INTRODUCTION AND AIM OF WORK

Hypercholesterolemia represents one of the most important risk factors for cerebrovascular and cardiovasecular disorders mainly due to its modulatory effect on various metabolic pathways and on some hormonal secretory patterns ***(Fryer and Kruszynska, 1993; Zhang et al., 1997 and Gamble, 2006).*** Inhibition of hepatic cholestesterologensis followed by an elevation in low density lipoprotein (LDL) cholesterol and decline in high density lipoprotein (HDL) fraction as one of the prominent features that characterize high cholesterol diet ***(Nervi et al., 1975 and Mahley and Holcombe, 1977).***

Gonzalez-Pacanowska et al. (1986) and Castillo et al. (1998 and 1999) reported that the chick has been recognized as a suitable model for studies on the cholesterol metabolism and transport because it is highly sensitive to dietary modifications.

Naber (1983) stated that the liver and ovary are the primary sites of cholesterol biosynthesis in laying hens and cholesterol biosynthesis primarly in the liver where it is regulated by both diets and drugs.

Brown and Goldstein (1981) and Caliskan et al. (2000) showed that the 3 hydroxy-3 methylglutaryl COA reductase inhibitors (HMG-COA) are fungal metabolites that are potent competitive inhibitors of (HMG COA) reductase, the rate controlling enzyme in the cholesterol biosynthetic pathway. These statin drugs are extremely effective in lowering plasma concentration of LDL- cholesterol. They appear to act by inhibiting

cholesterol synthesis in liver which in turn triggers a compensatory increase in the synthesis of hepatic LDL receptors and thereby causes a reduction in the concentration of plasma LDL.

Chicken eggs are a concentrated source of dietary cholesterol until recently, recommendations to limit cholesterol intake, and thus egg intake were based upon that dietary cholesterol increases plasma cholesterol, which in turn increases heart disease risk ***(McNamara, 2000)***.

Delbos et al. (2002) found that simvastatin prevented the development of hypertension and cardiovascular hypertrophy together with inhibition of the induced angiotensin II production in rat. Therefore, inhibition of HMG COA reductase by statins may have a beneficial effect on cardiovascular alterations through its antioxidant action in experimental angiotensin II dependent hypertension. Also, simvastatin target organ damage independent of cholesterol lowering effect.

Lantuejou et al. (2002) reported that statins inhibit the 3-hydroxy-3- methylglutaryl coenzyme A reductase, reduce the serum level of LDL-C and are extensively prescribed to prevent cardiovascular mortality and morbidity.

Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzeme A (HMGCOA) reductase have revolutioinized the treatment of hypercholesterolemia HMGR, the rate- limiting enzyme in the cholesterol synthetic pathway, has been considered to be a prime target for pharmacological intervention for several decades ***(Alberts, 1988; Endo, 1992; Tobert, 2003 and Steinberg, 2006)***. The first

breakthrough in efforts to discover a potent, specific, and competitive inhibitor of HMGR occurred in 1976, when Akira Endo and colleagues reported the discovery of mevastatin (also known as ML-236B, CS-500, and compaction), a fungal metabolite first isolated from cultures of *Pennicillium citrinum* ***(Endo, 1992)***. Since then, a plethora of statins have been developed and approved for human use in united states including lovastatin (in 1987) simvastatin (in 1988) and atorvastatin (1997) ***(Tobert, 2003)***.

Pahan (2006) stated that statins are structural analogues of HMG-COA and thereby inhibit HMG-COA reductase competitively with an affinity about 1000-10,000 times greater than that of natural substrate.

Wagner et al. (2000) demonstrated that statins can attenuate the formation of superoxide anion in endothelial cells, by preventing the prenylation of P21 Rac protein and also prevent LDL oxidation by preserving the activity of the endogenous antioxidant systems like supproxide dismutase. So statins increaseing the antioxidant potential in sera.

Cheung et al. (2001) demonstrated that antioxidants substances help to prevent the potentially damaging effect of oxygen on molecules in the body and they believe that taking supplements of these antioxidents, such as vitamin E and C and beta carotene, will help to reduce the developing of heart disease.

Vitamin E is a fat-soluble vitamin that serves as an antioxidant and decreases lipid peroxidation in cell membranes either by preventing the induction of peroxisomal B-oxidation

enzymes and the formation of excess hydrogen peroxide ***(Hennig et al., 1990)*** or by direct removal of free radicals ***(Halliwell, 1996)***.

Lovastatin is an inhibitor of 3-hydroxy-3 methylglutaryl-coenzyme A reductase (HMG COA reductase), an enzyme which catalyzes the conversion of HMG-COA to mevalonate. Mevalonate is a required building block for cholesterol biosynthesis and lovastatin interferes with its production by acting as a competitive inhibitor for HMG-COA which binds to the HMG-COA reductase ***Endo (1992)***.

Sever et al. (2003) suggested that statin treatment reduces cardiovascular events even in normocholesterolaemic patients and especially in those having normal low-density lipoprotein (LDL) cholesterol.

Endo (1992)., Dujovne (1997 and Steinberg (2006) found that HMGR inhibitors are currently the most effective and widely used class of blood cholesterol lowering drugs available for the treatment of hypercholesterolemia in humans.

Vaugham and Gotto (2004) demonstrated that statin therapy forms the main stay basis of contemporary drug treatment of coronary artery diseases.

Devaraj et al. (2006) and ***Guazzi et al. (2007)*** found that simvastatin caused reduction of the inflammatory process subjects with hypercholesterolemia.

