The Hypolipidemic and Antioxidant Effects of Probiotic, Prebiotics and Natural Lipotropic Factors on Hyperlipidemia and Fatty Liver in Broilers

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Fatty liver is considered a serious problem in broilers as liver is the main organ involved in metabolism of all absorbed substances. In addition, fatty liver has a direct impact on the growth and performance of the chicken. One of the modern approach in controlling metabolic upset, is the use of probiotics, prebiotics in controlling this problems. Here we tried to summarize the protective role of some probiotics, prebiotics and other natural lipotropic factors and their mode of action against hyperlipidemia and fatty liver in broilers.

Introduction

Mild degree of feed restriction may reduce incidence & degree of numerous pathologies by decrease energy expenditure and increase serum antioxidant enzymes in broiler but it depend on degree of restriction. Sever feed restriction cause stress on chicken (Xiaojing et al., 2010). The repeated cycles of fasting and refeeding has a negative effect on broiler performance and feed conversion rate. It is associated with changes in lipid metabolism such as increased serum TG, cholesterol levels and hepatic lipogenic enzymes such as (maleic enzyme, acetyl coA carboxylase & fatty acid synthase). Lipogenesis increased as a result of up-regulation of hepatic lipogenic enzyme through increase in mRNA gene expression level of these enzymes (Wang et al., 2009). These metabolic disorders are associated with lipid peroxidation and oxidative stress (Matsuzawa et al., 2007).

Livestock performance and feed efficiency are closely interrelated with the qualitative and quantitative microbial load of the animal gut (Huygebaert et al., 2011). It is generally accepted that the bacterial community of the intestinal tract has a major impact on gastrointestinal function, and can thereby also influence animal health and productivity. Considerable efforts have been made to modulate the intestinal microorganisms by dietary means in such a way that the health of the host is beneficially affected (Brady et al., 2000).
The major function of gut microbiota is digestion of non-digestible carbohydrates and production of short chain fatty acids (SCFA) which are sources for energy. These SCFA influence cell proliferation and differentiation, mineral absorption and inhibit growth of pathogenic microorganisms (Compare et al., 2012). The probiotics are defined as live organisms that, when ingested in adequate amounts, exert a health benefit to the host. The most commonly used probiotics are Lactobacilli and Bifidobacteria (Eamonn, 2010). The probiotics are used to replace the antibiotics growth promoters to overcome the antibiotic resistance which may develop due to over use of these antibiotics. The use of probiotics increase body weight and improve feed conversion rate. It also lowering the serum TG and TC level (Alkhalf et al., 2010).

Prebiotics is defined as selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health (Gibson et al., 2004). For a food ingredient to be classified as a prebiotic, it must fulfill the following criteria: (1) Neither be hydrolyzed nor absorbed in the upper part of the gastrointestinal tract. (2) Be selectively fermented by one or a limited number of potentially beneficial bacteria commensally to the colon, e.g. bifidobacteria and lactobacilli, which are stimulated to grow and/or become metabolically activated. (3) Prebiotics must be able to alter the colonic microflora towards a healthier composition (Gibson, 1998). Attempts have been made to increase the number of Bifidobacterium and Lactobacillus bacterial strains, which are considered to have health-promoting properties. These strains are also assumed to be stimulated by various types of non digestible carbohydrates (NDC) (Yu et al., 2005). The most commonly used prebiotics are fructooligosaccharide, galactooligosaccharide and mannanoligosaccharide. The prebiotics induce their action through modulation of gut microbiota and enhance their action. They decrease denovo fatty acid synthesis and have antioxidant effect. The use of mixture of prebiotics and probiotics (synbiotic) may improve the metabolic disorders and oxidative stress which are induced by irregular feeding.

