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Review Article: Oxidative Stress as Molecular Mechanism in Environmental Stress

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SUMMARY

The concept and manifestation of stress and forms of environmental stress which was defined as a biological response to an event that an individual perceives as a threat to its homeostasis are linked to the activation of the hypothalamo-pituitary-adrenal system. Under normal conditions, cells have well-developed antioxidants systems that minimize the pertubations caused by reactive oxygen species (ROS). However, when ROS generations are increased to an extent that they overcome the cellular antioxidants then oxidative stress results. Oxidative stress is seen as a battle between inducers (pro-oxidants) and protective factors (antioxidants), because ROS are partially products of oxygen; they have a high chemical reactivity with other bio-macromolecules that may lead to lipid peroxidation and oxidation. Due to this reactivity, oxidative stress is thought to play an important role in the pathogenesis of environmental stress.

KEY WORDS: Environmental stress, Reactive oxygen species, Oxidative stress, Antioxidants.

The Stress Concept

Stress can be defined as a biological response to an event that an individual perceives as a threat to its homeostasis (Moberg, 1993; Einarsson *et al.*, 2008). Perception of stressful stimuli leads to activation of the hypothalamo-pituitary-adrenal (HPA) system, which in turn results in the release of a variety of peptides, principally corticotrophin releasing hormone (CRH) and vasopressin from the hypothalamus (Buckingham *et al.*, 1993; Einarsson *et al.*, 2008). CRH stimulates the release of adrenocorticotrophic hormone (ACTH) and other proopiomelanocortin (POMC) derived peptides, such as β-endophin from the anterior lobe of the pituitary gland. ACTH acts on the adrenal glands and causes secretion of glucocorticoid hormones, e.g cortisol. ACTH also causes the release of other hormones from the adrenal glands e.g progesterone, possibly prostaglandin F2α metabolites (Madej *et al.*, 2005) and even inhibin α (Brandt *et al.*, 2007). Stress also involves the activation of the sympathetic nervous system and the adrenal medulla. The subsequent release of catecholamines e.g adrenaline and noradrenalin into blood stream, leading to an increase in the glucose supply by accelerating the degradation of glycogen in the liver (Velluci, 1997). The glucocorticoids release during stress also stimulate lipolysis and gluconeogenesis (the conversion of amino acids to glucose), which leads to an increased

metabolism that promotes the ability to cope with the stress (Razdan, 2003).

Selye (1975) divided the effect of stress into three stages: Alarm, Resistance and Exhaustion.

Alarm Stage: - This is the first stage; the body's stress response is in a state of alarm when the stressor is identified. In this stage, adrenaline is produced to bring about the fight or flight response. Cortisol is produced as a result of the activation of the HPA axis.

Resistance Stage: - This is the second stage of stress reaction. When stressor persists, the body begins to try to adapt to the demand of the environmental stressor and since the body cannot keep up with this indefinitely, its resources are gradually depleted.

Exhaustion Stage: - is the final stage; at this point, all of the body's resources are eventually depleted and the body is unable to maintain normal function. Autonomic nervous system symptoms like sweating, increased heart rate etc may be seen, but if this stage is extended, long term damage may result as the body and the immune system are exhausted and function is impaired resulting in decompensation.

An animal is under stress when it has to make extreme functional, structural, behavioural or immunological adjustments to cope with adverse aspects of its environment (Curtis, 2003). During stress stage, there is an increase in generation of reactive oxygen species (ROS), elevated to a level that overwhelms tissue antioxidant defense system (Adenkola and Ayo, 2010).