Hermier et al. (1989) and ***Elkin et al. (1993)*** reported that cholesterol balance in egg-laying fowl differs greatly from that in omnivorous mammals. Laying hens generally are not fed products of animal origin and usually obtained their bodies need for cholesterol entirely by *denovo* synthesis. In addition most of the cholesterol in laying hen plasma residue in the very low density lipoprotein (VLDL) fraction, whereas ***Kieft et al. (1991)*** showed that normolipidemic humans, micropigs, hamsters, rabbits, rat, guina pigs and dogs, LDL or HDL were the main carriers of cholesterol.

LDL lowering and supraphysiological concentrations of antioxidant vitamins can improve endothelial function, prevent coronary vascoconstriction and improve function of the resistance vessels of human ***(Egashira et al., 1994; Anderson et al., 1995; Levine et al.,1996*** and ***Tiefenbacher et al., 2004).***

Bolego et al. (2002) demonstrated that the statins offer important benefits for the large populations of individuals at high risk for coronary heart disease but may cause hepatotoxicity and myopathy.

Montgomery et al. (2005) stated that the effect of vitamin E did not depend on source or concentration of supplemental fat. Vitamin E is a fat-soluble vitamin that serves as an antioxidant and decreases lipid peroxidation in cell membranes by direct removal of free radicals.

Stone et al. (2005) suggested that lipid lowering with statins prevents adverse cardiac events. Both lipid lowering and

antioxidant therapies may favorably affect vasomotor function and thereby improve ischemia.

Ayala et al (2005) stated that chicken is a good animal model for the study of atherosclerosis research, since it presents lipoprotein levels similar to those in humans. It is a biped, and develops spontaneous and induced atherosclerosis mainly in response to high cholesterol diets (being stimulated by hormones and vitamin D), vascular injury or infections (mainly Mark's disease) in a similar way to how humans develop atherosclerosis. The chicken atherosclerosis model proved it self useful and very suitable for *in vivo* drug intervention studies. Also, they reported that atherosclerosis can be induced in normal chickens by feeding diets rich in cholesterol.

Gamble, (2006) reported that atherosclerosis is a dynamic multifaceted disease which affects the aorta and its major branches, characterized by the presence of lesions called a theromatous plaques. The plaque is a focal thickening of the intima caused by proliferation of smooth muscle cells and the deposition of cholesterol, other lipids, and fibrous connective tissue. They proposed that the determinant steep of the process which leads to the disease atherosclerosis is the calcium precipitation which traps cholesterol in the plaque precursor matrix which contains lipoproteins, calcium carbonate, triglycerides, albumin, and other proteins. .

Rodenburg et al. (2007) indicated that early initiation of statin treatment delays the progression of carotid IMT (Intima-

media thickness) in adolescents and young adults and might be beneficial in the preventation of atherosclerosis. .

Copper et al. (1980) found that a diet supplemented with cholesterol and coconut oil is atherogenin in dogs caused decline in hematocrit, value increase in osmotic fragility and increase in erythrocyte membrane cholesterol/ phospholipid molar ratio.

Pierno et al. (1995) showed that there was increases in erythrocyte and platelet Na^+ concentrations during statin treatment in rats.

Pereira et al. (1998) reported that in Beef Heifers fed diet that contained various concentrations of vitamin E showed non significant differences in the hemoglobin content and hematocrit value.

Buchwald et al. (2000) postulated that the plasma cholesterol concentration, in equilibrium with the RBC membrane cholesterol content, influence O_2 transport and in turn, tissue O_2 availability.

Caliskan et al. (2000) observed significant increases in erythrocyte membrane phospholipids, while cholesterol/ phospholipids ratio decreased significantly in rat administered simvastatin. They concluded that simvastatin change the composition and also the functions of red cell membrane lipids and blood ATP concentration.

The involvement of inflammatory cells such as monocytes or macrophages cells and subsets of T-lymphocyte was critical to the progression of atherosclerosis. Statins have anti-inflammatory

properties, including inhibition of leukocyte-endothelium interactions and reduction of inflammatory cell number within atherosclerotic plaque ***(Liao, 2002)***.

Akahane et al. (2002) hypothesized that rats feeding on diet containing cholesterol for 2 weeks showed hemolytic anemia and decrease in erythrocyte count, hematocrit and hemoglobin concentration.

Kempaiah and Srinivasan. (2002) indicated that RBCs of hypercholesterolemic rats were relatively fragile, compared to normal controls.

Koter et al. (2003) found that the erythrocyte membranes of hyperlipidemic patients contain large concentrations of cholesterol and lower level of Na^+ K^+ ATPase compared to the control group and statin treatment decreases cholesterol content of erythrocyte membranes in hypercholesterolemic patients.

Choi and Pai (2004) hypothesized that there were no significant differences in the mean values of red cell indices and leukocyte counts between the subjects with and without hypercholesterolemia.

Dalgic et al. (2006) reported that there was increasing of blood parameters caused by dietary cholesterol in food consumption and increasing of body weight gain. Treatment with simvastatin makes hemoglobin, hematocrite, leucocyte normal.

Luneva et al. (2007) observed that erythrocytes from patients with cardiovascular disease exhibit changes in the conformation of

hemoglobin (Hb) hemoporphyrin (Hp), reflecting its lower O_2 transport capacity.

Blood respiratory functions have pecularities that are related to each individuals activity, habit, habitat, evasions of the environmental factors and metabolic rate ***(El- Shafey, 1997).***

Talbott and Frayser (1963) noted a decrease in arterial oxygen saturation was following the intravenous infusion of cotton seed oil.

Steinbach et al. (1974) demonstrated that high blood cholesterol concentrations were associated with reduced blood O_2 transport i.e. high blood cholesterol effectively shifted the Hb dissociation curve to the left.

Cooksey and Reilly (1977) suggested that blood lipids, triglycerides or cholesterol interfere with the blood's transportation of oxygen. Also, they found no effect of high-fat diet induced hypercholesterolemia on the oxygen-hemoglobin dissociation curve, P_{50} and oxygen-hemoglobin kinetics of rabbits.

