SUPPLEMENTATION OF BILE ACIDS AND LIPASE IN BROILER DIETS FOR BETTER NUTRIENT UTILIZATION AND PERFORMANCE: POTENTIAL EFFECTS AND FUTURE IMPLICATIONS – A REVIEW

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Abstract

Bile acids are used for better emulsification, digestion and absorption of dietary fat in chicken, especially in early life. Similarly, exogenous lipases have also been used for the improvement of physiological limitation of the chicken digestive system. Owing to potential of both bile acids and lipases, their use has been increased in recent years, for better emulsification of dietary fat and improvement of growth performance in broilers. In the past, pancreatic lipases were used for supplementation, but recently, microbial lipase is getting attention in poultry industry as a hydrolysis catalyst. Bile acids strengthen the defence mechanism of body against bacterial endotoxins and also play a key role in lipid regulation and sugar metabolism as signaling molecules. It has been demonstrated that bile acids and lipases may improve feed efficiency by enhancing digestive enzyme activity and ultimately leading to better fat digestion and absorption. Wide supplemental range of bile acids (0.004% to 0.25%) and lipases (0.01% to 0.1%) has been used in broiler diets for improvement of fat digestibility and their performance. Combinations of different bile acids have shown more potential to improve feed efficiency (by 7.14%) even at low (0.008%) levels as compared to any individual bile acid. Lipases at a lower level of 0.03% have exhibited more promising potential to improve fat digestibility and feed efficiency. However, contradicting results have been published in literature, which needs further investigations to elucidate various nutritional aspects of bile acids and lipase supplementation in broiler diet. This review focuses on providing insight on the mechanism of action and potential application of bile acids and lipases in broiler diets. Moreover, future implications of these additives in poultry nutrition for enhancing nutrient utilization and absorption are also discussed.

Key words: bile acids, broiler, digestibility, fat, lipase enzyme, meat quality

Lipids are hydrocarbons (highly reduced molecules) which are generally insoluble in water and soluble in organic solvents (Gunstone, 2012). They are consid-

ered principal energy reserves in the animal body. Oxidation of fats releases 2.25 times more energy than sugars and proteins. Generally, lipids are classified together as ether extract during proximate analysis of feedstuff, known as crude fats (Wu, 2018). Fats are also called triacylglycerols (TAGs) or triglycerides because all three hydroxyl groups of glycerol are esterified to a glycerol backbone (Mead, 1986). In most of the animal feeds, dietary fat makes up 98% of total lipids (Pond et al., 2004). Fats have protective functions in the skin and subcutaneous tissues. Fat contents of the body are low at birth but increase with age (Conde-Aguilera et al., 2013). Among fats, TAGs are the main form of energy storage in animal body (Ridgway and McLeod, 2016).

Higher energy requirements of broilers compel nutritionists to design diets with high oil contents. Generally, animal fats and vegetable oils are used in broiler diets to increase their energy density (Leeson and Summers, 2005; Fascina et al., 2009; Abudabos, 2014). Energy is considered as a major dietary component, which can affect the utilization of nutrients at different densities (Cho et al., 2012). According to Wu (2018), fat contents (on as-fed basis) in diets of broiler vary at different stages of age; for example, 5% for starter phase (1–21 days), 6% for grower phase (22–35 days) and 8% for finisher phase (36–49 days). It can also vary according to different environmental conditions.

Addition of fat in poultry diets improves palatability, provides energy, increases absorption of fat-soluble vitamins and nutrients in the gastrointestinal tract through reduced passage rate of digesta (Mateos et al., 1982; NRC, 1994; Baião and Lara, 2005; Febel et al., 2008; Firman et al., 2008). Diets with similar nutritive values containing oil have shown better growth than birds fed diets without oil (Elzobier et al., 2016). However, adding fat in broiler diets in their early age also poses negative impacts due to the limited ability of broiler to digest them and thus compromises growth performance (Wiseman and Lewis, 1998). Adverse effects on other nutrients intake and body composition of broiler have also been observed in response to higher dietary fat. Immature physiological function in early life of broilers can lead to low production of bile acids and pancreatic lipase that makes them vulnerable to negative impacts of dietary fat (Wiseman and Lewis, 1998; Classen, 2017; Pantaya et al., 2020). That is why improving fat utilization is essential for better performance of broiler.

This review aims to highlight the nutritional relevance and advantages of supplementation of bile acids and lipases in broiler diets to improve nutrient utilization and growth performance, especially in high-density diets.

Strategies to improve fat utilization

Ability of young birds to digest and absorb fat is comparatively less than mature ones (Wiseman and Salvador, 1989). The lower ability of fat digestion during early age is mainly due to insufficient production of pancreatic lipase and endogenous bile (Figure 1).

Figure 1. Relationship between age and endogenous lipase activity in broilers (data from Krogdahl and Sell, 1989; Nir et al., 1993; Dunnington and Siegel, 1995; Maiorka et al., 2004; Scanes, 2015 were used to generate this figure)

Fats emulsification is required for activity of a lipolytic enzyme because it is insoluble in aqueous solution of gastrointestinal tract (GIT). Therefore, emulsifiers supplementation is required in early life of poultry birds. Exogenous bile acids and lipase enzymes are used for proper fat utilization (Siyal et al., 2017).

Use of emulsifiers

Emulsifying agents (emulsifiers) are commonly used as stabilizer in water and oil emulsion. Emulsifiers break down oil and help in dispersion of oil droplets throughout water to make micelle formation. Emulsification can be affected by different characteristics of fat such as fat saturation, fatty acid position and chain length in triglycerides (Gu and Li, 2003). Emulsifying agents can be categorized into natural and synthetic groups. Natural emulsifiers include components of food material and are synthesized in the animal body. Synthetic emulsifiers are synthesized chemically for emulsification process. Common emulsifiers used in the poultry industry are listed in Table 1. Different emulsifiers possess different abilities of fat emulsification and utilization. Bile salts or bile acids are also one of them, focused here as an emulsifier in broiler diet to enhance fat utilization.

Bile acids, their synthesis and biological functions

Bile acids (C24) are organic components of bile usually synthesized from cholesterol in hepatocytes. Different amphipathic acidic steroids are involved in the formation of bile acid pool (Marin, 2008; Hofmann and Hagey, 2014). Bile acids are conjugated with glycine and taurine, and in some animals, with sulfate (SO_4). Due to their conjugation, molecular weight of fat-soluble compounds increases, which make them further water-soluble and less prone to precipitate in a watery medium. Bile acids are considered as steroid molecules that are involved in digestion and absorption of fat and hydrophobic compounds in the intestinal lumen.

