table 3. Energy released and increase in fat digestibility with the use of emulsifier

Ingredients	Inclusion %	Energy released (kcal/kg)	Increase in fat digestibility (%)	References
Soybean oil + Tallow	3.6 + 1.5	80		(Srinivasan et al., 2022)
Soybean oil	3	100	2.48	(Ahmadi-Sefat et al., 2022)
Poultry fat	4.62	75	5.06	(Shoaib et al., 2021 c)
Soybean oil	2.5	50	8.92	(Saleh et al., 2020)
Tallow	4.0	100	3.56	(Hu et al., 2018)
Soybean oil	4.4	–	3.5	(Hosseini et al., 2018)
Tallow	3.90	100	–	(Mohammadigheisar et al., 2018)
Soybean oil	2.5	72	5	(Papadopoulos et al., 2018)
Soybean oil	2.4	–	2.7	(Allahyari-Bake and Jahanian, 2017)
Tallow	5.03	108	1.6	(Zhao and Kim, 2017)
Rice bran oil	4.5	106	2.6	(Tan et al., 2016)
Soybean oil Pig lard	5 5	56 182	1 3	(Jansen et al., 2015)
Soybean oil Tallow	2 2	– –	1.3 3.5	(Zosangpuii et al., 2015)
Soybean oil Poultry fat	4 4	62 81	– –	(Zhang et al., 2011)
Poultry fat	4.5	–	4.6	(Neto et al., 2011)

Effect of emulsifier on nutrient utilisation in broilers

Liu et al. (2020) reported that fat digestibility was higher in birds receiving 0.1% lecithin (97% de-oiled) in basal diet. Addition of 0.1 and 0.2% exogenous emulsifier in broilers' diet had improved energy digestibility (An et al., 2020). Kamran et al. (2020) observed that birds fed diet supplemented with PGPR at 0.035 in soy oil based diet had improved digestibilities of dry matter (DM; 71.75 vs 70.66%) and EE (79.01 vs 76.58%). This is because PGPR increases fat digestion in gastrointestinal tract.

Siyal et al. (2017) used two levels (0.05 and 0.1%) of soy lecithin in diet containing palm oil as energy source. Birds fed on soy lecithin at 0.1% had higher digestibilities of gross energy and EE, while digestibilities of DM and CP remained unaffected. Emulsifier (0.05%) inclusion with rice bran oil in broilers' diet caused higher fat (88.66 vs 86.1%) digestibility (Tan et al., 2016). Kaczmarek et al. (2015) used two levels (SE = standard energy, LE = low energy) of apparent metabolisable energy to evaluate the response of emulsifier by using blend of rapeseed oil and tallow. The emulsifier supplementation improved the digestibility of gross energy only in low energy diets (72.2 vs 66.8%). This is due to increased fat digestibility by emulsifier. Addition of emulsifiers in broilers' diets containing poultry fat had improved EE digestibility than control group (Neto et al., 2011). Use of emulsifier caused greater apparent faecal digestibilities of OM (62 vs 58.6%), DM (59.7 vs 56.2%) and CP (77.4 vs 75.6%) than without emulsifier group (Dierick and Decuypere, 2004). Emulsifier (0.1%) group had no effect on digestibilities of DM, crude fibre and CP with non-emulsifier group (Zampiga et al., 2016). There was no interaction between emulsifier and different oil sources (soybean oil, poultry fat and tallow) on DM, CP and AME in broilers (Zhang et al., 2011). Supplementation of emulsifier in broilers' diet containing different oil sources had no influence on digestibilities of EE, CP and OM with non-emulsifier group (Allahyari-Bake and Jahanian, 2017).

Effect of emulsifier on blood haematology and gut health in broilers

Liu et al. (2020) observed higher HDL (109 vs 101 mg/dl) and lower LDL (33 vs 43 mg/dl) cholesterol in birds receiving 97% de-oiled lecithin in basal diet. Serum cholesterol, LDL and triglycerides concentrations were lower in birds fed diet containing emulsifier (lysophospholipids) (Zhao and Kim, 2017). Two levels of energy and supplementation of emulsifier had no effect on cholesterol, triglycerides, LDL and HDL (Aguilar et al., 2013). Birds fed diet having 0.05% emulsifier in low density diet had lower triglyceride concentrations than those fed high density diet (Cho et al., 2012). Use of emulsifier (glyceryl polyethylene glycol ricinoleate) in broilers' diet had no effect on HDL and LDL cholesterol compared to control diet (Roy et al., 2010). In contrast, cholesterol content was not affected by use of emulsifier (GPGR) in different oil based diets (soybean, palm oil and lard) (Zosangpuii et al., 2015).