What are Free Radicals

Free radicals are an atom, molecule or compound that is highly unstable because of its atomic or molecular structure (Wu, 2003). If an atom or molecule contains one or more unpaired electrons and is capable of independent existence, it is referred to as "free radical" (Halliwell and Gutteridge, 1989). Atoms possess electron that are usually associated in pairs. Each pair moves in a defined space around the nucleus referred to as the atomic/molecular orbital. One electron of the pair has spin quantum number +1/2 and the other -1/2. When the electrons are in opposite spins, the electronic state is referred to as "ground state." Electrons with the same spin are "triplet state" but if singlet molecules absorb energy without changing spin, the molecule is in "excited singlet state" (Kochever, 1993). Free radicals can be generated as products of homolytic, heterolytic, or redox refactions, producing either charged or uncharged radical species (Powers and Jackson, 2008). As a result, free radicals are very reactive as they attempt to pair up with other molecules, atoms or even individual electrons to create a stable compound. To achieve a more stable state, free radicals can "steal" a hydrogen atom from another molecule, or interact in various ways with other free radicals (Wu, 2003). A paradox in metabolism is that while the vast majority of complex life requires oxygen for its existence, oxygen is a highly reactive molecule that damage living organisms by producing reactive oxygen species (ROS) (Davies, 1995; Leeuwenburgh and Heinecke, 2001) and this has demonstrated to have important roles in cell signaling and homeostasis (Devasagayam *et al.*, 2004). It should be noted that ROS is a general term that refers to not only oxygen-centered radicals but also includes non radical but reactive derivatives of oxygen (Halliwell and Guteridge, 1989). ROS are oxygen-containing molecules that are capable of either accepting or donating a free electron, thus they are, to some extent unstable and react with other molecules (Tkaczyk and Vizek, 2007). Also the term reactive nitrogen species (RNS) refers to both nitrogen radicals along with other reactive molecules where the reactive centre is nitrogen. Reactive oxygen and nitrogen species (RONS) can be used collectively for both ROS and RNS and this includes both free radicals and non-free radical species (Power and Jackson, 2008).

Free radical generation is a natural consequence of living in an oxidizing environment. Cells generate small amount of free radicals or ROS while performing their normal metabolic functions (Mates *et al.*, 1999). A free radical prefers to "steal" electrons from the lipid membrane of a cell, initiating free radical attack on the cell known as lipid peroxidation. ROS target the carbon-carbon double bond of polyunsaturated fatty acids. The double bond on the carbon weakens the carbon-hydrogen allowing for easy dissociation of the hydrogen by a free radical (Halliwell and Gutteridge, 1985). A free radical will "steal" the single electron from the hydrogen associated with the carbon at the double bond. In turn this leaves the carbon with an unpaired electron and hence becomes a free radical. In an effort to stabilize the carbon-centered free radicals, molecular arrangement occurs. The newly arranged molecule is called a conjugated diene which easily reacts with oxygen to form a peroxy radical. The peroxy radical "steals" an electron from another lipid molecule in a process called propagation. This process then continues in a chain reaction (Halliwell and Gutteridge, 1985). Thus, a free radical causes oxidative damage to cell components (Avellini *et al.*, 1995; Lecarpentier, 2007) such as lipids (Buettner, 1993), carbohydrates (Greenwald and Moy, 1980), proteins (Staldman, 1986), and deoxyribonucleic acid (Imlay and Linn, 1986). These deleterious effects of ROS are involved in many molecular, haematological and biochemical changes occurring during stress-induced disease conditions (Akinwande and Adebule, 2003) and adaptation (Meerson, 1986). The mechanism of damage involves oxidative stress which leads to lipid peroxidation and destruction of cell membranes with the release of intracellular components, such as lysosomal enzymes leading to further tissue damage (Demir *et al.*, 2003).

What is Oxidative Stress

The term oxidative stress was first defined in 1895 as "a disturbance in the pro-oxidant-antioxidant balance in favour of pro-oxidant (Sies, 1985). Under normal conditions, cells have well-developed antioxidant system that minimize the perturbations caused by ROS, however during the period of oxidative stress, pro-oxidants overwhelm the antioxidant defenses in cells and damage cellular constituents (Powers and Jackson, 2008). Therefore oxidative stress may be viewed as a continuous battle between pro-oxidants and protective factors (antioxidants). Oxidative stress results from the generation of enormous free radicals and other reactive oxygen species (ROS) to a level that cannot be handled by the tissue antioxidant defense system (Powers and Jackson 2008, Ambali 2010).