Table 1. Emulsifiers utilized in broiler diets

	Main Impacts	References
Natural emulsifiers		
Bile salt or bile acid	Improved body weight gain Improved ADG and FCR Improved performance and the digestion Increased metabolizable energy	(Alzawqari et al., 2011; Hemati Matin et al., 2016; Lamasak et al., 2018)
Soy-lecithin	Improved performance, HDL, triglycerides, insulin Reduced, total serum cholesterol and LDL cholesterol Improved weight gain and carcass characteristics	(Huang et al., 2008; Zhu et al., 2008; Sival et al., 2017)
Lysophosphatidylcholine or lysolecithin (lecithin)	Improved weight gain, FCR and fat digestibility Increased villous height of small intestine Improved carcass quality and dressing percentage	(Azman and Ciftci, 2004; Zhang et al., 2011; Hosseini et al., 2018; Mohammadighetsar et al., 2018)
Phospholipid or lysophospholipid	Improved fat digestibility Enhanced villous height to crypt depth ratio of ileum and jejunum Promoted growth performance, nutrient utilization, gut health and anti-inflammation Improved feed efficiency Had no effect on digestibility	(Boontiam et al., 2016; Zampiga et al., 2016)
Milk-derived casein	Improved performance, enhanced ether extract digestibility and pancreatic lipase No effect on carcass traits, serum cholesterol, HDL and triglycerides	(Guerreiro Neto et al., 2011)
Globin	Improved digestibility and nutrient utilization efficiency only during starter phase	(Dabbou et al., 2019)
Synthetic emulsifiers		
Glycerol polyethylene glycol ricinoleate	Increased body weight and ADG Increased total cholesterol and HDL cholesterol Enhanced pellet quality Improved FCR and fat digestibility	(Cheah et al., 2017; Bontempo et al., 2018)
Sodium stearoyl-2-lactylate (SSL)	Increased the body weight gain only during starter phase Improved ADG and FCR Reduced serum cholesterol	(Cho et al., 2012; Ali et al., 2017)
Nonionic (Liprex® poultry)	Increased the body weight and liver weight Improved performance, intake and utilization efficiency of fat, CP, ME	(Aguilar et al., 2013)

Bile acids have specified features: the steroid nucleus, derived from a saturated tetracyclic hydrocarbon perhydrocyclopentanophenanthrene system formed by three six-membered rings (A, B and C) and a five-membered ring (D), shortened side chain (C5) in comparison of cholesterol (C8), and are acidic in nature, usually because of the carboxylic group which terminate the side chain branched to ring D (Monte et al., 2009; Marin et al., 2016). Sodium and potassium salts of bile acids are known as bile salts and give alkaline milieu to bile. While, these two terms, bile acids and bile salts are typically used interchangeably, bile salts are usually considered to be better emulsifying agents than bile acids in chicken. To increase solubility of water bile salt, the terminal carboxyl group of bile acids (approximately 98%) is conjugated with taurine or glycine before secretion from the liver (Lindsay and March, 1967). Different animal species have a different amount of glycine and taurine (Washizu et al., 1991). Bile acids are conjugated with glycine in herbivores, taurine in carnivores, and both taurine and glycine in omnivores (Agellon, 2008). Rate of biliary secretion is faster (24.2 $\mu\text{L}/\text{min}$) in broiler (Lisbona et al., 1981).

Bile acids as amphipathic molecules have a hydrophilic side (α -side) on one end and hydrophobic side (β -side) on another, which gives distinctive detergent features to bile acids. Bile acids form mixed micelles with biliary phospholipids to stimulate biliary lipid secretion, which allows solubilization in bile of cholesterol and other lipophilic compounds (Coleman, 1987; Coello et al., 1996). Besides, bile is required for intestinal absorption of vitamin D₃ in chicks, and this indirectly improves absorption of calcium as sparingly soluble calcium hydrogen phosphate (Webling and Holdsworth, 1965; Sanyal et al., 1994). Secretion of bile acids into bile canaliculi generates an osmotic pressure that accounts for the so-called bile-acid-dependent fraction of bile flow. At the intestinal level, bile acids are known to modulate secretion of pancreatic enzyme and cholecystokinin release (Koop et al., 1996). Other lipids secreted into bile are lecithin (phosphatidylcholine), free cholesterol and bile pigments (bilirubin, glucuronides). Furthermore, continuous flow of bile acids forms a potent antimicrobial barrier, avoiding both bacterial penetrations of biliary tree and in the small intestine (Begley et al., 2005; Engelking, 2011).

Bile acids play an essential role in the defence mechanism against bacterial endotoxins (Kocsar et al., 1969). Moreover, bile acids can reduce endotoxin absorption (Sheen-Chen et al., 2002), repair physical damage to the intestinal mucosa (Kamiya et al., 2004), and inhibit noxious bacteria, such as *E. coli* and *Clostridium botulinum* (Huhtanen, 1979). In addition to their role in managing nutrients, bile acids also play a key role in lipid regulation and sugar metabolism as signalling molecules (Watanabe et al., 2006; Russell, 2009). Recent studies have revealed that bile acids could regulate the expression of hepatic lipogenic genes and enhance intestinal lipase activity in broilers (Piekarski et al., 2016; Ge et al., 2018), but the underlying mechanism is still unknown.

Bile acids are synergistically produced by endogenous metabolic and symbiotic intestinal microbiota. The process is associated with the catalytic oxidation of cholesterol in liver and transformation of intestinal microbiota. Complex scenario related to bile acid biosynthesis, enterohepatic circulation and interactions with ileal and liver receptors are shown in Figure 2. Complex molecular mechanisms involve

different nuclear receptors, such as farnesoid X receptor, retinoid X receptor, small heterodimer partner, liver receptor homologous-1 and liver X receptor (Garruti et al., 2012). In liver, primary bile acids (CA, CDCA) are mainly synthesized from cholesterol by the rate-limiting microsomal enzyme (alternative pathway). Bile acids are conjugated to taurine or glycine mainly via bile acid CoA synthase and BA-CoA-amino acid N-acetyltransferase, secreted into bile. Many intracellular reactions of various organelles (mitochondria, endoplasmic reticulum, cytosol, and peroxisomes) of hepatocytes are involved in bile acid synthesis from cholesterol (Lefebvre et al., 2009).