The hydrogen ion and carbon dioxide concentration changes play a significant physiological role in regulating blood oxygen affinity in birds ***(Kiley et al., 1982; Arad and Marder, 1983; Gleeson and Brackenbury, 1984 and Isaacks et al., 1986)***.

Droubay and Puppione (1980) showed no significant differences in PO_2, PCO_2, or pH in 11 male subjects fed fat-induced lipemia, whether significant changes in PO_2 would occur with higher level of lipemia or with increased oxygen demand. Also,

they reported that the dietary fat- induced lipimia in patients, caused decreased in pulmonary diffusing capacity and decreased arterial oxygen saturation.

Hirst et al. (1987) showed that antilipedmic drugs produced a significant increase in the P_{50} of the blood in mice.

Birds blood Co_2 , HCO_3^- , pH and plasma lactate change to ameliorate any disturbances in blood acid base balance ***(Eissa et al., 1990a & b; Odom and Ono, 1991 and Koelkebeck and Odom (1994)***.

Wideman and Buss (1985) reported that blood pH low and HCO^-_3 concentrations impair the egg shell thickness of laying hens.

Hatta and Frei (1995) showed that at both high and low PO_2, beta-carotene can not inhibit LDL oxidation whereas alphatocopherol has moderate protective effect in protection of human LDL-C.

Balnave and Muheereza (1997) found that in laying hens restore blood pH and HCO^-_3 concentrations at high temperature to improve the egg shell quality.

Buchwald et al. (2000) reported that the cholesterol of RBCs membrane act as barrier to O_2 diffusion and delayed O_2 entry into the RBCs during saturation in analysis of 93 patients divided according to their cholesterol concentration into five groups. The percentage in blood O_2 diffusion was inversely proportional to the cholesterol concentration. The RBCs membrane cholesterol was in equilibrium with the plasma cholesterol concentration. As the

plasma cholesterol increased, the RBCs membrane become impaired and O_2 transport reduced. The implications of this new perspective on O_2 transport include the ability to increase tissue oxygenation, by lowering plasma cholesterol.

Morgan and Leak (1993) found that in the renal patient present metabolic acidosis, which generally begins as early as the creatinine clearance diseases below around 30ml/min. Acidosis by itself, may predispose to the oxidation of LDL, which is important for the involvement of macrophages and the development of the atherosclerotic lesion.

Jiang and Roman (1997) found that in young (4- week- old) spontaneously hypertensive rats (SHR), treatment with lovastatin (10mg/ kg per day for 4 weeks) attenuated the development of hypertension as did hydralazine but remodeling of intrarenal arterioles corrected only slight reduction in arterial pressure.

Menchaca et al. (1998) showed that in an experiment using the cholesterol-supplemented group by rabbits showed higher plasma PO_2 levels during the saturation phase and lower plasma PO_2 levels during the desaturation phase. It also had a markedly increased RBCs membrane cholesterol content.

Rosenson (1999) reported that the nonlipid effect of statins may contribute to alleviation of tissue ischemia and prevention of acute cardiovascular disease, also to can improve endothelial functions.

Korte et al. (1999) showed that low PCO_2 in the venous blood may be a useful tool for genetic selection, whereas a high PCO_2 may be a useful indicator of poor welfare.

Hass et al. (2001) stated that low blood pH in hypercholesterolemic rabbits associated with enhanced DNA binding activity of a transcriptional repressor of the apoplipoprotein A (1) gene, suggesting that acidosis may negatively interfere in the synthesis of apo A. They suggested that acidosis may be an important factor contributing to the atherogenesis.

Depinieu et al. (1996) and Goli et al. (2002) reported that statin drugs work by inhibiting (HMG- COA) reductase and reduce cholesterol synthesis in human so there was a decrease in the production of other non – sterols such as coenzyme Q_{10} (CO Q_{10}; ubiquinone) which is as essential carrier in the mitochondrial respiratory chain that participates in oxidative phosphorylation.

Menchaca et al. (2004) reported that plasma and RBC membrane cholesterol levels are inversely associated with the transmembrane O_2 diffusion rate of the RBC. As blood cholesterol levels decreased by the administration of simvastatin, the blood oxygen diffusion increase.

Fukuzaki et al. (1975) stated that subjects with angina pectoris dietary fat-induced postprandial lipemia showed depressions 5 to 10% of PO_2.

Buchwald et al. (2000) demonstrated that high blood cholesterol concentration of patients were associated with reduced

blood O_2 transport; in essence, the hemoglobin dissociation curve was shifted to the left.

Tovar et al. (2007) observed no significant difference in serum pH and bicarbonate concentrations in rabbit fed hypercholesterolemic diet compared to those fed normal diet.

Miller et al. (1975) proposed that a reduction of plasma HDL concentration may accelerate the developing atherosclerosis by imparing the clearance of cholesterol from the arterial wall. This hyposis was supported by various epidemiologic studies in different population groups, where plasma HDL, cholesterol was found to be inversely related to the prevalence of coronary heart disease. Hypolipidemic drugs are intended to prevent coronary heart disease on serum HDL-C concentration.

Mol et al. (1982) found that the cholesterol-induced hypercholesterolemia in chicken showed significant increases in serum total cholesterol, VLDL and LDL cholesterol.

Pittman and ***Steinberg (1984)*** stated that cholesterol esters represent the main component of HDL cholesterol.

Thompson et al. (1986) suggested that mevinolin was highly effective in controlling hypercholesterolemia refractory to cholesterolamine by decreasing in total serum cholesterol. This decrease was accompanied by only small changes in serum triglyceride and HDL cholesterol and was largly due to reduction in LDL cholesterol.