Thus oxidative stress in biological systems is often characterized by the following parameters:

(1) Increase in the formation of radicals and other oxidants
(2) Decrease in small-molecular-weight and or lipid soluble antioxidants
(3) Disturbance in cellular redox balance
(4) Oxidative damage to cellular components (i.e., lipids, proteins, and or DNA).

Reactive oxygen species (ROS) are continuously produced during cell metabolism (Pandey *et al.*, 2009). Under normal conditions they are scavenged and converted to nonreactive species by different intracellular, enzymatic and non enzymatic antioxidants systems such as catalase, peroxidase, superoxide dismutase, vitamins and reduced glutathione (Hyman *et al.*, 2005). Over production or an ineffective elimination of ROS may induce oxidative stress and cause damage to all types of molecules such as proteins, lipids and nucleic acids (Drooge, 2002; Akinwande and Adebule, 2003).

The oxidative modification of the erythrocyte

membrane has been shown to increase the fragility of the RBC (Langsdorf and Zydney, 1993). Lipid peroxidation, which is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) when it happens in biological membranes causes impairment of membrane function and structural integrity (Gutteridge and Halliwell, 1988),decrease fluidity and inactivation of a number of membrane bound enzymes and protein receptors (Sidhu *et al.*, 2004). Erythrocytes are frequently used to evaluate oxidative stress; this is because their membrane is rich in polyunsaturated fatty acids which is a primary target for reaction involving free radicals and is susceptible to lipid peroxidation (Brzezinska-Slebodzinska, 2003). Oxidative stress in animals during transportation stress has been quantified indirectly by the shifting of the fragiligram to the right side indicating increased haemolysis (Adenkola and Ayo, 2009a, Adenkola *et al.*, 2010a).

The constant exposure of erythrocyte to high oxygen tension coupled with high level of iron and richness in Polyunsaturated Fatty Acids (PUFA) (Kollanjiappan *et al.*, 2002) and also their inability to possess nucleus and other organelles (Doroevic *et al.*, 2008) have made erythrocyte a centre of free radical attack. Process of lipid peroxidation decreases hydrophobic characteristics of bilayer membrane of erythrocytes, altering affinity and interaction of proteins and lipids, thereby impairing the functioning and homeostasis of erythrocytes membrane (Dargel, 1991). ROS can equally affect the proteins resulting in modification of enzymes activity, and damage to the membrane transport proteins may produce disturbed cellular ionic homeostasis, leading to alterations in intracellular calcium and potassium that triggers a series of changes in the cell (Kerr *et al.*, 1992). ROS can directly affect the conformation and/or activities of all sulfhydryl-containing molecules, by oxidation of their thiol moiety (Wilcox *et al.*, 2001).

Environmental Stress

The productivity and health of livestock are being affected by adverse meteorological conditions (Ayo *et al.*, 1998a & b; Adenkola *et al.*, 2009b) as well as the quality of meat (Adenkola *et al.*, 2011a) prevailing in the tropical and subtropical countries which make animals often subjected to environmental stress (Vathana *et al.*, 2002). The most important climatologically thermal conditions are heat stress during the hot season and the wind chill factor during the cold season (Broucek *et al.*, 2007). In Nigeria, three distinct season are known; rainy season, hot-dry season and harmattan season. Heat stress is common during dry season, occurring between November and May (Igono *et al.*, 1982), with a mean monthly rainfall of less than 51mm (Walter, 1969). The harmattan season is characterized by marked fluctuations in ambient temperature (AT) with high AT in the afternoon hours of the day and relatively low temperature of about 10 ^{0}C in the evening and early morning hours of the day. The season is associated with a dry cold and dust-laden wind that blows from Sahara desert and low relative humidity (RH) (Igono *et al.*, 1982; Oladele *et al.*, 2003). The hot-dry season is also characterized by high AT and RH and long duration of sunshine. These seasons (hot-dry and harmattan) constitutes environmental stress to livestock (Adenkola and Ayo, 2009b; Adenkola *et al.*, 2011b) by impairing the homeostatic mechanism of the body. Stress is the responses of the body to extraneous stimuli that disturb the normal physiological equilibrium or homeostasis (Khansari *et al.*, 1990; Mstl and Palme, 2002). This condition causes decrease in the concentration of antioxidant vitamins, lipid peroxidation as a result of free radicals formation. This increase in the plasma and tissue lead to damage of cell membrane (Sahin *et al.*, 2001).