CA = cholic acid; CDA = chenodeoxycholic acid; DCA = deoxycholic acid; LCA = lithocholic acid; UDCA = ursodeoxycholic acid; FXR = farnesoid X receptor; RXR = retinoid X receptor; SHP = small heterodimer partner; LRH-1 = liver receptor homologous-1; LXR = liver X receptor; BACS = bile acid CoA synthase; BAAT = bile acid CoA-amino acid N-acetyltransferase; BSEP = bile salt export pump; MRP = multidrug resistance-associated protein; CCK = cholecystokinin; VIP = vaso-intestinal peptide; FGF = fibroblast growth factor; GPBAR = G protein-coupled receptor; PYY = peptide YY; GLP = glucagon-like peptide; ASBT = apical sodium dependent bile acid transporter; I-BABP = ileal bile acid binding protein; OST = organic solute transporter; JNK/ERK = Jun N-terminal kinase/extracellular signal regulated kinase.

Figure 2. Bile acid biosynthesis, enterohepatic circulation and function through bile receptors in the liver and intestine (adapted from Di Ciaula et al., 2018)

Enterohepatic circulation involves recirculation of bile acids (95%) into liver from terminal ileum (Hurwitz et al., 1973). Remaining 5% bile salts (acids) goes into large intestine for further modifications by bacterial enzymes (Devlin, 2006). Here, biochemical reactions mediate deconjugation (removal of glycine or taurine) and removal of one hydroxyl group from the primary bile acids. Resultant products of

dehydroxylation are called secondary bile acids (Wu, 2018). Usually, bile acids are comprised of primary bile acids and secondary bile acids. Almost all mammals, birds and some fish have bile acids of C24 compounds, but ancient mammals (elephants and manatees), reptiles and some aquatic animals have bile acids of C27 compounds (Hofmann, 1999; Agellon, 2008; Hagey et al., 2010; Kurogi et al., 2011). The C27 bile acids contain the C8 side chain of cholesterol, while the C24 bile acids have a truncated C5 side chain. Due to complex anatomy of birds, relatively insufficient information is recognized about biliary secretion. Primary bile acids in chicken and turkeys are chenodeoxycholytaurine and cholytaurine, while in ducks, chenodeoxycholytaurine and phocaecholytaurine are considered as predominant bile acids (Elkin et al., 1990). A list of different bile acids is shown in Table 2, according to their prevalence in different species.

Table 2. Occurrence of bile acids in different species

Trivial Name	Type	Occurrence (Species)	References
1	2	3	4
Chenodeoxycholic acid	P	Bear, hamster, horse, human, pig	(Hofmann et al., 2010)
Cholic acid	P	Bear, cat, cattle, chicken, hamster, human, rodents	(Pedersen and Gustafsson, 1980; Hagey et al., 1997)
Hyocholic acid	P	pigs	(Cantafora et al., 1986; Guertin et al., 1995)
β -Muricholic acid	P	Rodents	(Rodrigues et al., 1996; Kakiyama et al., 2004)
Lithocholic acid	S	Bear, hamster, human, pig	(Rossi et al., 1987)
Deoxycholic acid	S	Bear, cat, cattle, chicken, dog, hamster, human, rodents, rabbit,	(Washizu et al., 1991; Guertin et al., 1995; Wang et al., 2003)
Ursodeoxycholic acid	S	Bear	(Hagey et al., 1997)
Hyodeoxycholic acid	S	Pig	(Cantafora et al., 1986)
Phocaecholic acid	P	Sea mammals, birds	(Murphy et al., 2001)
Haemulcholic acid	P	Fish	(Hoshita, 1967; Anderson et al., 1980; Goto et al., 2003; Hagey et al., 2010)
Bitocholic acid	S	Snakes	(Bergström et al., 1960)
Lagodeoxycholic acid	S	Nutria, bear	(Hagey et al., 1997)
Ursocholic acid	P, S	Humans, bear	(Ridlon et al., 2006)
α -muricholic acid	S	Rodents	(Merrill et al., 1996)
β -muricholic acid	P	Rodents	(Kuramoto et al., 1987)
ω -muricholic acid	P, S	Rodents	(Borgstrom et al., 1986)
Murideoxycholic acid	S	Rodents	(He et al., 2003; Kakiyama et al., 2004)
Vulpecholic acid	P	Marsupials	(Lee et al., 1987; Kakiyama et al., 2007)

Table 1 – contd.

1	2	3	4
Cygnocholic acid	P	Swans	(Kakiyama et al., 2006)
Avicholic acid	P	Birds	(Hackett et al., 2008)
Avideoxycholic acid	S	Birds	(Livezey and Zusi, 2007)
Norchenodeoxycholic acid	P	Pinnipeds	(Hellou et al., 1988)
Allochenodeoxycholic acid	P	Reptiles	(Meyer and Zardoya, 2003; Moschetta et al., 2005)
Allocholic acid	P	Reptiles	(Vidal and Hedges, 2009)
Allodeoxycholic acid	S	Rat, rabbit	(Hofmann and Mosbach, 1964; Kallner et al., 1967)
Alloavicholic acid	S	Birds	(Hagey et al., 1994)

P = primary bile acid (formed in hepatocyte); S = secondary bile acid (formed in intestine by bacteria).

Exogenous lipases and their mechanism of action

Exogenous enzymes have great popularity among poultry and livestock industry (Beauchemin et al., 2003; Sarica et al., 2005). Avian liver health and function can be improved using exogenous enzymes; however, these aspects require further investigation (Zaefarian et al., 2019). Lipases are defined as triacylglycerol acyl hydrolases (EC 3.1.1.3) that are involved in the hydrolysis of fats and oils to yield glycerol and free fatty acids. As lipases are produced in the digestive tract to hydrolyze absorbed triglycerides, their synthesis might be activated by a hormone-sensitive regulation system in case of higher energy demands that ultimately initiates degradation of reserve triglycerides. Young animals with immature digestive capability for complete absorption of lipids could particularly benefit from dietary lipases (Pleiss et al., 1998; Fickers et al., 2011).

Lipases, like other enzymes, cannot tolerate high temperatures, extreme pH, high ionic strength and organic solvents. Owing to these limitations like inactivation by gastric acidity, degradation by digestive tract proteases and physiological concentration of bile salts in the small intestine, most of the lipases cannot be applied to animal feed (Moreau et al., 1988; Zentler-Munro et al., 1992). Fungal lipase (*Aspergillus niger*) showed better stability, in terms of pH and temperature, when exposed to conditions associated with the glandular stomach compared to bacterial (*Chromobacterium viscosum*) and crude porcine lipase. The optimal pH for *Aspergillus niger* lipase and *Chromobacterium viscosum* lipase were 5 and 6–8, respectively. Exposure of lipases to 40°C and pH 7 for 30 min has been shown to reduce the activity of all lipases except *Aspergillus niger* lipase. According to Wang et al. (2018), *Yarrowia lipolytica* (YL) lipase formulated with spray-drying with skimmed milk powder and starch, exhibited better stability under low pH with proteases and bile salts. The YL lipase supernatant displayed the best stability under low pH values compared to other lipases derived from different fungi (*Rhizopus oryzae*, *Candida rugosa* and *Thermomyces lanuginosus*). Therefore, in order to improve the stability and utilization, functional enzyme preparations with better efficacy spectrum are required.