Chen et al. (2019) revealed that use of 0.05% lysophospholipid in broilers' diet had improved VH (2257 vs 2028 mm) and VH/CD (12.35 vs 9.25) and reduced CD (188.97 vs 228.02 mm). Brautigan et al. (2017) showed that addition of lysolecithin in broiler diet increased villus height and width of jejunum of broilers. However, Zosangpuii et al. (2015) found no effect of emulsifier (glycerol polyethylene glycol ricinoleate: GPGR) at 0.04% on villi length of duodenum, jejunum and ileum.

Effect of lipase on production performance in broilers

Lipase is an enzyme that catalyses the hydrolysis of triglycerides into fatty acids and glycerol. Most of the fat present in the diet is digested in the duodenum by the action of pancreatic lipase. Lipase enzyme is water-soluble, so it can act only on the surface of fat molecules. Emulsification by bile salt breaks down the bigger fat molecules into smaller droplets and greatly enhances the surface area of fat molecules to be acted on by pancreatic lipase. Triglycerides are then hydrolysed to diglycerides, monoglycerides, fatty acid and glycerol (Arshad et al., 2020). Use of 0.03% lipase in broilers fed reduced energy diet had improved FCR (1.38 vs 1.48). On the other hand, body weight gain was not affected (Hu et al., 2018). Soya lecithin and lipase (100000 IU/ton) in broilers' diet had higher FI (3810 vs 3508 g), WG (2050 vs 1757 g) and better FCR (Nagargoje et al., 2016). On the contrary, Al-Marzooqi and Leeson (1999) reported that 0.714% lipase enzyme had negative effect on FCR and FI. Enzyme activity was claimed to be 25 units USP/mg. Lipase addition at 0.02% had no effect on growth performance of broilers fed various sources of oil (canola oil and beef tallow) (Meng et al., 2004). Feed intake and weight gain was increased with increasing the levels (0, 0.375, 0.750, or 1.125%) of lipase in broilers' diet during starter phase (Al-Marzooqi and Leeson, 2000). Supplementation of lipases may improve fat digestibility (Preston et al., 2001). Al-Marzooqi and Leeson (1999) showed that increasing concentration of lipase in the diet improves fat digestibility and resultantly FCR. Al-Marzooqi and Leeson (2000) demonstrated that addition of lipase did not affect motility and morphology of gut.

Effect of lipase on nutrient utilisation in broilers

Birds fed diet with 0.03% lipase enzyme had higher digestibilities of DM (76.12 vs 74.66%) and EE (79.95 vs 77.20%) than control and reduced energy diet (Hu et al., 2018). Addition of lipase had improved apparent ileal digestibility of OM (62 vs 58.6%), DM (59.7 vs 56.2%) and CP (77.4 vs 75.6%) (Dierick and Decuypere, 2004). In contrast, lipase supplementation at 0.02% in broilers' diet had no effect on digestibilities of fat, starch and nitrogen in broilers (Meng et al., 2004). According to Alzawqari et al. (2011), fat digestibility was higher in birds that received 0.25 and 0.5% desiccated ox bile during starter phase. Similarly, during finisher phase, use of 0.25% and 0.50% desiccated ox bile had improved fat digestibility. Tan et al. (2000) demonstrated that dietary supplementation of lipase caused a numerical increase in fat digestibility and AMEn of birds. Al-Marzooqi and Leeson (1999) reported that 0.0714% lipase had improved fat digestibility (84 vs 76%) and AME_n (2974 vs 2814 kcal/kg) compared to control at 12 days.