Goldstein and Brown (1990) suggested that the level of plasma LDL is regulated by the LDL receptor, a cell surface glycoprotein that removes LDL from plasma by receptor mediated endocytosis. Defects in the gene encoding the LDL receptor, which occur in patients with familial hypercholesterolemia, elevated the plasma LDL, produce premature coronary atherosclerosis. The physiologically important LDL receptor are located principally in the liver, where their number was regulated by the content of cholesterol of the hepatocyte, when the cholesterol content of hepatocytes was raised by ingestion of diets high in saturated fat and cholesterol, LDL receptors fall and plasma LDL levels raised. Conversely, ingestion of drugs that inhibit cholesterol synthesis (Mevinolin or compactin) or prevent the neutilization of bile acids (cholestyramine or colestipol) stimulated LDL receptor production and lowered plasma LDL levels. The normal process of receptor regulation can therefore be exploited so as to reserve hypercholesterolemia and prevent atherosclerosis.

Beyer and Jensen (1992) reported that the level of plasma cholesterol shows great variation, suggesting that this effect could represents the synthesis and excretion of cholesterol through the liver associated with the diet.

Liscum and Underwood (1995) studied the intracellular cholesterol transport and compartmentation, they stated that cholesterol metabolism in the liver is complex with close regulation of both the compartmentalization and cellular concentrations of cholesterol.

Adamopoulos et al. (1996) showed that rats which feed cholestoral with diet were significantly increased in plasma total cholesterol, total lipids and triglycerides as compared with those of the control rats.

Ness et al. (1998) stated that atorvastatin increased the rate of degradation and presumably cycling of the hepatic LDL receptor. In atorvastatin-treated rats, the half-life of the receptor was decreased by over 60% and showed reduction in hepatic VLDL-C by feeding diet containing 0.04% atorvastatin.

Hennessy et al. (1992) and ***Mori et al. (1999)*** reported that elevation in chick plasma lipid caused by saturated fat or cholesterol feed, the lipid lowering drugs (probucol, gemfibrozil and lovastatin) caused reduction in plasma lipids of laying hen.

Weder et al. (1991) reported that lovastatin significantly lowered total and low density lipoprotein cholesterol and raised high density lipoprotein cholesterol in hypercholesterolemic patients.

Engeseth et al. (1993) found that one way to increase the oxidative stability of lipids and cholesterol in food is to increase the amount of natural antioxidants such as, α tocopherol (vitamin E) or B carotene in the diet feeding.

Prasad and Kalra (1993) showed that vitamin E is a potent antioxidant and so, it prevents oxidation of polyunsaturated fatty acids in human cell membrane.

Thompson et al. (1986)., Tobert (1987) and ***Sehayek et al. (1994)*** reported that mevinolin was high effective in controlling hypercholesterolemia than cholesterolamine by decreasing in serum total cholesterol. This decrease was accompanied by only small changes in serum triglyceride and HDL cholesterol and was largely due to reduction in LDL – C.

Cindy et al. (1988) found that lovastatin caused a 36% reduction in plasma total cholesterol, in LDL cholesterol level and 17% increasing HDL- C level, lovastation lowered both total and low density lipoprotein level in normal and hypercholesterolemic subjects with few acompanying side effects.

Huff and Telford (1989) showed that no significant effect was seen in the LDL apoliporotein fractional catabolic rate in atorvastatin treated animals in addition, the fractional catabolic rate for the direct catabolism of VLDL apolipoprotein and IDL (intermediate density lipoprotein) apolipoprotein was unchanged by atorvastatin treatment.

Poernama et al. (1990) reported that a spontaneous sex-linked HDL deficiency syndrome in chickens. These mutant chickens called WHAM (Wisconsin hypo- alph mutant) have a 70-90% reduction in plasma HDL cholesterol and apolipoprotein concentrations. They concluded that HDL deficiency syndrome in chickens is not associated with an increased susceptibility to atherosclerosis.

Berglund et al. (1994) found that altered apoplipoprotein metabolism in VLDL from lovastatin-treated guinea pigs and

explanation for the lack of effect on LDL apoliporotein fractional catabolic rate would be the synthesis of an LDL particle of altered composition such that it interacts less efficiently with upregulated LDL receptors.

Bujo et al. (1995) proved the presence of a spontaneous mutation in the family of genes for the LDL receptor in hens, the resulting syndrome included altered ovulation, hypercholesterolemia and severe atherosclerosis.

Conde et al. (1996) found that hypocholesterolemic actions of atorvastatin are associated with alterations on hepatic cholesterol metabolism and lipoprotein composition LDL clearance may become apparent at higher doses of atorvastatin in guinea pigs.

Mendonca (1996) indicated that plasma cholesterol concentration of laying hen is not related to egg yolk lipid content, although synthesized in the liver and transported by the blood. He suggested that the primary action of atorvastatin is through reduced microsomal cholesterol availability for either lipoprotein surface or as a substrate for acylcoenzyme a transferase, thereby reducing cholesterol ester availability for lipoprotein formation.

Huff et al. (1985) found that mevinolin and cholestyramine inhibit the direct synthesis of LDL-C in minature pigs due to a decrease in LDL apopliporotein production.

Berglund et al. (1989) studied the effect of lovastatin therapy on guinea pig. They found that treatment with lovastatin had no effect on VLDL or LDL apo B production but did increase hepatic expression of LDL receptors.

Huff et al. (1992) studied the effect of dietary fish oil plus lovastatin in miniature pigs. They found that modulation of apolipoprotein synthesis and secretion appears to be an important mechanism where by HMG- COA reductase inhibitors showed reduction in the plasma concentration of apolipoprotein containing lipoprotein and also decreases in both VLDL and LDL apoliporotein production.