Lipases naturally catalyze the hydrolysis of triacylglycerols by attacking ester bonds. Mono- and diacylglycerols and free fatty acids are products of this enzymatic hydrolysis. They are also active on a broad range of substrates. In all cases, this reaction is carried out at the interface of a biphasic system (Pérez et al., 2019). This biphasic system originates from an immiscible organic phase, containing the hydrophobic substrate, in water. Lipases are also capable of expressing other related activities such as phospholipase, lysophospholipase, cholesterol esterase, cutinase, or amidase activities (Bora et al., 2013).

Lipase-mediated catalysis starts from formation of enzyme-substrate complex (Figure 3). It is accomplished in two steps: the first step is acylation, in which enzyme-substrate complex is formed through covalent bonding performed by proton transfer from aspartate and histidine to OH group of serine, which on activation attacks carbonyl group resulting in negative charge on the oxygen of carbonyl as an intermediate 'oxyanion'. This oxyanion is stabilized with hydrogen bonding with histidine where serine acts as a nucleophile and aspartate or glutamate as a catalytic acid residue that forms hydrogen bonds with amino acids present at active sites. In second step, deacylation is initiated by nucleophilic attack of a water molecule on enzyme-substrate complex at oxyanion site and fatty acid releases on leaving active enzyme freed for next reaction (Casas-Godoy et al., 2018).

Figure 3. Catalytic mechanism of lipases (adapted from Casas-Godoy et al., 2018)

Sources and application of lipases

There are many sources of lipases available, and they are also extracted from various organisms such as animals, plants and microbial species in the food industry (Fallahi et al., 2018; Negi, 2019). Different sources of lipases have different properties and limitations. For example, pig pancreatic lipase is polluted by traces of trypsin which impart a bitter taste (Sharma and Kanwar, 2014). Other impurities include animal viruses and hormones. Therefore, due to the ease of production and abundance, frequently studied and industrially used lipases are obtained from mi-

crobial sources. Moreover, compared to bacterial sources, lipases from generally recognized as safe (GRAS) yeast sources are widely accepted and used in several industries, including food processing (Johnson, 2013). Some of the major lipases used in industrial processes are listed in Table 3.

Table 3. Major lipases and their applications

Type	Source	Application	References
Bacterial	<i>Staphylococcus haemolyticus</i>	Food industry	(Jo et al., 2014)
	<i>Pseudomonas alcaligenes</i>	Hydrolysis	(Andualema and Gessesse, 2012; Rios et al., 2018)
	<i>Serratia marcescens</i>	Hydrolysis	(García-Silvera et al., 2018)
	<i>Bacillus licheniformis</i>	Oil and fat industry	(Rashid et al., 2013)
	<i>Pseudomonas mendocina</i>	Hydrolysis Detergents	(Zhang et al., 2019)
	<i>Chromobacterium viscosum</i>	Lipolysis organic synthesis	(Lang et al., 1996; Bajaj et al., 2010)
Fungal	<i>Yarrowia lipolytica</i>	Hydrolysis, lipid absorption	(Brígida et al., 2014; Wang et al., 2018)
	<i>Aspergillus oryzae</i>	Lipolysis oleochemistry	(Sánchez et al., 2002; Abe et al., 2006)
	<i>Rhizomucor miehei</i>	Flavour and fragrance detergents	(Chang et al., 2003)
	<i>Pecillium chrysogenum</i>	Waste cooking oil transformation	(Kumar et al., 2012)
	<i>Rhizopus chinensis</i>	Flavoured milk products	(Xiao et al., 2015)
	<i>Aspergillus niger</i>	Feed, aquaculture supplement	(Collar et al., 2000)
	<i>Thermomyces lanuginosus</i>	Transesterification	(Fernandez-Lafuente, 2010; Dantas et al., 2019)
Yeast	<i>Geotrichum candidum</i>	Hydrolytic kinetic resolution	(Brabcová et al., 2013; de Morais Júnior et al., 2018)
	<i>Williopsis californica</i>	Food	(Negi, 2019)
	<i>Candida antarctica</i>	Food, pharmaceuticals	(Kapoor and Gupta, 2012; Primožič et al., 2016)
	<i>Candida rugosa</i>	Food processing, quick drying oils	(Dhake et al., 2013)
Animal	Pig pancreatic lipase	Hydrolysis, transesterification	(Caballero et al., 2009; Zheng et al., 2014)
	Calf, kid and lamb	Hydrolysis	(Villeneuve et al., 1996; O'Connor et al., 2001)
	Chicken	Hydrolysis	(Borrelli and Trono, 2015)
Plant	Almond	Oil	(Huang et al., 2017)
	Coconut	Coconut oil	(Zin et al., 2017)
	Castor beans	Vegetable oils	(Salaberria et al., 2017)

Some techniques have been developed to obtain higher conversions for highly specific enzymes for each application, improving the possibility of industrial applications of lipases (Soccol and Vandenberghe, 2003; Franken et al., 2010). *Candida rugosa* lipases have great significance for their diverse biotechnological potentials (Pandey et al., 1999). Existence of *C. rugosa* lipase isoforms has been reported by several authors (Jaeger et al., 1994; Benjamin and Pandey, 1998). *Rhizopus* species is mainly divided into three groups, *Rhizopus oryzae*, *Rhizopus microsporus*, and *Rhizopus stolonifer* which generally produce *R. oryzae* lipase, *Rhizopus delemar* lipase, and *Rhizopus javanicus* lipase, respectively (Minning et al., 1998). Lipase gene from *R. stolonifer* possesses 84% sequence homology (amino acid) with *R. oryzae* lipase. However, there is no report on molecular characterization of lipase from *R. microspores* (Yu et al., 2009).

The lipase from YL is an ideal candidate for enzyme replacement therapy due to its unique biochemical properties: It shows the highest activity at low pH values and is not repressed by bile salts. The YL belongs to the same gene family as *Thermomyces lanuginosus* lipase, a well-known lipase with many applications in the field of detergents and biotechnological processes (Aloulou et al., 2007). Although these two lipases show a high sequence identity of 30.3%, they have relatively different biochemical properties.