L-carnitine is a non-protein amino acid (Mardones et al., 1999). L-carnitine is synthesized from the essential amino acids lysine and methionine with the assistance of vitamin C and this occurs in the liver, as well as in the kidneys and the brain (Lohninger et al., 1987). It is an endogenous cofactor that enhances carbohydrate metabolism and reduces the intracellular accumulation of toxic metabolites under ischaemic conditions (Qi et al., 2004) and plays an important role in the transport of long-chain fatty acids (Bremen, 1983). Under certain conditions, the demand for l-carnitine may exceed an individual’s capacity to synthesize it, making it a conditionally essential nutrient (Gross and Henderson, 1984). In carnitine deficiency, fatty acid oxidation is reduced and fatty acids are diverted into triacylglycerol synthesis, particularly in the liver. This process is reversible with the addition of sufficient quantities of carnitine to the diet (Dowell, 1989).

**Role of liver in lipid metabolism.**

In avian species, the liver is the main site of de novo fatty acid synthesis, accounting for 95% of all lipid production in young chicks, while the adipose tissue serves only as a storage tissue (Griffin et al., 1992; Leveille 1969). So there is now an apparently general assumption that almost all the fat that accumulates in broiler adipose tissue is synthesized in the liver or derived from the diet (Griffin et al., 1992). This organ supplies energy substrates to peripheral tissues by the Cori cycle and glycogen catabolism, and is important for detoxification (Yang et al., 2008).

Lipid metabolism in poultry differs from that in mammals with the liver being the main organ involved in its metabolic activity (compared to adipose tissue in mammals). The synthesis and decomposition of fatty acids is an important part of lipid metabolism. The catabolism of fatty acids (beta-oxidation) is carried out in the mitochondria, whereas fatty acids are synthesized in the cytoplasm of the hepatocyte being the principle location in poultry (Chen et al., 2012).

As one of the main constituents of the body, adipose tissue plays an important role in energy storage and mobilization, as well as, endocrine regulation of animal energy homeostasis (van et al., 1992). In poultry, triglyceride is synthesized mainly in the liver, then transported and stored in subcutaneous fat, visceral fat, and other organs, such as liver and muscle (Hirsch et al., 1998).

Lipogenesis and fatty acid oxidation are important metabolic pathways in controlling liver triacylglycerol accumulation. In general, body fat accumulation may be considered the net result of the balance among dietary absorbed fat,
endogenous fat synthesis (lipogenesis) and fat catabolism via beta-oxidation (lipolysis). Thus, if the amount of absorbed fat is the same, lower body fat deposition may be attributed to increasing fat catabolism or diminishing endogenous fatty acid synthesis or both processes (Sanz et al., 2000).

The decrease in serum triglyceride in animals derives from the reduction of very low density lipoprotein–triglyceride secretion and the inhibition of hepatic lipogenesis through the reduction of activity and gene expression of lipogenic enzymes (Delzenne et al., 2001). Hepatocytes are the main place for lipid metabolism and there are many substances affecting lipid metabolism such as the hepatic lipid metabolism enzymes and lipid receptors (Xiaofei et al., 2011).

**Hazard effects of fatty liver on broiler performance and growth.**

For several decades, the commercial poultry industry has focused on enhancing bird growth rate, but little attention has been paid to the concomitant abdominal fat deposition. Obesity have a negative effect on productivity. In meat-type chickens, excess fat deposition could decrease feed efficiency during rearing and the yield of lean meat after processing (Leclercq et al., 1990). Excessive abdominal fat deposition not only declines chicken quality, but also decreases feed conversion rate (Zerehdaran et al., 2004).

Fatty liver is associated with hyperlipidemia and hyperuricemia. There is a relationship between the fatty liver and the lipemia. AST, ALT, cholesterol, triglyceride and LDL-C concentrations were significantly higher and HDL-C was significantly lower in fatty liver than in normal liver (Chi et al., 2003). The pathogenesis of liver damage in hyperlipidemia consists of triglyceride and fatty acid accumulation in the liver, oxidative stress and inflammation of the liver (Capeau, 2008).

**Hyperlipidemia and oxidative stress.**

The high energy diet can be associated with increased oxidative stress and free radical species produced in cellular membranes (Prasad and Kalra, 1993). Dyslipidemia is characterized with abnormalities in lipid metabolism such as increase in serum TG, TC, LDL and decrease of HDL levels (Koruk et al., 2003). As a consequence of dyslipidemia, lipid peroxidation occurs (Matsuzawa et al., 2007). Hypercholesterolemia (HC) induces the production of ROS such as superoxide (Munzel et al., 2010). High plasma lipid levels can harden the arteries or speed up the process of atherosclerosis which increases the risk of heart disease (Wei et al., 2009).