Effect of Environmental Stress in Livestock

During times of environmental stress ROS levels can increase dramatically

(Devasagayam *et al.*, 2004). This may result in significant damage to cell structures. This cumulates into a situation known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation (Devasagayam *et al.*, 2004). Temperature stress is a phenomenon that can impart physical and economical losses to livestock production in temperate, sub tropic and tropical regions of the world (Nisa *et al.*, 1999). Temperature stressed animals undergo a series of metabolic and physiological changes. These changes are necessary for adaptability and survivability of the animal. Nutritional balance of the animal is an important factor in thermal stress, the disturbance of which may be deleterious to performance (Christopherson and Kennedy, 1983). When environmental temperature move out of the thermo neutral zone, livestock begin to experience either heat stress or cold stress. Thermal stress in livestock results in increased demand for net energy for maintenance and subsequent reduction in energy for tissues growth and production (Ames *et al.*, 1994). Thermal stress has negative effect on food consumption and metabolic activities (Nisa *et al.*, 1999). Cold stress (below the thermo neutral zone) increased maintenance requirements of livestock (Hidriroglou and Lessard, 1970). Environmental stress can occur when an animal's environment changes so as to provoke reaction for instance when environmental temperature falls below the critical level or when the animal itself changes in relation to a given environment (Curtis, 2003).

Environmental stress is not limited to climatic factors but extends to nutrition, housing and any stimuli that demand a response from the animal to adapt new circumstances. Low energy and low or excessive protein levels in the diet are detrimental to reproduction (Lee, 1993). Also, high ambient temperature and humidity alter the intricate balance of endocrine profiles resulting. When stressors become so aversive that an animal is unable to adapt, it enters a state of distress, where its physiology and behavior become maladaptive, productivity is also affected due to difficulty in coping with stress (Selye, 1975).

Road Transportation Stress

Road transportation is another form of stressor that livestock are inevitably subjected to (Buckham Sporer *et al.*, 2008; Adenkola *et al.*, 2009b Adenkola *et al.*, 2011c) as a result of marketing and the need to slaughter them for meat in abattoirs which is often located outside places where the animals are reared (Chandra and Das, 2001; Voslarova *et al.*, 2007). The stress may be more or less severe depending on a number of factors such as crowding, temperature, feed and water deprivation and length of travel (Ritter *et al.*, 2007; Adenkola *et al.*, 2009a; Adenkola, 2011). Transportation disrupts normal patterns of feeding and drinking in animals. It is associated with exposure to novel environments, sometimes involving mixing with unfamiliar and closely confined animals, noise, vibration and extremes of AT and humidity (Warris, 2004).