Intestinal digestion and absorption of fat

Majority of fats emulsified by pancreas and bile salt-stimulated lipases are digested in the lumen of small intestine (Liao et al., 1984; Hamosh et al., 1989). Fat globules made up of multiple lipids, including triglycerides, are emulsified by bile salts in intestine (Figure 4). The emulsion droplets are hydrolyzed by lipases releasing free fatty acids and monoglycerides. Small micelles absorbed by the intestinal epithelium, form free fatty acids and monoglycerides with bile salts. After re-esterification of free fatty acids and monoglycerides in intestinal cells and subsequent packing into chylomicrons, they are secreted into the lacteal and lymphatic circulation of the intestine.

Figure 4. Mechanism of action of both bile acids and lipases (adapted from Martin, 2015)

Effect of dietary bile acids on feed intake and FCR

Response to dietary bile acids at an earlier age in broiler was highly variable in different studies (Table 4). In many studies better FCR ($P < 0.05$) was observed in response to bile acid supplementation at same feed intake during both starter and finisher phases (Atteh and Leeson, 1985; Maisonnier et al., 2003; Ge et al., 2018). These findings provide evidence that broilers have a limited amount of endogenous bile during an early age, which can be overcome by using dietary bile acids. However, in some studies it has been reported that dietary bile acid improved FCR only during the finisher phase (Alzawqari et al., 2011; Lai et al., 2018 a, b). On the contrary, a decrease in feed intake has been observed in response to inclusion of chenodeoxycholic acid in broiler diet (Polin et al., 1980; Hemati Matin et al., 2016; Piekarski et al., 2016). Reduction in feed intake, firstly might be due to synergistic effects of some components of natural bile acids that are not present in synthetic or purified bile acids, and secondly due to low plasma glucose level. The most abundant bile acids in swine include chenodeoxycholic acid and α -hyodeoxycholic acid, which is exclusive to swine, while chenodeoxycholic acid and cholic acid predominate in broiler biliary acids. It appears that chenodeoxycholic acid had little effect on performance in pigs, while it can decrease feed intake of broiler. A recent study has shown that exogenous emulsifiers/salts (phosphatidyl choline, lysophosphatidyl choline and polyethylene glycol ricinoleate) have potential to improve FCR of broilers fed low energy diet (< 50 kcal/kg ME) compared to the basal diet (Saleh et al., 2020). Variable results about the effect of bile acids may be due to different source of bile acids feed ingredients used in broiler diets.

Effect of dietary lipase on feed intake and FCR

Dietary lipases are claimed to be effective in improving feed efficiency in broilers (Nagargoje et al., 2016; Hu et al., 2018). Significant improvement in FCR has been reported in broilers with no effect on feed intake in response to supplementation of different levels of lipase enzyme (Al-Marzooqi and Leeson, 2000; Wang et al., 2018). These studies revealed that chicken might have a limited amount of endogenous pancreatic lipase during an early age, which can be augmented by using dietary lipase. However, in some studies addition of lipase in broiler diet has also shown no significant changes in feed intake and FCR (Polin et al., 1980; Meng et al., 2004). No effect of inclusion of dietary lipase on the performance of chicken suggests that insufficiency of pancreatic lipase production may not contribute to the lower-fat digestibility of tallow-containing diets. On the contrary, Al-Marzooqi and Leeson (1999) have reported that addition of lipase to broiler diet resulted in significant depression in feed intake and FCR (Table 5).

Effect of dietary bile acids on growth rate and body weight

Improvement in body weight (BW) gain in broilers in response to supplementation of emulsifiers in their diet has been reported in many studies (Table 4). Addition of bile acids to diets has exhibited growth-promoting effect on broilers during first 21 days of rearing period (Maisonnier et al., 2003; Parsaie et al., 2007; Ge et al., 2018). However, during the finisher phase (22–42 days) ADG remained unaffected.

Similarly, Atteh and Leeson (1985) reported that supplementary chenodeoxycholic acid and cholic acid had higher ($P<0.05$) ADG (2.67 vs 2.51 g) and BW gain (2001 g vs 1937 g) in broilers. They suggested that improved performance of birds was mainly due to enhanced dietary metabolizable energy and nutrients (especially fat) digestibility. On the other hand, some researchers reported that supplementation of different bile acids in broiler diets exhibited no effect on BW gain during first 21 days of rearing (Lai et al., 2018 a, b). However, during the finisher phase (22–42 days) a significant increase was observed in ADG. Bile acids used in this study were composed of 8% hyocholic acid, 70.67% hyodeoxycholic acid and 19.1% chenodeoxycholic acid. Alzawqari et al. (2011) observed that supplementation of 0.25% and 0.50% desiccated ox bile in broiler diet exhibited a significant effect on BW gain only during 42 days of the rearing period. Significant increase in BW gain of birds fed bile might be due to the higher availability of energy derived from enhanced fat absorption. However, no possible explanation of non-significant effects on BW during the first 21 days was given. On the contrary, Piekarski et al. (2016) reported that supplementing 0.01% and 0.5% bile acids (chenodeoxycholic acid) resulted in a decrease in body weight by 3–6% and 7–11% respectively, as compared to control group. These changes were accompanied by a significant decrease in plasma glucose levels. Reduced growth performance (decrease in feed intake and BW) in chicken might have been due to modulation of feeding-related hypothalamic neuropeptides and hepatic lipogenesis-related genes.

Table 4. Effect of bile acids on the performance of broiler

Trivial name	Dose rate	Experimental duration	FCR	Feed intake	BW gain	References
1	2	3	4	5	6	7
Mixture of phosphatidyl choline, lysophosphatidyl choline and polyethylene glycol ricinoleate	500 g/ton of feed in reduced energy diet	35 days	↑	=	↑	(Saleh et al., 2020)
Combination of hyocholic acid, hyodeoxychoic acid and chenodeoxycholic acid	0	1–21 days	=	=	=	(Lai et al., 2018 b)
	0.004%		=	=	=	
	0.006%		=	=	=	
	0.008%		=	=	=	
	0	22–42 days	=	=	=	(Ge et al., 2018)
	0.004%		=	=	=	
	0.006%		↑	=	↑	
	0.008%		↑	=	↑	
	0.006%	1–21 days	↑	=	↑	
	0.008%	22–42 days	↑	=	=	
	0	1–21 days	=	=	=	(Lai et al., 2018 a)
	0.008%		=	=	=	
	0.04%		=	=	=	
	0	22–42 days	=	=	=	
	0.008%		↑	=	↑	
	0.04%		=	=	=	

Table 4 – contd.