Naoumova et al. (1996) studied the effect of atorvastatin therapy on homozygous familial hypercholesterolemia. They demonstrated that in familial homozygous hypercholesterolemia, plasma mevalonate concentrations (an indirect measure of cholesterol biosynthesis) were decreased significantly longer following a single dose of atorvastatin, compared with a similar dose of simvastatin. Alternatively, atorvastatin could influence triglyceride and/ or phospholipids synthesis in addition to its effect on cholesterol synthesis.

Macri et al. (1995) suggested that atorvastatin increases the rate of intracellular degradation and decreases apolipoprotein translocation.

Shand and West (1995) studied the effects of simvastatin and cholestyramine, alone and in combination on hepatic cholesterol metabolism in the male rat. They found that both inhibitors of HMG- COA reductase have been shown to decrease the activity of acyloenzyme A: cholesterol acyltransferase, preventing the esterification of newly synthesized cholesterol.

Watts et al. (1995) found that simvastatin treatment on heterozygous familial hypercholesterolemia decreased the VLDL apo B absolute secretion rate. This reduction correlated with the change in plasma LDL cholesterol but not with those of triglyceride or mevalonic acid.

Burnett et al. (1997) believed that the mechanism whereby HMG- COA reductase inhibitors decrease LDL cholesterol concentrations is by enhanced catabolism via upregulation of hepatic LDL receptors. Hepatic cholesterol synthesis inhibition would be expected to decrease hepatic cholesterol concentrations, thereby increasing the expression of LDL receptors. Plasma LDL cholesterol and its precursors would decrease consequent to the resulting increase in apopliporotein fractional catabolic rate.

Burnett et al. (1998) studied the effect of atorvastatin drug on miniature pigs fed a fat (34% of calories; polyunsaturated to monounsaturated to saturated ratio, 1: 1: 1) and cholesterol (400mg/day cholesterol ; 0.1%; 0.2 mg/ kcal) containing pig chow-based diet. Treated by atorvastatin (3 mg/ kg per day) for 21 days they found that treatment by atorvastatin significantly reduced both VLDL and LDL cholesterol.

Scheen (1998) stated that the dietary cholesterol induced hypercholesterolemia was associated with increase in serum cholesterol, decline in HDL and elevation in LDL fraction. The decline in HDL occurs secondary to the developed hyperlipidemia.

Caliskan et al. (2000) found that there were significant reduction in rat plasma cholesterol, triglyceride after 4 weeks of simvastatin therapy.

Heibashy (2000) stated that rats fed a high cholesterol induced hypercholesterolemia which caused significant increases in total serum lipids, cholesterol, triglyceride, LDL-C and VLDL cholesterol.

Anton et al. (1989)., Elkin et al. (1999) and ***Kim et al. (2004)*** reported that simvastatin significantly reduced total serum cholesterol, LDL – C in patients with hypercholesterolemia, 0.03 % and 0.06% of atorvastatin, lovastatin and simvastatin were fed to white leghorn hens for 5 weeks lowered plasma total cholesterol concentration by both doses of atorvastatin and simvastatin. Forty four brown layers of laying hen which were fed lovastatin, simvastatin or pravastatin at 0.03% or 0.06% for 4 weeks found that total plasma cholesterol was significantly reduced by 0.06% lovastatin and 0.03% and 0.06% simvastatin whereas, pravastatin had no effect, respectively.

Hallman et al. (2004) found that several epidemiological studies have shown inverse correlation between HDL cholesterol concentration and the incidence of cardiovascular disease.

Jonathan and Tobert (1986) and Puicini et al. (1995) reported that lovastatin and simvastatin produced a moderate reduction in serum triglycerol with hypercholesterolemic subject. Also, ***Anton et al. (1989)*** reported that simvastatin significantly reduced serum triglyceride in patients with hypercholesterolemia.

In humans, Edwards and Moore (2003) developed randomized, double blind study using statins intrials for 12 weeks or longer and showed that different statins at a range of doses reduced total cholesterol by 17-35% and LDL cholesterol by 24-49% from baseline. Lower doses of statins generally produced less cholesterol lowering, though for most statins in trials of 12 weeks or longer there was at best only a weak relationship between dose and cholesterol reduction. Duration of treatment and baseline total cholesterol concentration did not alter the amount of the benefit attained. Also they concluded that statins are effective drugs and confer benefit to patients in terms of primary and secondary prevention of coronary heart disease. Reductions in total cholesterol of 25% or more and LDL cholesterol of more than 30% were recorded for fixed doses of simvastatin (40 mg), atorvastatin (10mg) and rosuvastatin (5mg and 10mg).

Hypertriglyceridemia leads to decrease in HDL due to transfer of cholesterylesters from HDL into VLDL ***(Nestel et al., 1979). Mori et al. (1999)*** reported that dietary lipid-lowering drugs (0.1 % probucol, 0.025 % gemfibrozil and 0.005 %, 0.001%, 0.0015% lovastatin) on laying hen for 12 weeks showed depression in triglyceride concentration approached statical significantly only for lovastatin (0.001%) and they stated that triglyceride reduction by HMG- COA reductase inhibitors parallels LDL cholesterol reduction.

Elkin et al. (1999) reported that in atorvastatin – treated birds, there were parallel reductions in the levels of circulating cholesterol and triglycerides with no change in VLDL.

Heibashy (2000) observed that rats fed extra cholesterol in diet for five months led to significant increases in serum total lipid, total cholesterol, triglycerides, VLDL-C, LDL-C and HDL-C.