Adenkola *et al.* (2009c) demonstrated that road transportation induced leucocytosis, neutrophilia, lymphocytosis, eosinophilia in control pigs that were not supplemented with ascorbic acid while total protein, alkaline phosphatase, aspatate amino transferase and neutrophil lymphocyte ratio was found to decrease significantly in pigs administered with ascorbic acid as compared to control group after 4-h of road transportation during harmattan season in Northern Guinea Savanna zone of Nigeria in pigs. This finding indicates for the first time the beneficial effect of ascorbic acid administration on haematology and serum biochemistry of pigs transported during the harmattan season. In another study, Adenkola and Ayo (2009a) reported that administration of ascorbic acid prior to transportation of pigs is beneficial as it protects the integrity of the erythrocyte membranes in experimental pigs following road transportation and thus may alleviate the risk of

increased haemolysis due to road transportation stress in pigs during the harmattan season. It is, therefore, recommended that ascorbic acid be administered to pigs before transportation in order to reduce the adverse effects of road transportation stress on erythrocytes.

Antioxidants

Originally, the term antioxidant specifically referred to a chemical that prevented the consumption of oxygen (Mattill, 1947). ROS are produced during normal cellular functions and are generated as by-products of cellular metabolism, primarily in the mitochondria. ROS include hydroxyl radicals (OH), superoxide anion (O_2), hydrogen peroxide (H_2O_2) and nitric oxide (NO). The cell has developed a powerful, complex defence system that limits its exposure to these agents, and these are the so-called antioxidants (Chaudiere and Ferrari-Illiou, 1999; Powers and Jackson, 2008). Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity (German, 1999). Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C, and E, as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Jacob, 1996; Knight, 1998).

Antioxidants are substances that either directly or indirectly protect cells against the adverse effects of xenobiotics, toxicants, drugs, and carcinogens. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions, they do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols (Sies, 1997).

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membrane from lipid peroxidation (Sies, 1997). These compounds may be synthesized in the body or obtained from the diet Vertuani *et al.* (2004).

Classification of Antioxidants

Several antioxidants are enzymes or essential nutrients (Machin and Bendich, 1987). An essential nutrient is a compound that must be supplied in the diet because the organism is unable to synthesize it. Based on this characteristic, some authors classify antioxidant as non-enzymatic and enzymatic (Larkins, 1999; Chaudiere and Ferrari-Illiou, 1999). However, another frequently used classification is based on the protective mechanism used by the antioxidant, grouping them into those that prevent free radical generation and those that trap free radicals (Cheeseman and Slater, 1993).

Antioxidants can therefore be divided into three categories:

(1). Dietary (nutritional) antioxidants are low molecular weight compounds ingested in the diet such as vitamin C, vitamin E, the carotenoids, flavonoids, other plant phenolics and wine phenolics.

(2) Intrinsic molecules such as glutathione, albumin, bilirubin and uric acid.

(3) Enzymes that specifically metabolize ROS precursors, such as catalase, superoxide dismutase (SOD), glutathione peroxidase, and peroxiredoxins.

They are molecules that prevent unlimited generation of free radicals or inhibit their reaction with biological structures (Yu, 1994). Antioxidants are agents that scavenge physiologically
ROS and RNS, and thereby prevent oxidative damage to important biological macromolecules such as deoxyribonucleic acid, lipids and proteins (Akinwande and Adebule, 2003; Lecarpentier, 2007). Antioxidants protect lipids from peroxidation by radicals. They are effective because they readily give up their electron to free radicals. When a free radical gains an electron from an antioxidant, it can no longer attack the cell and the chain reaction of oxidation is broken (Dekker, 1996). After donating an electron, an antioxidant becomes a free radical. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive (Adenkola and Ayo, 2010).

Ascorbic acid

Ascorbic acid or "vitamin C" is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. As one of the enzymes needed to make ascorbic acid has been lost by mutation during primate evolution, humans must obtain it from the diet (Smirnoff, 2001). Most other animals are able to produce this compound in their bodies and do not require it in their diets (Linster and Van Schaftinger, 2007). Ascorbic acid is required for the conversion of the procollagen to collagen by oxidizing proline residues to hydroxyproline. In other cells, it is maintained in its reduced form by reaction with glutathione, which can be catalysed by protein disulfide isomerase and glutaredoxin (Wells *et al.*, 1990; Meister, 1994). Ascorbic acid is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide (Padayatty *et al.*, 2003).