1	2	3	4	5	6	7
Chenodeoxycholic acid	0.01% 0.5%	21 days	= =	↓ ↓	↓ ↓	(Piekarski et al., 2016)
Desiccated ox bile	0 0.25% 0.50%	1–21 days	= = =	= = =	= = =	(Alzawqari et al., 2011)
	0 0.25% 0.50%	22–42 days	= ↑ ↑	= = =	= ↑ ↑	
Bile salts (sodium taurocholate)	0.3%	7–21 days	↑	=	↑	(Maisonnier et al., 2003)
Cholic acid	0.05% 0.2%	1–18 days 1–56 days	= ↑	↑ =	↑ ↑	(Parsaie et al., 2007) (Atteh and Leeson, 1985)
Cholic acid and chenodeoxycholic acid	0.04%	1–7 days	↑	↓	↑	(Polin et al., 1980)

↑ = improve; ↓ = decrease/deteriorate; = no effect.

Table 5. Effect of lipase on the performance of broiler

Trivial name	Dose rate	Experimental duration	FCR	Feed intake	BW gain	References
<i>Yarrowia lipolytica</i> lipase	0 4U/g 6U/g	42 days	↑	=	=	(Wang et al., 2018)
Microbial lipase	0.015% 0.03%	1–14 days	↑	=	↑	(Hu et al., 2018)
Lipase	100000 IU/ton of feed	42 days	↑	=	=	(Nagargoje et al., 2016)
Lipase (pancreatic)	0.02%	1–18 days	=	=	=	(Meng et al., 2004)
Lipase (pancreatic)	0% 0.375% 0.750% 1.125%	1–21 days	↑	↓	↓	(Al-Marzooqi and Leeson, 2000)
Lipase (pancreatic)	0.714%	1–12 days	↓	↓	↓	(Al-Marzooqi and Leeson, 1999)
Lipase	0% 0.01% 0.1%	1–21 days	=	=	=	(Polin et al., 1980)

↑ = improve; ↓ = decrease/deteriorate; = no effect.

Effect of dietary lipase on growth rate and body weight

Lack of response on growth performance to exogenous lipase enzyme has been reported in many studies in broilers during a period of 42 days (Polin et al., 1980; Wang et al., 2018). However, it does not necessarily mean that enzyme products fail to work on their specific substrates (Cowieson and Adeola, 2005). Meng et al. (2004) reported that supplementation of 0.02% lipase caused no difference in ADG and BW gain during a period of 18 days (Table 5). On the other hand, Hu et al.

(2018) reported that providing a reduced energy diet had decreased ($P<0.05$) BW gain compared to basal diet during first 14 days of rearing, though reduced BW gain was compensated with supplementation of 0.015% and 0.03% lipase in reduced energy diets. Therefore, due to the overwhelming complexity of full function of co-enzyme and other compounds, further research is required to confirm the effects of feed enzyme preparations. In previous studies (Al-Marzooqi and Leeson, 1999, 2000), adverse effects of enzymes on growth performance of the broiler have been reported. The reason for reduced performance may be due to contamination of lipase enzyme with cholecystokinin, which influences satiety signals ultimately affecting feed intake (Antin et al., 1975; Savory and Gentle, 1980).

Effect of dietary bile acids on carcass characteristics

Bile acids can improve absorption of dietary lipids which are not stored in abdominal fat (Table 6). According to Lai et al. (2018 b) and Ge et al. (2018), dietary bile acids possess significant potential to improve dressing percentage and carcass characteristics in broiler partly through a reduction of abdominal fat. The abdominal fat pad is a reliable indicator for judging total body fat contents owing to its direct association with total body fat contents in avian species (Becker et al., 1979; Thomas et al., 1983). Supplementation of bile acids has also shown desirable effects on breast muscles index. However, liver and thymus indices were reduced by bile acids (Ge et al., 2018). Parsaie et al. (2007) reported a significant decrease in liver weight due to dietary cholic acid. Liver is a principal site for detoxification and bile production, and its size is directly associated with functional load.

Effect of dietary lipase on carcass characteristics

Dietary lipase enzyme failed to bring change in abdominal fat percentage of broilers fed tallow based diet (Nagargoje et al., 2016; Hu et al., 2018) (Table 7). This effect may be attributed to dietary fat and fatty acid composition of the diets. Sanz et al. (2000) reported that broilers fed diets containing unsaturated fat exhibited less abdominal fat and fatty acid synthesis than those fed diets containing saturated fat. Increasing level of lipase enzyme resulted in higher liver weight ($P<0.05$) at 21st day of age (Al-Marzooqi and Leeson, 2000). Improvement in liver weight might be due to increased metabolic activity associated with lipid utilization.

Effect of dietary bile acids on fat digestibility

Energy-yielding potential of fat is markedly influenced by its chemical structure (Freeman, 1984; Krogdahl, 1985). Fatty acids composition, their chain length and saturation degree of the carbon chain all impact digestion and absorption of fats. Degree of saturation of fatty acid has a major influence on the AME of fats (Wiseman et al., 1991). Animal fats containing high amounts of long-chain saturated fatty acids (palmitic and stearic acids) are poorly digested and absorbed by poultry (Danicke, 2001; Leeson and Summers, 2001). Saturated fatty acids require bile salts to emulsify them and to form micelles before digestion. Garrett and Young (1975) reported that solubilization and absorption of saturated fatty acids are more negatively affected by the absence of bile salts than those of unsaturated fatty acids. Both pal-

mitic and stearic acids are non-polar and cannot spontaneously form mixed micelles. They require the presence of conjugated bile salts and unsaturated fatty acids to form mixed micelles. On the other hand, vegetable oils contain high concentrations of unsaturated fatty acids that are easily emulsified and better digested than tallow (Sklan, 1979). Usually, high energy diets exhibit poor digestibility of fat, due to lower bile acids and lipase synthesis in poultry birds. Supplementation of bile acids (2.5 g/kg) in broiler diet significantly improved apparent ileal digestibility of fat (Atteh and Leeson, 1985; Alzawqari et al., 2011; Hemati Matin et al., 2016; Lammasak et al., 2018). Similarly, Maisonnier et al. (2003) reported that supplementation of 0.3% bile salts (sodium taurocholate) improved ($P < 0.05$) lipid digestibility (89.4% vs 81.4%), but combination of bile salts with 0.5% guar gum had negative effect on lipid digestibility (85.7% vs 89.4%). The negative effect of guar gum on lipid digestibility was mediated mainly by higher viscosity hindering absorption of bile salts and fatty acids, leading to reduced intestinal pool size of bile salts and reduced lipid digestibility (Table 6).