Abnormal lipid metabolism is a main cause of dyslipidemia which is a major risk factor for cardiovascular disease, obesity, cholestasis, and overall mortality (Rizvi et al., 2003). The high levels of fat increase the fat-mediated oxidative stress and decrease antioxidative enzyme activity (Slim et al., 1996) and so the antioxidants are used in protection against dyslipidemia induce oxidative stress (Mary et al., 2003; Gorinstein et al., 2006).

The pathogenesis of liver damage in hyperlipidemia (HLP) consists of triglyceride and fatty acid accumulation in the liver, oxidative stress and inflammation of the liver. Both conditions can be induced by high energy diets which are believed to play a key role in insulin resistance and dyslipidemia, resulting in increased oxidative stress and a proinflammatory environment (Capeau, 2008). When the reactive oxygen species (ROS) production increases, an oxidative stress may occur. the very low density lipoprotein (VLDL) may overload the antioxidant system. HLP had lower plasma superoxide dismutase (SOD) activity and higher ROS production. The plasma triglyceride concentration was positively correlated to ROS release (Araujo et al., 1995).

**Origin and identity of ROS (reactive oxygen species).**

ROS are generated in eukaryotic organelles such as mitochondria (respiration), chloroplasts (photosynthesis) and peroxisomes (fatty acid degradation) and in the electron transfer chains. ROS can also be produced extracellularly, by the action of NADPH oxidases in phagocytes and colon epithelial cells (Gara et al., 2010). ROS include the hydroxyl radical, the superoxide radical, the singlet oxygen and hydrogen peroxide. Among these, the OH is the most reactive and dangerous species and immediately attacking virtually any molecule in it's neighborhood. In contrary, H₂O₂ is much more stable and it is able to cross membranes than OH radical (Van et al., 2011).

ROS attacks various biomolecules (e.g. DNA, RNA, proteins, lipids, cofactors in enzymes) damaging them and disturbing normal cellular metabolism. Stress generally increases ROS production. This can potentially be detrimental to the cell leading to cell death. However, the generated ROS (e.g. H₂O₂) can
also be used as signaling molecules for initiating ROS defence mechanisms to sustain ROS homeostasis (Van et al., 2011).

Many diseases (such as artherosclerosis, cancer, type 2 diabetes and neurodegenerative diseases) are believed to be initiated by ROS. Conversely, Recent data strongly suggest that sugars and sugar metabolising enzymes can regulate ROS formation in mitochondria, contributing to ROS homeostasis (Xiang et al., 2011).

**Mechanism of action of ROS.**

Oxidative stress–related factors cause impairment of the liver function. ROSs have toxic effects on hepatocytes by damaging DNA, lipids, and proteins, leading to a disruption in cellular homeostasis and aggravating metabolic syndrome features (Kohen and Nyska 2002; Raval et al., 2006). Hyperlipidemia is associated with high level of MDA and subsequent lipid peroxidation.

Lipoperoxidation is a biological reaction initiated by reactive oxygen species (ROS) from fatty acids (FA) (Niki et al., 2005). Lipoperoxidation severely alters structure and function of the cells and may produce toxic metabolites (Esterbauer, 1993). The ROS are rapidly neutralised by antioxidants. Therefore, the susceptibility of lipids to peroxidation in tissue depends on the proportion of poly unsaturated fatty acids (PUFA) in lipid bilayers, the amount of ROS produced and the level of antioxidants which can be of endogenous or nutritional origin. Each of these factors may be modulated leading to an enhancement or a reduction of lipoperoxidation. Indeed, ROS are overproduced under oxidative stress conditions which are frequent in domestic animals (Aurousseau, 2002) and the PUFA content of lipids in tissues can be increased by feeding PUFA rich diets which is a strategy more and more applied to improve the health value of animal product (Wood et al., 2004, Scollan et al., 2005). These increase the susceptibility to lipoperoxidation in tissue. lipoperoxidation can be prevented by increasing the intake of nutritional antioxidants. The liver is susceptible to reactive oxygen species (ROSs) damage (Hamelet et al., 2007).