Melatonin

Melatonin is a powerful antioxidant (Tan *et al.*, 2007). Melatonin easily crosses cell membranes and the blood-brain barrier (Reiter *et al.*, 2009). Unlike other antioxidants, melatonin does not undergo redox cycling, which is the ability of a molecule to undergo repeated reduction and oxidation. Redox cycling may allow other antioxidants (such as vitamin C) to act as pro-oxidants and promote free radical formation. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Tan *et al.*, 200).

Tocopherols (Vitamin E)

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat –soluble vitamins with antioxidant properties (Herrera and Barbas, 2001; Packer *et al.*, 2001). Of these, α-tocopherol has been most studied as it has the highest bioavalability, with the body preferentially absorbing and metabolising this form (Brigelius *et al.*, 1999).
It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant, and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrera and Barbas, 2001; Taber and Atkinson, 2007). This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidised α-tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol (Wang and Quinn, 1999). This is in line with findings showing that α-tocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death (Seiler *et al.*, 2008). GPX4 is the only known enzyme that efficiently reduces lipid-hydroperoxides within biological membranes.

Antioxidant Enzyme

Glutathione peroxidase

This is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Epp *et al.*, 1983; Ran *et al.*, 2007).

Glutathione is actually made up of amino acids, gamma - glutamic acid, cysteine and glycine and is considered a tri-peptide and is found in large concentrations is the liver. Glutathione not only protects us against free radical attacks but is instrumental in a well functioning immune system. As we age, our levels of glutathione declines, which many researchers believe may be why we are more vulnerable to disease. Glutathione helps maintain the integrity of red blood cells, as well as protecting white blood cells, assists in carbohydrate metabolism and breaking down oxidized fats.

Superoxide Dismutase

This enzyme was discovered in 1969 by Irwing Fridovich and Joe McCord (McCord and Fridovich, 1969) and is a class of enzyme that catalyzes dismutation of superoxide into oxygen and hydrogen peroxide, as such they are important antioxidant defense in nearly all cell exposed to oxygen (McCord and Fridovich, 1988). In mammals, three isomers of superoxide dismutase (SOD) exit (SOD1, SOD2, SOD3), and all require a redox active transition metal in the active site to accomplish the catalytic breakdown of the superoxide anion (Culotta *et al.*, 2006).

Several forms of superoxide dismutase (SOD) exist: they are protein co-factored with copper and zinc, or manganese, iron or nickel

REFERENCES

ADENKOLA, A.Y and AYO, J.O. (2009a). Effect of road transportation on erythrocyte osmotic fragility of pigs administered ascorbic acid during the harmattan season in Zaria, Nigeria. *J. Cell Anim. Biol.* 3(1): 004-008.

ADENKOLA, A.Y. and AYO, J.O. (2009b). Effect of ascorbic acid on rectal temperature Fluctuations in indigenous turkeys during the hot-dry season. *Int. J. Poul. Sci.* 8(5): 457 – 461.

ADENKOLA, A.Y., AYO, J.O and SACKEY, A.K.B. (2009a). Ascorbic acid-induced modulation of rectal temperature in pigs during the harmattan season. *J. Thermal Biol.*, 34(3): 152 -154.

ADENKOLA, A.Y., AYO, J. O., SACKEY, A. K. B., ADELAIYE, A. B., MINKA, N. S. (2009b). Excitability scores of pigs administered ascorbic acid and transported during the harmattan season. *Vet. Arhiv.* 79 (5): 471-480.

ADENKOLA, A.Y., AYO, J.O. SACKEY, A.K.B and ADELAIYE, A.B. (2009c). Haematological changes in pigs administered with ascorbic acid and transported by road for four hours during the harmattan season. *J. Cell Anim. Biol.* 3(2): 021- 028.