Effect of dietary lipase on fat digestibility

Studies evaluating supplemental lipases are scanty and in general, have not to date yielded similar outcomes (Table 7). For example, Meng et al. (2004) found no effect of lipase addition on fat digestibility or AME in young broilers. Researchers suggested that insufficiency of pancreatic lipase synthesis may not be a significant factor contributing to incomplete fat digestion in young birds. On the contrary, fat digestibility was significantly improved due to supplementation of lipase in broiler diets (Polin et al., 1980; Brenes et al., 2008; Hu et al., 2018). On the other hand, Al-Marzooqi and Leeson (1999) reported that supplemental lipase in the diet was effective in increasing animal fat digestibility, although it is suspected that reduced feed intake may be due to contaminants like cholecystokinin hormone.

Effect of dietary bile acids on serum lipid metabolites

Concentrations of lipoproteins and plasma lipids are considered diagnostic markers in the metabolism of lipids. Synthesis of adipose tissue and fat deposition in poultry is dependent on available serum triglycerides (TG). Most fatty acids are synthesized in the liver and carried in adipose tissue as triglycerides via LDL or chylomicron (Hermier, 1997). In contrast, HDL promotes uptake and transport of cholesterol to liver for catabolism from peripheral tissues (Miller and Miller, 1975). It has been reported that bile acids possess hypocholesterolemic properties (Ge et al., 2018). Recently, Saleh et al. (2020) showed that plasma total cholesterol, HDL-cholesterol, total protein and globulin contents were lower in the low energy diets (<50 Kcal/kg). However, their concentration seemed to be increased with supplementation of exogenous emulsifier/salts. Hemati Matin et al. (2016) reported birds fed a diet supplemented with bile acids resulted in a lower serum concentration of TG and LDL-C, whereas serum concentrations of HDL-C and total cholesterol (TG) were unaffected. Supplementation of bile acids failed to change serum TG, total cholesterol, HDL-C and LDL-C content (Alzawqari et al., 2011; Lai et al., 2018 a). It might be due to failure of cholesterol transportation from peripheral tissues to the liver.

Table 6. Effect of bile acids on carcass characteristics and fat digestibility of broiler

Trivial name	Dose rate	Experimental duration	Dressing percentage	Abdominal fat (%)	Pancreas Wt (%)	Liver Wt (%)	Thymus	Bursa of Fabricius	Fat digestibility (%)	References
Combination of hyocholic acid, hyodeoxychoic acid and chenodeoxycholic acid	0	42 days	=	=	=	ND	ND	ND	ND	(Lai et al., 2018 b)
	0.004%		=	=	=					
	0.006%		=	=	=					
	0.008%		↑	↓	=					
	0.008%	42 days	↑	↓	=	↓	↓	=	ND	(Ge et al., 2018)
Bile acids (pig)	0	42 days	ND	ND	=	=	=	=	ND	(Lai et al., 2018 a)
	0.008%				=	=	=	=		
	0.04%				=	=	=	=		
	0.25%	21 days	ND	ND	ND	ND	ND	ND	↑	(Lammasak et al., 2018)
Bile acids (sodium deoxycholate)	0.15%	21 days	ND	ND	ND	ND	ND	ND	↑	(Hemati Matin et al., 2016)
Desiccated ox bile	0	21 days	ND	ND	ND	ND	ND	ND	=	(Alzawqari et al., 2011)
	0.25%								↑	
	0.50%								↑	
	0	42 days							=	
	0.25%								↑	
	0.50%								↑	
Bile salts (sodium taurocholate)	0.3%	7–21 days	ND	ND	ND	ND	ND	ND	↑	(Maisonnier et al., 2003)
Cholic acid	0.05%	1–18 days	↑	↑	=	↓	ND	ND	ND	(Parsaie et al., 2007)
	0.2%	1–56 days	ND	ND	ND	ND	ND	ND	↑	(Atteh and Leeson, 1985)

ND = not determined; ↑ = increase; ↓ = decrease; = no effect.

Table 7. Effect of lipase on carcass characteristics and fat digestibility of broiler

Trivial name	Dose rate	Experimental duration	Dressing percentage	Abdominal fat (%)	Pancreas Wt (%)	Liver Wt (%)	Thymus	Bursa of Fabricius	Fat digestibility (%)	References
Microbial lipase	0.015% 0.03%	14 days	ND	=	=	=	=	=	↑	(Hu et al., 2018)
Lipase	100000 IU/ton of feed	42 days	=	=	ND	ND	ND	=	↑	(Nagargoje et al., 2016)
Lipase (pancreatic)	0% 0.375% 0.750% 1.125%	21 days	ND	ND	=	↑	ND	ND	ND	(Al-Marzooqi and Leeson, 2000)
Lipase (pancreatic)	0.714%	12 days	↓	ND	ND	ND	ND	↓	↑	(Al-Marzooqi and Leeson, 1999)
Lipase	0% 0.01% 0.1%	1–21 days	= = =	ND	ND	ND	ND	ND	= = ↑	(Polin et al., 1980)

ND = not determined; ↑ = increase; ↓ = decrease; = no effect.

Effect of dietary lipase on serum lipid metabolites

Studies on the effect of supplemental lipases on serum lipid metabolites are limited. Triglycerides are fatty acid trimesters of glycerol that are derived from food as well as produced by the animal body. They were secreted from the liver into the blood by TG-rich lipoproteins. Brenes et al. (2008) reported that dietary lipase has hypocholesterolemic activity in broilers. In a further study, dietary lipase significantly reduced total, TG and LDL-C, whereas HDL-C remained unaffected (Hu et al., 2018). It has been suggested that faster rates of absorption and metabolism of ingested fat may be the reason for lower serum TGs in broiler fed lipase. However, further studies are warranted to reveal the mechanism affecting lipase in serum profiles.

Effect of dietary bile acids on enzyme activities

Intestinal lipase activity can be an indicator of lipid utilization in animals. Hormone-sensitive lipase (HSL) is an intracellular neutral lipase and is activated when the body needs energy derived from lipid mobilization. It is the rate-limiting enzyme in the degradation of triacylglycerol to diacylglycerol and free fatty acids (Duncan et al., 2007). The activity of HSL was decreased in 42 d old broiler fed 60 and 80 mg/kg bile acids indicating that bile acids could improve the efficiency of fat digestion and absorption, leading to reduce requirements of dietary fat. Energy density of diets is also associated with total endogenous lipase activity in the gut. It has been reported that high-energy diets decreased ($P<0.05$) hepatic LPL activity at 42 d, which was increased by supplemented bile acids (Ge et al., 2018). Different dietary fibres present in poultry diets have been also shown to interact with supplementation of bile acids in birds. Supplementation of 0.15% bile acids in the diet decreased pancreatic lipase activity (PLA) (Hemati Matin et al., 2016). Similarly, Knarreborg et al. (2003) reported that lipase activity of proximal part of the small intestine was low due to increased level of unconjugated bile acids (sodium deoxycholate) in broilers.