Effect of lipase on carcass characteristics, blood chemistry and gut health in broilers

Soya lecithin and lipase (100000 IU/ton) caused higher carcass yield (77.64 vs 73.62%) in broilers than control group (Nagargoje et al., 2016). Feeding birds a diet containing 0.015 and 0.03% lipase enzyme in reduced energy diet had no effect on muscle pH, drip loss and water holding capacity (Hu et al., 2018). Birds fed diet having lipase (1.125%) had increased liver weight at day 21 (Al-Marzooqi and Leeson, 2000). Similarly, abdominal fat weight/eviscerated weight was improved (1.14 vs 1.45%) with the supplementation of bile acids (Lai et al., 2018 b). Al-Marzooqi and Leeson (2000) reported that with an increasing level of lipase enzyme, liver weight was increased at day 21, but it was unaffected at 42 days of age. However, pancreas and heart weight were unaffected due to dietary treatments. Abdominal fat was unaffected by the supplementation of lipase in broilers fed diets having 25% high oleic acid (Brenes et al., 2008). In contrast, Hu et al. (2018) reported that 0.015% and 0.03% lipase enzyme in low energy diets reduced abdominal fat percentage at 28 days of age. According to Hu et al. (2018), lipase enzyme had no effect on meat quality and sensory evaluation parameters. Birds fed diet with 0.03% lipase enzyme in low energy diet had lower triglycerides (78.7 vs 85.6 mg/dl) and LDL (20 vs 23 mg/dl) than control diet (Hu et al., 2018). Hu et al. (2018) concluded that supplementation of lipase caused higher villus height (911 vs 778 µm) and VH:CD (8.59 vs 6.82) in broilers reared to 100 kcal/kg reduced energy diet. Histological examination of the small intestine was not influenced by different levels (0, 0.268, 0.536, 0.804, 1.071 and 1.339%) of lipase supplementation (Al-Marzooqi and Leeson, 2000).

Effect of bile acid on growth performance in broilers

Bile salts are considered to be natural emulsifiers produced in the body of animals (Noy and Sklan, 1995) and some naturally derived exogenous emulsifiers are also used as a supplement in poultry diets (Soares and Lopez-Bote, 2002). Naturally, bile salts are released from liver for emulsification and micelle formation of fats. As described earlier, secretion of lipase is less at the early age of birds and increases with their age (Noy and Sklan, 1995).

Shoaib et al. (2022) reported that use of bile acids had improved weight gain, FCR and protein efficiency ratio than lysolecithin and lysophospholipid. Kwak et al. (2022) used three levels (0.0005, 0.001 and 0.0015%) of Sophorolipid, a glycolipid emulsifier and observed that body weight was higher in grower phase with addition of 0.001% Sophorolipid in broiler diet. Shoaib et al. (2021 a) showed that use of bile acid at 0.05% in broilers' diet caused higher weight gain 2042 vs 1899 g) and better FCR (1.58 vs 1.66). Shoaib et al. (2021 c) concluded that use of lipase and bile acid in reduced energy diet caused higher weight gain (2146 vs 1961g) and better FCR (1.58 vs 1.77). Lai et al. (2018 b) observed increase in ADG (73.86 vs 70.93 g) due to supplementation of 0.006% and 0.008% bile acids as compared to control. Alzawqari et al. (2016) showed that supplementation of bile salts had no pronounced effect on production performance.

Parsaie et al. (2007) reported that addition of exogenous bile salts and xylanase on a wheat based diet had improved WG and FI but feed consumption ratio remained unaffected throughout the trial. Villus length of duodenum of birds fed on bile acid decreased in comparison to the birds fed on xylanase enzyme. Nazir (2014) reported that weight gain was not affected by different levels of bile acids (0, 0.03% and 0.06%). In contrast, Maisonnier et al. (2003) reported that 0.3% bile salts had improved growth performance (WG: 440 vs 399 g) in broiler chickens (Table 4).

