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LYSOPHOSPHOLIPIDS: Role in Nutrient Enhancement

Bird performance has shown consistent improvement over the past few decades. This change has occurred due to improved genetics, precise nutrition and better farm management practices. The industry has undergone a remarkable growth over the last 30 years. Today we see 3 Kg broilers at 40 days of age and egg layers are capable of producing more than 330 eggs in 52 weeks of lay. Nutrition plays a vital role in supporting the desired growth and production performance of birds. Provision of good quality feed with essential nutrients must be ensured. Further, nutrients supplied through feed have to be effectively digested and absorbed. If the nutrients are not absorbed within the time limit, they are attacked by bacteria in the large intestine or excreted as waste, defeating the purpose for which they are fed and reflected in terms of poor growth and productivity.

Feed accounts for 65-70% of the total costs in animal production. With the rise in feed costs internationally; the birds' ability to absorb nutrients optimally is a very important aspect of overall performance efficiency. Nutritionists are therefore now emphasizing much on optimizing the feed efficiency and reducing feed cost.

Lysophospholipids (LPLs) besides providing a superior emulsification are also proven to be very effective in enhancing the flux rate of nutrients across the gut membrane, thereby improving the absorption and reducing the nutrient loss through faeces.

Phospholipids & Fluidity of the Bilayer Membrane

Membranes define the boundaries of the cell and its organelles and act as permeability barriers. One remarkable feature of all biological membranes is their flexibility; their ability to change shape without losing their integrity and becoming leaky.The"Fluid-Mosaic" model (Singer & Nicolson, 1972) of cell membrane indicates that membranes are made up of lipid bilayer and membrane proteins (peripheral and integral).

The lipid component of the membrane is a two dimensional fluid in which the constituent molecules are free to move laterally. The Lipid bilayer functions as both; a solvent for integral proteins and a permeability barrier. Peripheral membrane proteins are anchored to the surface of the membrane, while integral membrane

proteins contain trans-membrane regions that pass completely through the bilayer.These two classes of membrane proteins contribute to the "mosaic" aspect of the fluid-mosaic model.

Phospholipids (PLs) are the most abundant lipids found in membranes. These are amphipathic in nature, having hydrophilic as well as lipophilic properties. PLs are characterized by a glycerol backbone to which a polar hydrophilic phosphodiester (head) group and two lipophilic hydrocarbon tails are linked. The tails are usually fatty acids derived acyl residues that can differ in length (normally contain 14 to 24 carbon atoms). One tail usually has one or more cis-double bonds (unsaturated), while the other tail does not (saturated).

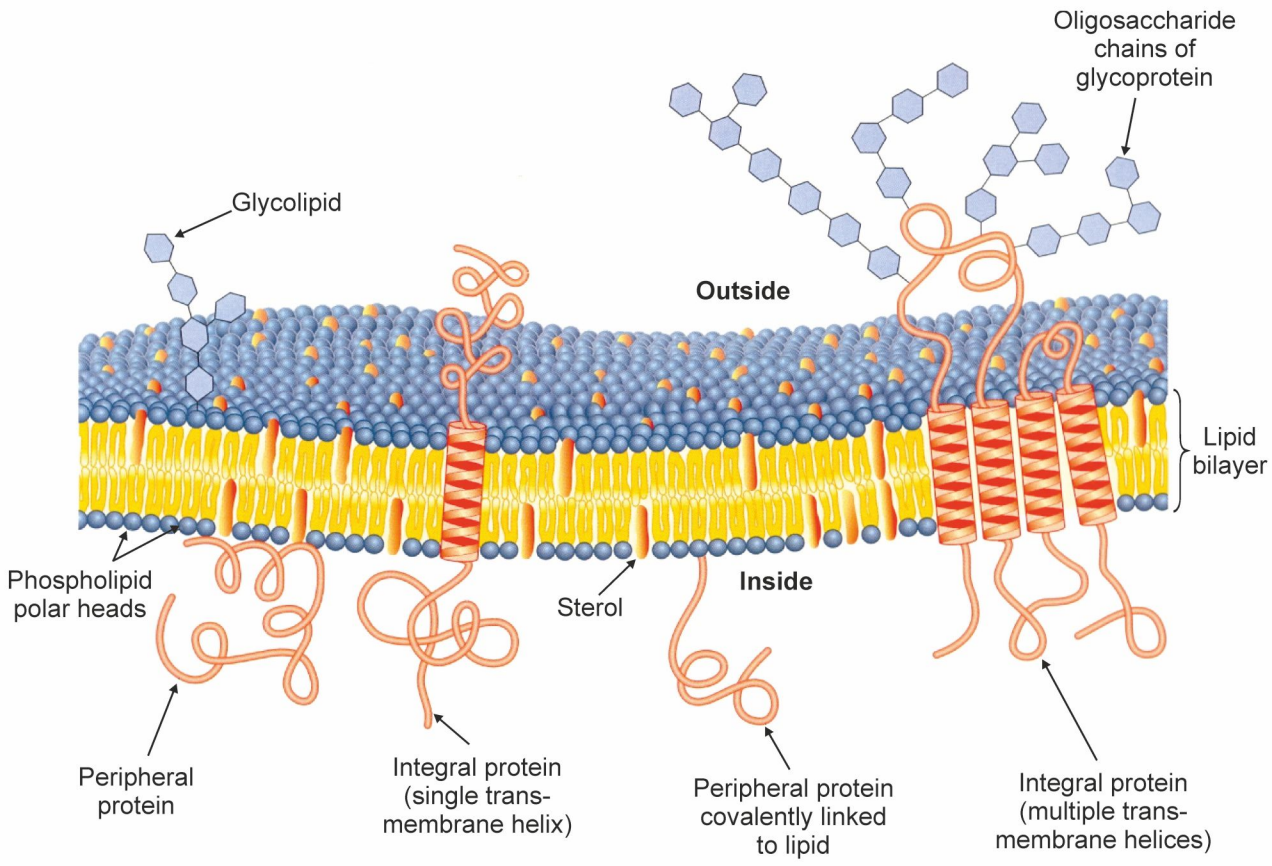
The structural diversity of PLs is attributable to different polar head groups. Based on the different polar head groups, phospholipids are Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylinositol (PI), Phosphatidylserine (PS) and Phosphatidic acid (PA). Among these, PC and PE are the predominant phospholipids.