**Natural antioxidants (NANTs).**

ROS homeostasis in cells is reached at the balance between ROS production and ROS scavenging by antioxidants. The latter act as electron donors, reducing ROS to less harmful molecules. The oxidized products formed in this process are not very reactive or harmful. Higher eukaryotic organisms use an array of enzymatic and nonenzymatic mechanisms to detoxify ROS (Apel and Hirt, 2004). Non-enzymatic mechanisms involve ascorbate (Vit C), glutathione, tocopherol (Vit E), polyphenols, alkaloids and carotenoids. The enzymes involved include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR) (Foyer and Noctor, 2009).

**Probiotics Definition.**

Probiotics, derived from the Greek and meaning “for life”, are defined as live organisms that, when ingested in adequate amounts, exert a health benefit to the host. There are several commercially available supplements containing viable microorganisms with probiotic properties. The most commonly used probiotics are Lactobacilli and Bifidobacteria (Eamonn, 2010). They inhibit pathogen adhesion (Laparra and Sanz, 2010), reduce the risk of colon carcinogenesis (Pool and Sauer, 2007), enhance mineral absorption (Scholz-Ahrens et al., 2007), modulate lipid metabolism (Brighenti, 2007) and modulate the secretion of gastrointestinal peptides involved in the appetite regulation (Delzenne et al., 2002).

**Probiotics and lipid profile.**

The probiotic have cholesterol decreasing effect on broilers. At 42 days of age, chicken groups fed with various levels of probiotic showed a significant decrease in cholesterol concentrations when compared to the control group (Alkhalf et al., 2010).

Mohan et al., (1995) reported that probiotic supplementation resulted in lowering of the serum cholesterol level in White Leghorn layers from 176.5 to 114.3 mg/dl serum. Also, Mohan et al., (1996) mentioned that chickens that received 75, 100 and 125 mg probiotic/kg diets had lower serum cholesterol content (93.3 mg/100 ml) compared to the control birds (132.2 mg/100 ml). Arun et al., (2006) found that serum total cholesterol and triglycerides were reduced significantly by dietary supplementation of probiotic (100 mg per kg diet).

The significant reduction in serum cholesterol of broiler chickens fed probiotic supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract (Mohan et al., 1995, 1996). The mechanism by which a probiotic can lower the serum cholesterol has been declared by
Fukushima and Nakano, 1995. He demonstrated that probiotic microorganisms inhibit hydroxymethyl-glutaryl-coenzyme A. Lactobacillus acidophilus reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis (Abdulrahim et al., 1996). Lactobacillus has found to have a high bile salt hydrolytic activity which is responsible for deconjugation of bile salts (Surono, 2003). Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and is more likely to excrete in faeces (Klaver and van der Meer, 1993). The using of acidophilic probiotics lowers the pH of the environment it occupies.

Prebiotics Definition.

Prebiotics is defined as “selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health” (Gibson et al., 2004).

Prebiotics as functional food ingredients.

The efficacy of prebiotics is depend on their ability to withstand digestive processes before they reach the colon and preferably persist throughout the large intestine such that benefits are apparent distally (Gibson et al., 2004).

For prebiotics to serve as functional food ingredients, they must be chemically stable to food processing treatments such as heat, low pH, and Maillard reaction conditions. That is a prebiotic would no longer provide selective stimulation of beneficial microorganisms if the prebiotic was degraded to its component mono- and disaccharides or chemically altered so that it was unavailable for bacterial metabolism (Yanbo, 2009).

However, the effect of a prebiotic is, essentially, indirect because it selectively feeds one or a limited number of microorganisms thus causing a selective modification of the host’s intestinal (especially colonic) microflora. It is not the prebiotic by itself but rather the changes induced in microflora composition that is responsible for its effects (Yanbo, 2009).

Prebiotics can be used for either their nutritional advantages or technological properties, but they are often applied to offer a double benefit, an improved organoleptic quality and a better-balanced nutritional composition (Franck, 2002).