Table 4. Effect of fat mobilisers on production performance in broilers

Fat mobilisers	Dose	Result	Reference
1	2	3	4
Lysolecithin, lysophospholipid and bile acids	0.05%	Bile acids addition improved weight gain, FCR and protein efficiency ratio	(Shoaib et al., 2022)
Sophorolipid, a glycolipid emulsifier	0.0005, 0.001 and 0.0015%	Body weight was higher in grower phase with addition of 0.001%	(Kwak et al., 2022)
Emulsifier blend (phosphatidyl choline, lysophosphatidyl choline and polyethylene glycol ricinoleate)	0.1 and 0.2%	Higher weight gain and better FCR	(Ahmadi-Sefat et al., 2022)
Lysolecithin	0.050, 0.075 and 0.10%	Lower feed intake and better FCR	(Mahmood et al., 2022)
Herbal emulsifier, AV/PFE/15	0.025%	Improved growth performance	(Gole et al., 2022)
Blend of lysophospholipids and phospholipids	0.025%	Higher weight gain, lower feed intake and better FCR	(Srinivasan et al., 2022)

Table 4 – contd.

1	2	3	4
Lysophospholipid (Lipidol)	0.1%	Higher weight gain, lower feed intake and better FCR	(Kamel et al., 2022)
Bile acid	0.05%	Higher weight gain and better FCR	(Shoaib et al., 2021 a)
Bile acid + lipase	0.05% and 0.015%	Higher weight gain and better FCR	(Shoaib et al., 2021 c)
Lysophospholipid	0.05%	Improved weight gain and FCR	(Shahid et al., 2021)
Lysophospholipid (Lipidol)	0.1%	Improved weight gain and FCR	(An et al., 2020)
Polyglycerol polyricinoleate	0.025, 0.035 and 0.045%	Improved FCR	(Kamran et al., 2020)
Mixture of phosphatidyl choline, lysophosphatidyl choline and polyethylene glycol ricinoleate	0.05%	Improved weight gain and FCR in 50 kcal/kg reduced energy diet	(Saleh et al., 2020)
Lysophospholipid	0.05%	Improved feed intake and FCR	(Chen et al., 2019)
Orffa energizer	0.035%	Improved body weight gain and FCR	(Kulkarni et al., 2019)
Lipase	0.03%	Improved FCR in low energy diet	(Hu et al., 2018)
Bile acids	0.006 and 0.008%	Increase in ADG	(Lai et al., 2018 b)
Soy lecithin	0.05 and 0.1%	Better average WG and FCR	(Siyal et al., 2017)
Lysophospholipid (Lipidol)	0.1%	Improved FCR	(Zhao and Kim, 2017)
Lecithin	0.035%	Better FCR	(Abbas et al., 2016)
Bile salts	0.05%	Improved weight gain and FCR	(Alzawqari et al., 2016)
Soya lecithin + Lipase	50% of oil and 100000 IU/ton	Higher FI, WG and better FCR	(Nagargoje et al., 2016)
Lysolecithin	0.1%	Improved FCR	(Zampiga et al., 2016)
Polyethylene glycol ricinoleate	0.05%	Improved weight gain	(Tan et al., 2016)
Sodium stearoyl-2-lactylate (Prosol®)	0.05%	Higher feed intake	(Cho et al., 2012)
Ox bile	0.5%	Improved weight gain and FCR	(Alzawqari et al., 2011)
Bile salts	0.05%	Improved WG and FI	(Parsaie et al., 2007)
Bile salts	0.3%	Better body weight gain	(Maisonnier et al., 2003)
Lipase	0, 0.37%, 0.75%, 1.12%)	FCR was improved with increasing levels of lipase enzyme	(Al-Marzooqi and Leeson, 2000)