Cheung et al. (2001) demonstrated that patients receiving simvastatin had significant reductions in their cholesterol, triglycerides, and LDL (bad) cholesterol levels, while therapy. Those receiving simvastatin plus niacin had greater increases in their HDL (good) cholesterol compared to those receiving simvastatin plus niacin and the antioxidants. The antioxidants appeared to blunt the positive effects of the statin therapy.

Kempaiah and Srinivasan (2002) found that serum cholesterol, triglycerides, LDL-C and VLDL-C were increased and HDL-C was decreased in hyperlipidemic rats.

Simonen et al. (2000) showed that cholesterol absorption and synthesis were related to respective serum sex hormone binding globulin, glucose and insulin values.

Ahin et al. (2002) who studied the effect of vitamin E supplementation at various concentrations (0, 62.5, 125, 250 or 500 mg/ kg of diet on cobb- 500 male broilers under heat stress 32^{o}C. They showed that increased supplementation vitamin E linearly decreases serum triglycerides and cholesterol.

Souza and Sliva (2006) observed that dietary vitamin E (100 mg, 200mg, 400mg/ kg diet caused reduction of 30% in the cholesterol level of pig meat and cooked ham. Supplementation of 200mg of vitamin E/ kg diet or more maintained cholesterol esters values below 10ug/g during 116 days before slaughter.

Tovar et al. (2007) found that in hypercholesterolemic rabbits the values of cholesterol, triglyceride and glucose were significantly higher at the end of 8^{th} week of feeding cholesterol.

Guazzi et al. (2007) reported that total and LDL cholesterol significantly reduced after statin therapy in post-myocardial infarction patients.

Ahin et al. (2002) studied the effect of vitamin E supplementation at various concentrations (0, 62.5, 125, 250 or 500 mg/ kg of diet) on cobb- 500 male broilers under heat stress $32^{o}C$. They found that protein and albumin concentrations increased linearly when dietary vitamin E supplementation increased.

Gamba et al. (2001) found that proteinuria began to rise in rats from the 8^{th} month in the groups ingesting diets containing 5% lipid enriched diet, while in the control group it increased significantly (above 10mg/ 24h) only after 10 month and showed a significant progressive decline in glomerular filtration rate (GFR) in hypercholesterolemic rats.

El Fiky (1999) and ***Kaya et al. (2001)*** reported that hypercholesterolemic rats and laying hens suffered a significant elevation in circulating plasma glucose respectively.

Simonen et al. (2002) found that the higher blood glucose level, the higher the cholesterol synthesis and cholesterol absorption efficiency was lower. Cholesterol synthesis was higher in obese subjects with diabetes than in those without diabetes.

Ahin et al. (2002) who studied the effect of vitamin E supplementation at various concentrations (0, 62.5, 125, 250 or 500 mg/ kg of diet) on cobb- 500 male broilers under heat stress 32^{o}C. They found that increased supplemental vitamin E linearly decreases in serum glucose.

Matos et al. (2005) found that there was no difference in serum albumin and glucose levels in hypercholesterolemic rats compared to control one.

Ozanosy et al. (2005) reported that simvastatin treatment decreased plasma lipids and provided a relatively better glucose metabolism in diabetic animals.

Milne (1996) reported that minerals play an important role in the regulation of body fluids, acid-base balance and metabolic processes.

Rubatu et al. (1993) found that hypercholesterolemia in rats caused reduction of urinary sodium excretion, while urinary potassium/ sodium ratio was increased.

El Fiky (1999) found that in rats dietary cholesterol induced hypercholesterolemia showed elevation in circulating levels of phosphorus, decline in calcium level and potassium level and no change in sodium level.

Ahin et al. (2002) studied the effect of vitamin E supplementation at various concentrations (0, 62.5, 125, 250 or 500 mg/ kg of diet) on cobb- 500 male broilers under heat stress 32^{o}C. They found that increased supplemental vitamin E linear increased in serum concentration of calcium and phosphorus.

Abdl-El Reheem (2003) found a significant increase in cholesterol and a significant decrease in calcium in the serum of rats fed vegetable oil compared to the control one.

Simarks et al. (2004) observed that hens at the ovulation have significantly higher calcium level than no reproductive female, sodium is present mainly in the extracellular fluid and is primarily responsible for determining the volume of the extracellular fluids and its osomatic pressure. The normal range of serum sodium in mature birds were 130-150 mmol/L.

Thompson et al. (1986)., ***Jonathan*** and ***Tobert (1986)., Cindy et al. (1988)*** found that mevinolin elevated serum transaminases enzyme in familial hypercholesterolemia. Transaminase levels particularly of (ALT) tend to rise slightly during treatment with lovastatin. The elevations were greater than the upper limits of normal values multiplied by 2. The same phenomenon had been reported with most lipid lowering drugs, including cholestyramine since bile acid sequestrants are not absorbed. Small increase in transaminases may be general response to change in lipid metabolism rather than a direct effect of lipid lowering drugs on the liver, elevations of levels of transaminase three folds (AST), (ALT) levels were uncommon biochemical

abnormalties in patients treated with lovastatin. The adverse effects of simvastatin were primary composed of increase in serum transaminases, elevations in AST and ALT values more than three folds with pravastatin treatment in men with hypercholesterolemia.

Ahin et al. (2002) who studied the effect of vitamin E supplementation at various concentrations (0, 62.5, 125, 250 or 500 mg/ kg of diet) on cobb- 500 male broilers under heat stress 32^{o}C. They found that AST and ALT were not influenced by dietary vitamin E supplementation.

Tarumi et al. (1989) and ***Walsh et al. (1996)*** hypothesized that there was elevation of HMGR activity in dogs fed up to 80 mg/ kg atorvastatin for 12 weeks or 25 mg/ kg pravastatin for 14 weeks, respectively.