Membrane fluidity means the relative freedom of motion for membrane components, especially phospholipids. Two properties of PLs are especially important in determining the fluidity of bilayer membrane:

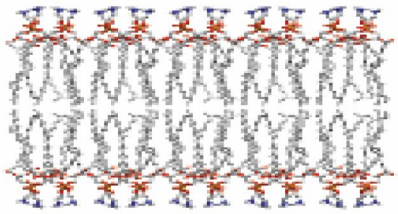
- The length of the fatty acid side chain, and
- Their degree of unsaturation.

Fluidity is also determined by the acyl chain swinging movement and phospholipid rotation. Short chains and double bonds in acyl chains of phospholipids create spaces in the bilayer and promote membrane fluidization.

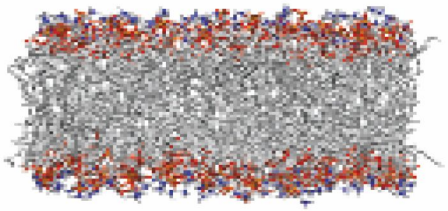
Along with phospholipids, the animal cell membrane contains significant amount of sterols also, mainly cholesterol, which is necessary for maintaining and stabilizing the membrane by acting as a fluidity buffer. Though the lipid bilayer



1.2 Membrane components. Membranes contain lipids, proteins and carbohydrates as glycolipids and glycoproteins. Nelson, D.L., and M.M. Cox (eds.), Lehninger Principles of Biochemistry, 4th ed., W.H. Freeman, 2005, p. 372. © 2005 by W.H. Freeman and Company. Used with permission.