Table 5. Effect of fat mobilisers on nutrient digestibility in broilers

Fat mobilisers	Dose	Result	Reference
1	2	3	4
Lysolecithin, lysophospholipid and bile acids	0.05%	Bile acids addition increased CP and EE digestibility	(Shoaib et al., 2022)
Emulsifier blend (phosphatidyl choline, lysophosphatidyl choline and polyethylene glycol ricinoleate)	0.1 and 0.2%	Improved protein, fat and energy digestibility	(Ahmadi-Sefat et al., 2022)
Glyceryl polyethyleneglycol ricinoleate	0.035%	Improved digestible energy	(Tenório et al., 2022)
Lysolecithin	0.050, 0.075 and 0.10%	Improved fat digestibility	(Mahmood et al., 2022)
Bile acid + lipase	0.05 and 0.015%	Higher digestibilities of EE and CP	(Shoaib et al., 2021 c)
Lysophospholipid	0.05%	Improved EE digestibility	(Shahid et al., 2021)
Lecithin (97% de-oiled)	0.1%	Fat digestibility was higher	(Liu et al., 2020)
Mixture of phosphatidyl choline, lysophosphatidyl choline and polyethylene glycol ricinoleate	0.05	Improved protein and ether extract utilisation	(Saleh et al., 2020)
Lysophospholipid (Lipidol)	0.1%	Improved energy digestibility	(An et al., 2020)
Polyglycerol polyricinoleate	0.035%	Improved digestibilities of dry matter and EE	(Kamran et al., 2020)
Globin	0.05%	Increased protein digestibility and energy efficiency	(Dabbou et al., 2019)
Lipase	0.03%	Higher digestibilities of DM and EE	(Hu et al., 2018)
Lysophospholipid (Lipidol)	0.1%	Improved digestibilities of gross energy and CP	(Zhao and Kim, 2017)
Soy lecithin	0.1%	Higher digestibilities of gross energy and EE	(Siyal et al., 2017)

Table 5 – contd.

1	2	3	4
Lecithin	0.035%	Higher digestibilities of DM and EE	(Abbas et al., 2016)
Dissected bile acid	0.05%	Fat digestibility was improved	(Alzawqari et al., 2016)
Polyethylene glycol ricinoleate	0.05%	Higher fat digestibility	(Tan et al., 2016)
Glyceryl polyethylene glycol ricinoleate	0.04%	Increased fat digestibility	(Kaczmarek et al., 2015)
Milk derived casein	0.05%	Improved EE digestibility	(Neto et al., 2011)
Ox bile	51.9 to 68.9% and 78.8%	Fat digestibility was improved	(Alzawqari et al., 2011)
Lysoforte	0.3%	Greater apparent faecal digestibilities of OM, DM and CP	(Dierick and Decuypere, 2004)
Bile salts	0.3%	Improved lipid digestibility	(Maisonnier et al., 2003)
Lipase	0.1%	Increase in fat digestibility and AMEn	(Tan et al., 2000)

Table 6. Effect of fat mobilisers on blood chemistry and gut health in broilers

Fat mobilisers	Dose	Result	Reference
Blood chemistry			
Lysophospholipid (Lipidol)	0.1%	Emulsifier also elevated the cholesterol level	(Kamel et al., 2022)
Herbal emulsifier, AV/PFE/15	0.025%	Increased HDL level	(Gole et al., 2022)
Blend of lysophospholipids and phospholipids	0.025%	No effect on biochemical parameters	(Srinivasan et al., 2022)
Lysolecithin	0.050, 0.075 and 0.10%	No effect on biochemical parameters	(Mahmood et al., 2022)
Bile acids	0.05%	Lower LDL concentration and atherogenic index	(Shoaib et al., 2021 a)
Bile acid + lipase	0.05 and 0.015%	Lower atherogenic index	(Shoaib et al., 2021 c)
Lysophospholipid	0.05%	Lower glucose content	(Shahid et al., 2021)
Lecithin (97% de-oiled)	0.1%	Higher HDL and lower LDL cholesterol	(Liu et al., 2020)
Mixture of phosphatidyl choline, lysophosphatidyl choline and polyethylene glycol ricinoleate	0.05	Reduced cholesterol, increased total protein and globulin	(Saleh et al., 2020)
Lipase	0.03%	Lower triglycerides and LDL	(Hu et al., 2018)
Lysophospholipid (Lipidol)	0.1%	Serum cholesterol, LDL and triglycerides concentrations were lower	(Zhao and Kim, 2017)
Sodium stearoyl-2-lactylate (Prosol®)	0.05%	Higher triglycerides concentrations	(Cho et al., 2012)
Dissected bile acid	0.05%	Increased cholesterol, triglycerides, HDL and LDL content	(Alzawqari et al., 2016)
Gut health			
Glyceryl polyethyleneglycol ricinoleate-	0.035%	No effect on intestinal histology	(Tenório et al., 2022)
Emulsifier blend (phosphatidyl choline, lysophosphatidyl choline and polyethylene glycol ricinoleate)	0.1 and 0.2%	Higher VH, VH/CD and villus surface area	(Ahmadi-Sefat et al., 2022)
Sophorolipid, a glycolipid emulsifier	0.0005, 0.001 and 0.0015%	Higher VH and VH/CD in birds that received 0.001 and 0.0015% Sophorolipid	(Kwak et al., 2022)
Bile acids	0.05%	Higher VH and villus surface area	(Shoaib et al., 2021 a)
Lysophospholipid	0.05%	Improved VH and VH/CD and reduced CD	(Chen et al., 2019)
Lipase	0.03%	Higher villus height and VH:CD	(Hu et al., 2018)
Lysolecithin	0.05%	Increased VH and width of jejunum	(Brautigan et al., 2017)
Lysophosholipid	0.1%	Increased jejunum VH and VH/CD	(Boontiam et al., 2017)