Ness et al. (1998) reported that atorvastatin caused less induction of rat hepatic HMGR mRNA than lovastatin, despite causing a large induction of reductase protein.

Elkin et al. (1999) found that (HMGR) activities were significantly elevated 1-2 folds in the livers of all (HMGR) inhibitor (atorvastatin- lovastatin or simvastatin) fed hens, relative to control.

Ness and Chambers (2000) stated that high basal expression of hepatic HMG-COA reductase, whether due to genetic or hormonal factors, appears to result in greater cholesterol buffering capacity and thus increased resistance to dietary cholesterol.

Yassin et al. (2005) found that control of body level of cholesterol depends on the rate of excretion in the bile as

cholesterol or bile salts in relation to the rate of synthesis Acetyl-co-A, which is regulated by feed back inhibition on HMGCOA reductase by excess cholesterol. High diet cholesterol causes decreased synthesis and the excess is excreted in bile. The excreted bile salts are more efficiently reabsorbed than dietary cholesterol.

Sturkie (1965) reported that high cholesterol diet is one of the potential mediators for persistent filtration rate arising from lipid accumulation and the consequent development of glomerulo-sclerosis.

Grone et al. (1989) found that serum uric acid of mature female thai indigenous chickens was higher than that of immature indigenous chickens, while serum uric acid of laying birds was lower than in non reproductive females.

El – fiky (1999) showed that elevation in urea and creatinine level as the result of hypercholesterolemia induced complications in male rates.

Hoyos et al. (2000) recorded increases in the uric acid, bilirubin and glucose levels of hypercholesterolemic rats.

Ahin et al. (2002) studied the effect of vitamin E supplementation at various concentrations (0, 62.5, 125, 250 or 500 mg/ kg of diet) on cobb- 500 male broilers under heat stress 32^{o}C. They found that increased supplementation vitamin E caused linearly decreases in serum uric acid.

Simaraks et al. (2004) reported that uric acid of birds is a major product of the catabolism of nitrogen. Age and diet may

influence the concentration of blood uric acid beside that hyperuricemia has been documented during ovulatory activity.

Daghini et al. (2006) found that hypercholesterolemic pigs showed significant increases in plasma uric acid.

Hermier et al. (1989) found that laying hen represents a physiological model in which the mechanisms of action of estrogen in lipid transport can be evaluated and elevation in (VLDL), triglyceride-rich lipoproteins. The hyperesterogenic state in the laying hen is associated with major medications in lipoprotein and apolipoprotein profile such modifications may be relevance to clinical disorders involving esterogen induced hyperlipdemia.

Rubatu et al. (1993) reported that in rats dietary cholesterol induced hypercholesterolemia which exerts a significant influence on adrenal steroid metabolism and electrolytes.

Elkin and ***Yan (1999)*** found that plasma esterogen or progesterone concentration were decreased as a result of HMGR inhibitors treatment.

Connor et al. (1965) and ***Andrews et al. (1968)*** studied the cholesterol metabolism in laying fowl. They found that the cholesterol is readily transferred from the blood across the ovarian membranes to developing ova and therefore most, if not all, egg yolk cholesterol originates from blood cholesterol.

Patients receiving supplemental dietary cholesterol from egg yolk exhibited a significant increase in plasma cholesterol levels

and a decrease in plasma levels of cholesterol precursors, which may be toxic ***(Linck et al., 2000)***.

Weggemans et al. (2001) reported that to avoid elevations in blood cholesterol and reduce the risk of coronary heart disease, consumption of no more than 300mg of cholesterol daily and limited consumption of eggs has been recommended.

Elkin et al. (2003) reported that each chicken egg contains about 215mg cholesterol. The effort at reducing the level of cholesterol in intact chicken eggs have included genetic selection, use of low fat and high fibre diets, administration of pharmacological agents.

Salma et al. (2007) reported that the cholesterol content and fatty acid composition of eggs have received increased attention due to the relationship between dietary lipid and the incidence of atherosclerosis.

Olawumi and Ogunlade (2008) found that in determination of the internal and external quality traits of the breeding eggs of Isa Brown layer breeder, the almost all internal quality traits of the eggs were influenced at significant levels depending on the change that occurred in the egg weight with respect to the external quality traits of the egg. They suggested that it was possible to use egg weight in determining the egg shell weight, egg length, egg width.

Chowdhury et al. (2002) stated that egg yolk cholesterol concentrations have been shown to vary depending on genetics of the laying hen which genetic selection of hens for lower egg

cholesterol has resulted in a slight reduction in egg cholesterol concentration.

Robert (2004) found that egg shell quality may be affected by the strain and age of hen; induced moult; nutritional factors such as calcium, phosphorus, vitamins, water quality, non starch polysaccharides, enzymes, contamination of feed.

Cook and ***Briggs (1977)*** stated that chicken eggs are an excellent food stuff from a nutritional standpoint due to their composition of high-quality protein, mono-and polyunsaturated fatty acids, minerals and vitamins.

Sulton (1984) studied the cholesterol metabolism in the laying hen as influenced by dietary cholesterol they showed that the nutrients influence egg cholesterol content.

Berrio and Hebert (1990) who studied the effect of adding cholesterol (0, 0.5, 1, 2, 4%) for 35 days to laying hen diets as powder or predissolved in fat. They showed that mixing cholesterol with the fat source before feed incorporation did not promote higher yolk or liver cholesterol levels and were essentially the same as the method in which powdered cholesterol was added directly to the feed. A linear increase in yolk and liver cholesterol was observed with 0.5 and 1% dietary cholesterol. Yolk cholesterol also increased linearly during the first 14 days of cholesterol administration. However increase in yolk cholesterol, were not obtained with either the higher levels of dietary cholesterol or extended feeding time.