Effect of dietary lipase on enzyme activities

Reports on the effect of lipase on enzyme activity in birds are limited. According to Brenes et al. (2008) addition of lipase enzyme improved pancreatic amylase activity and PLA. Ultimately, these activities resulted in improved performance of birds. Hu et al. (2018) reported that supplementation of 0.03% lipase enzyme had positive ($P<0.05$) effects on PLA. However, no differences were found in digestive enzyme activities with enzyme supplementation on d 28 of age. Researchers suggested age as a possible reason for no effect of lipase on digestive enzyme activities except pancreas lipase, because the digestive enzyme activities may be less affected by dietary manipulation in adult birds than in young birds (Liu and Kim, 2017). However, further investigations are required to provide mechanistic insights into this aspect.

Effect of dietary bile acids on intestinal morphology

Dietary bile acids have potential to improve intestinal morphology in terms of villus height, crypt depth and villus-to-crypt ratio of jejunum (Alzawqari et al., 2011). In contrast, Parsaie et al. (2007) reported that 0.05% dietary cholic acid decreased ($P<0.05$) height of duodenal villi (0.968 mm vs 1.086 mm) and depth of ileal

villi compared to control. Villi are considered as the most important sites for nutrient absorption. Longer villi provide more surface area and are capable of higher absorption of available nutrients (Caspary, 1992).

Effect of dietary lipase on intestinal morphology

Reports on the effect of lipase on enzyme activity in birds are minimal. Hu et al. (2018) reported that supplementation of 0.03% lipase in low energy diets increased ($P < 0.05$) villus height (911 vs 778 μm) and the ratio of villus height and crypt depth (8.59 vs 6.82). It indicated an increased digestive and absorptive capacity of small intestine in response to greater flow of nutrients for optimal growth of broilers. Additionally, this may also be responsible for improved nutrient digestibility. Villus height was associated with absorption capacity of enterocytes, and short villi may decrease surface area for nutrient absorption (Parsaie et al., 2007). Recently, Bansal et al. (2020) showed that dietary deoxycholic acid (1.5 g/kg) has potential to reduce *C. perfringens* luminal colonization in birds infected with *E. maxima* and *C. perfringens*. These infected birds had short villi, crypt hyperplasia and immune cell infiltration in the ileum; however, supplementation of deoxycholic acid effectively alleviated the ileal inflammation.

Effect of dietary bile acids on meat quality

Meat color is considered as one of the most important characteristics for customers and is often used to determine the economic value of food (Mugler and Cunningham, 1972). No report is available in literature regarding the effect of bile acids on meat quality in broilers. However, studies involving other emulsifiers are available, and a majority of them reported non-significant effects of emulsifier supplementation on meat quality parameters (Zhao and Kim, 2017; Bontempo et al., 2018; Upadhaya et al., 2018).

Effect of dietary lipase on meat quality

Limited reports are available regarding the effect of lipase on meat quality in broilers. Recently, Hu et al. (2018) reported that supplementation of lipase enzyme did not affect pH value, color (redness, yellowness and lightness) of breast muscle, water holding capacity and drip loss in broilers. Similarly, Nagargoje et al. (2016) reported that supplementation of lipase enzyme exhibited no effect on pH of breast and thigh muscles.

Future implications

Accelerated growth rate and body weight gain of broilers require highly nutritious and energy-dense diets to match body requirement and energy balance. This scenario demands to look for nutrients that can increase energy density of diets without compromising feed intake and feed costs. Dietary fats contain the highest caloric value of all other nutrients and are ideal to meet energy requirements of high performing birds. Nevertheless, the issue with dietary fat is that their digestion is a complex process as it involves many steps, including breakdown of fat droplet, emulsification, lipolysis and micelle formation.

Moreover, emulsification of fat requires endogenous bile acids secreted by liver and pancreatic lipase, which have limited activity in birds, especially at a younger age. Addition of fats in poultry diets to make high-density diets will further require the addition of exogenous bile acids and lipase for better emulsification and utilization of fats. In this case, these two additives for dietary fat utilization are crucial and an understanding of their role in efficient digestion and absorption of fats is warranted. A better understanding of both is inevitable, especially in the future scenario of increasing feed costs and competition for developing economical and energy efficient diets. Although bile acids and their derivatives appear to have positive effects on fat digestion and birds performance, it must be noted that the extent of improvement would be different for different bile salts. In the past they were expensive, but presently their use in poultry diets is cost-effective and is of practical interest. However, further investigations are required to critically evaluate the potential of bile acids and lipase enzyme in broiler nutrition with a specific focus on improving intestinal morphology, gut microbiome and meat quality.

Recent developments in omics technologies have made it possible to investigate effects of nutrients on gut microbiome and transcriptome, so it will be interesting to explore effects of these additives on metabolism, gut microbiota and ileal absorption capacity. Moreover, bile acids as signalling molecules have become a topic of increasing interest in mammals as they play a role not only as intestinal detergent molecules but also as metabolic regulators and molecular signatures that affect body lipid, glucose and energy metabolism (Thomas et al., 2008; Prawitt et al., 2011). Such effects are presently unknown in avian species. Furthermore, modulation of hypothalamic neuropeptides and genes associated with hepatic lipogenesis has opened up new pathways for further study to decipher the underlying downstream processes in chicken. Therefore, the effects of bile acid and lipase on global gene expression profiles and epigenetic signalling will provide new insights into the interaction of these compounds with nutrient's absorption, metabolism and cellular uptake.

Conclusions

Literature survey provided convincing evidence that dietary supplementation of bile acids and lipase improves in broiler performance owing to increased fat digestibility due to improved pancreatic lipase activity. However, due to the wide variety of available products, effective dosage of both supplements showed substantial variation in different studies. Studies have evaluated wide supplemental range of bile acids (0.004% to 0.25%) and lipases (0.01% to 0.1%) in broiler diets for improvement of fat digestibility and performance. However, combinations of different bile acids have shown more potential to improve feed efficiency even at low (0.008%) levels as compared to any individual bile acid. Likewise, lipases at a lower level of 0.03% have exhibited more promising potential to improve feed efficiency. Recent studies have shown that bile acids can effectively enhance digestive enzymatic activity and absorption capacity through improving intestinal morphology. These findings, along with a scope of bile acids as signalling molecule have opened a new horizon for future implications of bile acids in the poultry industry. Dietary supplementation of bile acids and lipases to enhance the efficiency of nutrient utilization through

modulation of intestinal morphology can have many far-reaching effects on performance and health of birds. However, further investigations are required to explore the potential interaction of bile acids and lipases with gut microbiome and immunity.

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