Gel State



Fluid State

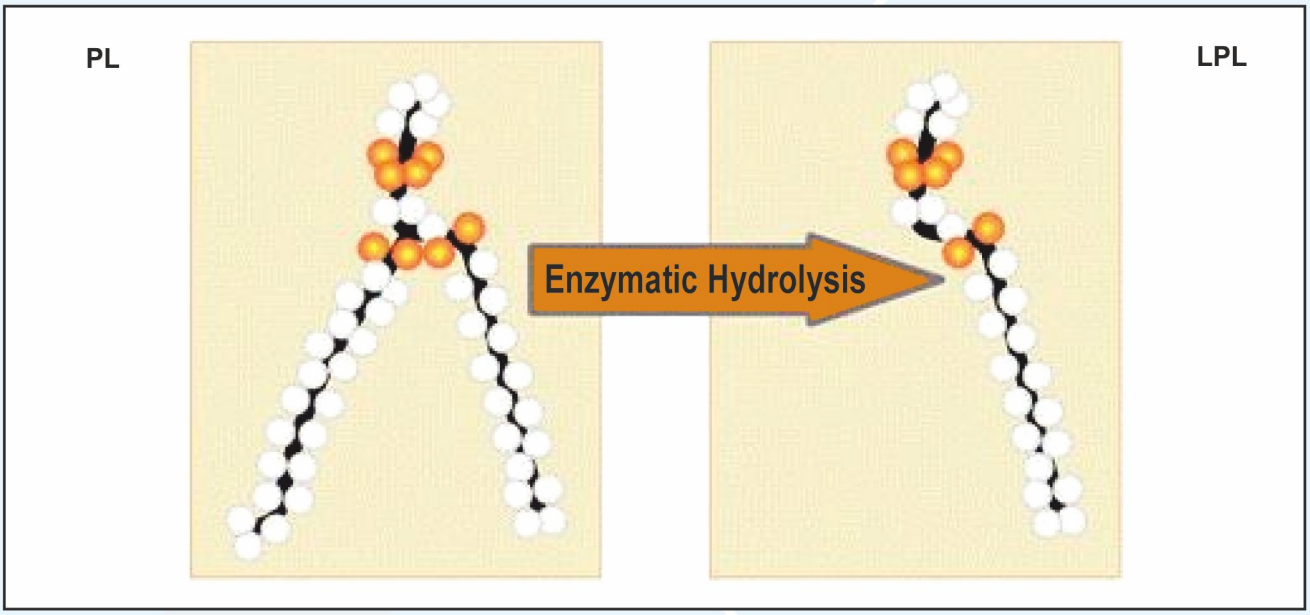
structure is quite stable, its individual phospholipid and sterol molecules have some freedom of motion. Besides their effect on membrane fluidity, sterols decrease the permeability of lipid bilayer to ions and small polar molecules and probably do so by filling in spaces between hydrocarbon chains of membrane phospholipids, thereby plugging small channels that ions and small molecules might otherwise pass through. Lysophosphatidylcholine (LPC) like molecules affect the fluidity of cell membrane by modifying cholesterol level in the membrane and thereby dynamics of the membrane.

Lipids in bilayer occur in different physical states depending on the temperature and the kind of lipids present:

1. Gel /semisolid state: In this state, all the types of motion of individual lipid molecules are strongly constrained; the bilayer is para-crystalline.
2. Liquid disordered state/Fluid state: In this state, individual hydrocarbon chains of fatty acids are in constant motion produced by rotation about carbon-carbon bonds of long acyl side chains. The interior of the bilayer is more fluid than solid and the bilayer is like a sea of constantly moving lipids.
3. Liquid Ordered State: In this state, there is less motion in the acyl chains of the lipid bilayer, but lateral movement in the plane of bilayer still takes place.

Lysophospholipids: The Fluidity Modulator

Phospholipids (PLs) have some effect on the emulsification of fat but have a little effect on the absorption of fat and other nutrients. Lecithin



serves as the source of phospholipids. The lecithin can be obtained from plant (soybean, sunflower and rape seed) or animal source (egg yolk). Generally lecithin is sourced from soybean due to its greater yield, easy availability and feasibility.

Lysophospholipids (LPLs) are glycerophospholipids in which one acyl chain is lacking and only one hydroxyl group of the glycerol backbone is acylated. LPLs which include lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidyl inositol (LPI) and lysophosphatidylserine (LPS) are prepared by enzymatic hydrolysis of natural soy lecithin through phospholipase A2.