Endo (1992) Elkin et al. (1999) found reduction of cholesterol content of eggs by the oral administration of statin drugs to laying hens.

Griffin (1992) stated that egg cholesterol quantity of laying hen was more correlated with VLDL/ LDL than with plasma total cholesterol concentrations.

Leonhardt et al. (1997) showed that egg was the source of vitamin E for human nutrition by dietary modification.

Surai et al. (1997) stated that egg yolk to tochopherol ingested as a part of the diet prevented lipid preoxidation, haemolysis and testicular atrophy. ***Elkin et al. (1999)*** reported that oral administration of pharmacological agents atorvastatin, simvastatin and lovastatin developed for hypercholesterolemic patients inhibitors of HMGR was effective in reducing egg cholesterol content by 46%, 22% and 7% in hens fed 0.06 % level of atorvastatin, simvastatin and lovastatin, respectively.

Mori et al. (1999) reported that dietary lipid – lowering drugs (0.1% probucol, 0.025% gemfibrozl and 0.0005% , 0.001 and 0.0015% lovastatin) upon plasma lipids and yolk cholesterol of laying hens. They found that the supplementation of the drugs didn't impair albumin and shell quality, while egg cholesterol content was significantly low in 0.0005% and 0.0015 lovastatin treated groups.

Meluzzi et al. (1999) studied the effect of dietary vitamin E on quality of eggs for brown hens. Four doses were supplemented with dI - α tocophyeryl acetate (0, 50, 100 and 200ppm) where diet of other groups were supplemented with 3% fish oil and the same

doses of vitamin E. They found that the performance of the hens and egg weight were not affected either by the type of lipid supplemented or by the vitamin levels. Groups supplemented with fish oil was not significantly different from non supplemented groups.

Elkin and Yan (1999) showed that cholesterol in chickens egg yolk is essential for embryonic development and they found that there was the relation between inhibition of mevalonate biosynthesis and reduced fertility in laying hens.

Shafey et al. (1999) found that dietary fat had little effects on cholesterol deposition in the egg yolk of laying hens.

Kaya et al. (2001) showed that zinc and vitamin A supplements did not influence the concentration of egg yolk cholesterol.

Robert (2004) studied the effect of atorvastatin on egg nutrient compositional changes and residue in eggs, tissue to laying hens. He found that egg yolks from treated hens contained greater amount of amino acids and reduced levels of total fatty acids and cholesterol. In contrast egg albumin and amino acid were unaffected by dietary treatments, this is favorable because the eggs from atorvastatin treated hens were lower in cholesterol and fat and relatively richer in high quality protein.

Kim et al. (2004) reported that the laying hens which were fed lovastatin, simvastatin or pravastatin at 0.03% or 0.06% for 4 weeks, its egg weight was significantly lowered with all statin

treatments. Oral administration with 0.06% pravastatin reduced egg cholesterol levels by almost 20% compared with control.

Elkin (2006) suggested that statins apparently lower egg cholesterol via an inhibition of hepatic VLDL production.

Bourre and Galea (2006) found that designed benefic eggs with multiple enriched eggs by feeding laying hens in the usual way but using additional autoclaved in seed, minerals, vitamins and lutin to provide the extra components. These eggs have greater nutrional value than standard, thus 100 g of these eggs contains 6 times more vitamin E, contain a less cholesterol and rich in vitamins, phosphorus, proteins. Also, they improve the blood concentration of omega 3- fatty acid, HDL, LDL cholesterol and triglyceride in human.

Asli et al. (2007) studied the effect of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature. They found that 200 mg of vitamin E per kg of diet showed significantly increased in yolk percent and increase in immune response. Antioxidant properties of vitamin E have been shown to enhance immunity of laying hens which it protect cells involved in immune response, such as lymphocytes, macrophages and plasma cells, against oxidative damage and to enhance the function and proliferation of these cells.

Elkin, (2007) indicated that feeding reductase inhibitors (statins) to laying hens could reduce egg cholesterol as 46 percent.

However, these were concerns with the effect on nutrient composition and potential transfer of drugs to the egg.

Ritchie et al. (1994) showed that ovulating hens have significantly higher calcium levels than non reproductive females.

The aim of the work was to investigate the effect of cholesterol administration and the most potent hypocholesterolemic drugs (atorvastatin, simvastatin and lovastatin) with vitamin E on some physiological aspects in laying hens and its eggs, including :

1- Blood parameters: White and red blood cell counts (WBCs and RBCs), hemoglobin content (Hb), hematocrit value (Hct) and calculated blood indices (mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH and mean corpuscular hemoglobin concentration, MCHC).

2- Respiratory functions of blood:

a) Arterial and venous blood gases including blood oxygen and carbon dioxide partial pressure and percent of blood oxygen saturation.

b) Blood acid-base status parameters comprising blood pH, blood bicarbonate, total carbon dioxide and base excess in both arterial and venous blood.

c) Blood oxygen equilibrium curve and half saturation pressure (P_{50}).

3- Biochemical assessment includes:

a) Metabolites: Serum total lipids, total cholesterol, high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very

low density lipoprotein (VLDL-C), triglyceride total protein, albumin, globulin and glucose, uric acid and creatinine concentrations were determined.

b) Electrolytes: Serum sodium, potassium, calcium and phosphorus were determined .

c) Enzymes:Serum aspartate amino transferase (AST), alanine amino transferase (ALT), and hepatic microsomal-3-hydroxyl-3 methylglutaryl coenzyme A reductase (HMGR) activities were determined.

d) hormones: Serum progesterone and esterogene levels were determined

4- Egg parameters: Egg, yolk, albumin and shell were weighted. Also, egg shell weight ratio, egg yolk cholesterol, egg yolk calcium and egg yolk phosphorus concentrations were determined.