Effect of bile acid on nutrient utilisation in broilers

Shoaib et al. (2021 c) reported that use of bile acids and lipase caused higher digestibilities of EE (67.65 vs 59.7%) and CP (69.32 vs 63.55%). Alzawqari et al. (2016) reported that addition of 0.05% dissected bile acid had improved fat digestibility. Maisonnier et al. (2003) concluded that supplementing bile salts (0.3%) resulted in higher fat digestibility. Nazir (2014) reported that 0.03% and 0.06% bile acids in broilers' diet caused higher fat digestibility (82 vs 86%). Alzawqari et al. (2011)

reported that desiccated ox bile (0.25 and 0.5%) caused higher fat digestibility (51% to 69%; Table 5).

Effect of bile acid on blood chemistry and gut health in broilers

The small intestine plays an important role in absorption of nutrients with the help of finger-like projection (Wang and Peng, 2008). Shoaib et al. (2021 a) observed that bile acids in broilers' diet caused lower LDL concentration (24.84 vs 41.11 mg/dl) and atherogenic index (0.23 vs 0.4). Shoaib et al. (2021 c) reported that use of bile acids and lipase in broilers' diet caused lower atherogenic index (0.45 vs 0.88). Lai et al. (2018 a) investigated the effect of high dosage of bile acids on health status of broiler chickens and concluded that 0.04% bile acids group had no effect on serum alanine transaminase and aspartate aminotransferase. Brautigan et al. (2017) evaluated the response of lysolecithin on the gut health of broiler chicken fed on soy oil diet. Two increasing doses of purified and commercial lysolecithin were added in the diet. Results of gene expression showed that commercial lysolecithin regulates and elicits gene expression leading to increased collagen deposition and villus length in the jejunal epithelium in comparison to pure lysolecithin. Supplementation of desiccated ox bile did not affect blood cholesterol, TG, HDL and LDL in both starter and finisher phase (Alzawqari et al., 2011). Hemati Matin et al. (2016) reported that LDL content was lower in birds that received bile acids, however, HDL and cholesterol were not affected. Alzawqari et al. (2016) showed that supplementation of dissected bile acid at 0.05% had increased cholesterol (122 vs 68 mg/dl), triglycerides (52 vs 35 mg/dl), HDL (86.6 vs 48 mg/dl) and LDL content (26.11 vs 19.44 mg/dl). Shoaib et al. (2021 a) revealed that use of bile acids in broilers' diet caused higher villus height (1268 vs 1022 µm) and villus surface area (1.23 vs 0.88 mm^2). Parsaie et al. (2007) reported that supplementing 0.05% cholic acid in wheat-based diet decreased height of duodenal villi (0.968 mm vs 1.086 mm) compared to control. However, height of jejunal and ileal villi was unaffected due to dietary bile acid. Depth of duodenal and jejunal villi was not different in both treatments. Further, depth of ileal villi was reduced by dietary bile acid treatment (Table 6).

Conclusion

According to previous research, the inclusion of oil in the diet of broilers is critical for meeting their energy requirements. Some fats contain a high level of unsaturated fatty acids and a low level of saturated fatty acids and are derived primarily from plant sources such as soybean oil, rice bran oil and palm oil, whereas other sources contain more saturated fatty acids and a low level of unsaturated fatty acids such as animal fats, beef tallow, mutton tallow and lard. Furthermore, the inclusion of emulsifiers and lipases in broiler diets improves growth performance, nutrient digestibility and intestinal histology.

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