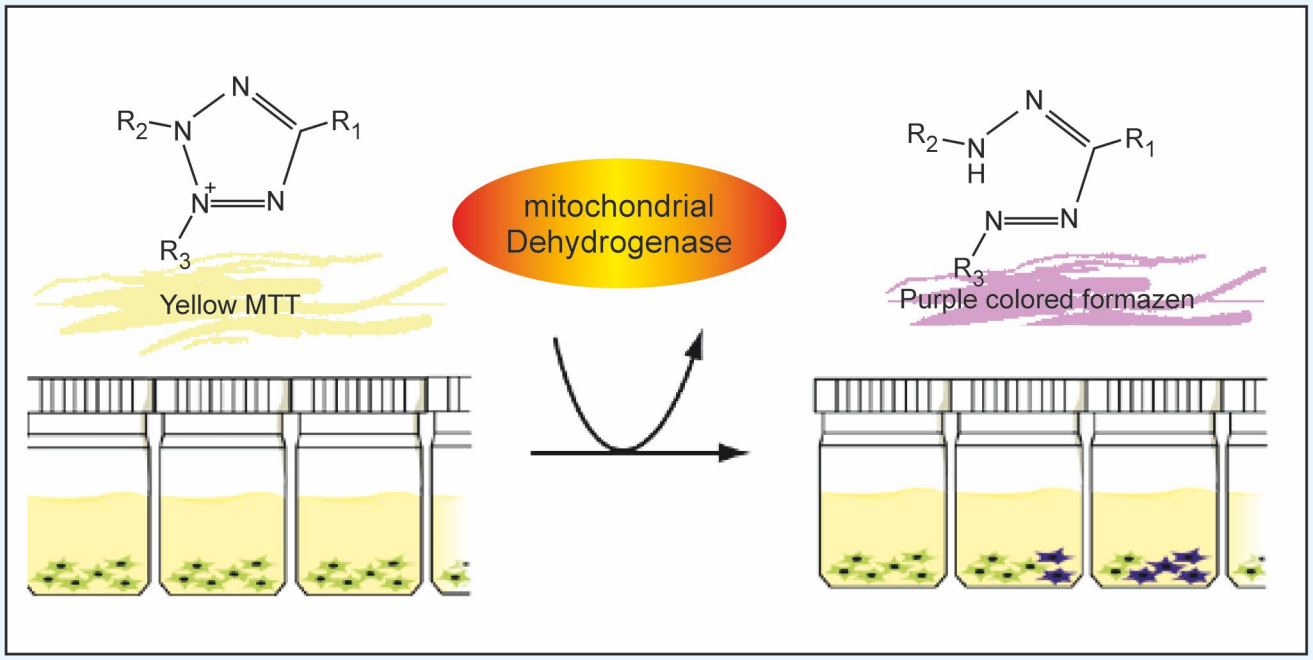
LPLs have superior emulsification properties than PLs due to lower CMC and optimum Hydrophillic-Lipophillic Balance (HLB) values. Unlike PLs, LPLs are found only in small amounts in biological cell membranes.

Each membrane at equilibrium contains pores and holes – these are best thought as gaps or vacancies where phospholipids are missing from lattice structure. Sometimes there are clusters of these vacancies of various sizes. When additional lysolipids are introduced it is this distribution that is affected and results in increase in both the number and the size of pores. Through the normal passive transport processes, nutrients of larger molecular weights can then pass more readily across the membrane. When the membrane comes into contact with certain ratio of LPLs, these exogenous lysolipids quickly get interdigitated into the bilayer membrane. The close packing between the PLs is disrupted (membrane perturbation) and the lipids go from order to disorder state and the membrane becomes more fluid i.e. the gaps or pores in the membrane form big clusters or larger vacancies in the matrix causing an increase in number and size of pores. This means that the nutrient absorption profile of the gut is beneficially altered with the passive flux hurdle temporarily lowered.

Also, LPLs have the ability to change the attraction between lipids and displace calcium ions. With this increased freedom of movement, lipids can aggregate closer together making existing holes larger so that larger molecules are easily absorbed.

Mere hydrolysis of the PLs would not serve the purpose as absorption enhancers; the enzymatic hydrolysis has to be standardized to yield consistent quantities of PLs and LPLs in the final product. A defined ratio has to be maintained between PLs and LPLs, and also between different LPLs to observe consistent and desired end results with the product.

That is how, Lysophospholipids (LPLs) help in improving the membrane permeability, increasing



the flux of various nutrients across the cell and act as an absorption enhancer. This is the key application of LPLs in animal nutrition because it means it is possible to extract more nutrient value from every kilogram of diet, even when such nutrients are normally poorly absorbed.

One of the ways in which the effect of any substance, like LPLs have on cell membrane permeability is the MTT assay which can be conducted in vitro.

MTT Assay

MTT (3-[4, 5-Dimethylthiazol-2-yl]-2,5 diphenyltetrazoliumbromide) assay is a colorimetric assay commonly being carried out to check the concentration of the live cells (viability) in the cell culture medium. This test is commonly used to screen the pharmaceutical compounds for their cytotoxic effect. This assay can also be carried out to assess the effect of a substance on cell membrane permeability.

Principle behind this assay is when MTT (a yellow colored dye) is taken up by a living cell, mitochondrial dehydrogenase enzyme cleaves the Tetrazolium ring (which is present in the MTT) and forms a water insoluble Formazan (purple/dark blue) within the cell. Live cells only can produce the enzyme (by the active mitochondria; an organelle present in the cytoplasm of the cell).

The intensity of the colored solution can be quantified by using spectrophotometer. The absorbance figure will provide an indication of the extent of cellular absorption.

Conclusion

Lysophospholipids act as membrane fluidity modulators. They increase the number and size of the pores by altering the mechanical properties of the membrane, thereby enhancing the flux rate at which nutrients of various molecular sizes pass across the membrane of the gut. Thus the absorption of breakdown products such as amino acids, simple sugars, fatty acids, vitamins, minerals and other additives is optimized leading to an efficient growth and production performance.

*(References are available with the company and can be furnished on request)

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