The use of inulin and non digestible oligosaccharides as fiber ingredients is straightforward and often leads to improved taste and texture. These specific forms of dietary fiber are readily fermentable by specific colonic bacteria, such as bifidobacteria and lactobacilli species, increasing their cell population with the concomitant production of short-chain fatty acids. These acids especially butyrate, acetate, and propionate provide metabolic energy for the host and acidification of the bowel (Sghir et al., 1998).

Prebiotic as antioxidants.

Natural antioxidants derived from plants become increasingly popular as functional food and feed ingredients. the natural non-structural carbohydrates, with focus on those plant-derived compounds have dual antioxidant and prebiotic properties. they have direct and in direct ROS scavenging processes in plants, food and in the gastrointestinal tract, counteracting oxidative stress and preventing pathogen outbreaks and the development of ROS-related diseases (Van et al., 2011).

NDCs as ROS scavengers.

It has become increasingly clear that a group of non digestible carbohydrates with “fibre-like properties”(NDCs: fructans, Raffinose Family Oligosaccharides or RFOs, arabinoylans, b glucans) and their breakdown products (e.g. fructosyl oligosaccharides or FOS) as well as sugar-sterols and sugar-phenols might act as important ROS scavengers (Nishizawa et al., 2008).

The antioxidant capacity of inulin type fructans can further be increased by grafting extra groups (e.g. phenols) on their structure (Ren et al., 2011). Although the ROS scavenging capacity of natural sugar preparations might in some cases be overestimated by contamination with other strong antioxidants (e.g. polyphenols), synthetically produced, pure carbohydrates were also shown to exert considerable antioxidant activity in vitro and they also counteracted lipid peroxidation processes in vivo (Li et al., 2007).

NDCs acting as prebiotics and antioxidants.

Prebiotics are a special class of carbohydrates and soluble fibers that are not digested in the upper gastrointestinal tract. In the colon, they are fermented and they selectively promote the growth of beneficial bacteria supporting overall good health. The criteria for being classified as prebiotics are strictly defined (Gibson et al., 2004).
Roberfroid (2007) defined “A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health”. Prebiotics are rapidly rising in popularity in functional food markets.

Functional foods include for instance probiotics (living bacteria), prebiotics (compounds selectively stimulating “beneficial” intestinal bacteria), and antioxidants (compounds counteracting oxidative stress caused by an imbalance of Reactive Oxygen Species: ROS) (Van et al., 2011).

About 15% of plants accumulate fructans, including inulins, levans, graminans and neokestose-derived inulins and levans (Van et al., 2004). They occur in vegetables such as chicory, onion, asparagus and garlic but also in cereals (Gara et al., 2003).

Inulin and fructo-oligosaccharides also termed oligofructose are the most widely used prebiotics (Roberfroid et al., 2010) and are commercially produced from chicory roots (Cichorium intybus L) or Jerusalem artichoke tubers (Helianthus tuberosus) (Van et al., 2004). NANTs as ROS scavengers in the stomach.

Kanner and Lapidot (2001) suggested that gastric fluid may be an excellent medium for enhancing the oxidation of lipids and other dietary constituents. The acid environment of the stomach (pH 1.5-2) can lead to a partial hydrolysis of inulin and oligofructose. Thus, dietary antioxidants could be involved in preventing lipid peroxidation in the stomach (Corder et al., 2006).

Inulin and FOS are well-known prebiotics. They have a selective stimulation on beneficial bacteria in the colon. These bacteria secrete extracellular b-fructosidases. The unique 3-D structure of the b-fructosidase of Bifidobacterium longum, the main inhabitants of the large intestine, has recently been unravelled (Bujacz et al., 2011).

Also Lactobacillus species are widely considered to exert a number of beneficial effects on health. The probiotic role of several lactobacilli has been attributed to the synthesis of prebiotic fructans by fructosyltransferases (ftfs) of family GH68 (Cerning, 1990).

**Synbiotics and immune modulatory effects.**

Synbiotics, is defined as a combination of a probiotic and a prebiotic, aim to increase the survival and activity of proven probiotics in vivo, as well as stimulating indigenous bifidobacteria and lactobacilli (Eamonn, 2010).

Prebiotics play an important role on the prevention and treatment of gastrointestinal diseases. Prebiotics consumption could result in some advantages to the host, due to their selective metabolism in the intestinal tract (Gibson et al., 2004).

The metabolites produced by healthy microbiota interact with the immune system in the prevention or reduction of intestinal inflammatory diseases (Damaskos & Kolios, 2008). The establishment of normal microbiota is fundamental for the development of the immune system. In germ-free animals, for example, there is a dramatic reduction in the number and function of lymphocytes in both the gut-associated lymphoid tissue (GALT) and spleen and peripheral lymph nodes (Grethel et al., 2011).

Short chain fatty acids (SCFA) generating from bacterial fermentation involved in suppression of pathogenic intestinal bacteria, immune system modulation, energy supply for colonic epithelium and modulation of cholesterol and lipid metabolism as well as gut microbiota composition (Topping and Clifton, 2001).

Several studies have highlighted the importance of intra luminal bacteria in maintaining the integrity of intestinal mucosa by modulating the inflammatory process in this microenvironment. Certain compositions of intestinal bacteria with severe acute pancreatitis, including depletion of beneficial intestinal bacteria such as lactobacillus and colon proliferation of pathogens (Hegazi, 2010). Supplementation of prebiotics mix (insoluble and soluble fibers) improved clinical outcome and dampened systemic inflammatory disease in patients with severe acute pancreatitis (Karakan et al., 2007).

The administration of prebiotics has been associated with immune modulatory effects encompassing innate, adaptive immunity as a result of the interaction with the microbiota (Vulevic et al., 2008). Inulin consumption increases the phagocytic capacity of macrophages and the production of secretory immunoglobulin A (IgA-s), which plays an important role in the defense of the gastrointestinal tract (Van, 2004).

**Lipotropic factors and their effects.**

The lipotropic factors prevent the liver from excess fat deposits by hastening fat removal,
limiting fat uptake, increasing fatty oxidation and reducing fatty acid and triglyceride synthesis. They involve methyl donation for methionine synthesis to favour hepatic phospholipid synthesis, these latter being constitutive of VLDL/LDL that export excess triglycerides outside the liver. They reduce the lipogenic enzyme activities and activate the fatty acid oxidation enzymes. In addition, the gene expression of PPARa (peroxisome proliferator-activated receptor) and SREBP (sterol regulatory element binding proteins), which both play a role in lipid metabolism regulation, may be, respectively, up- and down-regulated (Fardet and Chardigny, 2011).

L-carnitine.

L-carnitine (3-Hydroxy-4-N-trimethylamino butyric acid) acts as a carrier for translocation of long-chain fatty acids from the cytosol into mitochondria for beta-oxidation and hence it sustains the energy supply (Foster, 2004).

Indeed, L-carnitine and some of its acyl esters act as very potent reactive oxygen species scavengers (Liu et al., 2004) and are known to have immunomodulatory properties in mammalian as well as in avian species. It has a known antioxidant and protective effects against lipid peroxidation (Yapar et al., 2007).

Dietary carnitine significantly increases liver α-tocopherol which is inversely correlated to plasma triglyceride (TG). It is suggested that decrease L-carnitine level is associated with decrease plasma TG utilization and so increase TG accumulation and storage in tissues (Richard et al., 2007).

The balance between lipid synthesis and breakdown pathways within a cell is in part controlled by the transfer of fatty acids across mitochondrial, peroxisomal and endoplasmic reticulum membranes. Fatty acids cross these membranes as acyl-carnitine derivatives to enter pathways for oxidation, acylation, chain shortening or chain elongation-desaturation (Ramsay, 2000)

Conclusion

Finally, we can conclude that the repeated fasting and refeeding is associated with changes in lipid metabolism such as hyperlipidemia which may lead to oxidative stress and increase lipid deposition in liver. These metabolic disorders are negatively affecting on broiler performance and growth. The using of probiotics, prebiotics and L-carnitine can ameliorates the previous disorders through their hypolipidemic effect and antioxidant activities.

References


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