ADENKOLA, A.Y. and AYO, J.O. (2010). Physiological and behavioural responses of livestock to road transportation stress: A Review. *Afr. J. Biotech.* 9(31): 4845 – 4856.

ADENKOLA, A.Y. (2011). Behavioural responses in pigs administered with ascorbic acid and transported by road for eight hours during the harmattan season. *Vet. World.* 4(8): 353 – 357.

ADENKOLA, A.Y., LUGA, I.I., AGBEDE, O.O. and OKPE, J. (2011a). The Quality of Beef and Chevon during Harmattan Season in Makurdi, Nigeria. *Vet. Res.* 4(4): 117 – 120.

ADENKOLA, A.Y., AGBENDEH, J. and OKPE, J. (2011b). Comparative assessment of erythrocyte osmotic fragility of apparently healthy goat and cattle during the hot-dry and harmattan season in Makurdi, Nigeria. *J. Anim. Plant Sci.* 3: 1471 - 148.

ADENKOLA, A.Y., AYO, A.Y., SACKEY, A.K.B., ADELAIYE, A.B. (2011c). Eight hours road transportation and ascorbic acid administration effects on haematological parameters of pigs during the harmattan season. *Agric. Biol. J. North Amer.* 2(8): 1143 – 1150.

ADENKOLA, A.Y., AYO, J.O., SACKEY, A.K.B. and ADELAIYE, A.B. (2011d). Modulatory role of ascorbic acid on behavioural responses of pigs transported by road during the harmattan season. *Nig. J. Physiol. Sci.* 26: 061 – 065.

AKINWANDE, A.I. and ADEBULE, A.O.A. (2003). Ascorbic acid and beta-carotene alleviate oxidative effect of London Kingsize® cigarette smoke on tissue lipids. *Nig. J. Hlth. Biochem. Sci.* 2(1): 12 - 15.

AMBALI, S.F., ABUBAKAR, A.T., SHITTU, M., YAQUB, L.S., KOBO, P.I. and GIWA, A. (2010). Ameliorative effect of zinc on chlorpyrifos- induced erythrocyte fragility in wistar rats. *New York J.* 3(5): 117 – 122.

AMES, D.R., BRINK, D.R. and WILLIAMS, C.C. (1994). Adjusting protein in feedlot diets during thermal stress. *J. Anim. Sci.* 72: 598 – 605.

AVELLINI, L., SILVERSTRELLIi, M. and GAITTI, A. (1995). Training-induced modifications in some biochemical defences against free radicals in equine erythrocytes. *Vet. Res. Comm.* 19: 179 - 184.

AYO, J.O., OLADELE, S.B., FAYOMI, A., JUMBO, S.D. and HAMBOLU, J.O. (1998a). Body temperature, respiration and heart rate in the Red Sokoto goat during the harmattan season. *Bull. Anim. Produc. Hlth Afr.* 46: 161 - 166.

AYO, J.O., OLADELE., S.B., NGAM, S., FAYOMI, A. and AFOLAYAN, S.B. (1998b). Diurnal fluctuations in rectal temperature of the Red Sokoto goat during the harmattan season. *Res. Vet. Sci.* 66: 7 - 9.

BRANDT, Y., LUNDEHEIM, N., MADEJ, A., RODRIGNEZ – MARTINEZ, H., EINARSSON, S. (2007). Effects of ACTH injections during estrus on concentrations and patterns of progesterone, estradiol, LH and inhibin α and time of ovulation in the sow. *Dom. Anim. Endocrinol.* 32: 122 – 137.

BRIGELIUS-FLOHE, R. and TRABER, M.G. (1999). Vitamin E: Function and metabolism. *The FASEB J.* 13(10): 1145–55.

BROUCEK, J., RYBA, S., MIHINA, S., UHRINCAT, M., KISAC, P. (2007). Impact of thermal- humidity index on milk yield under conditions of different dairy management. *J. Anim. Feed Sci.* 16: 329–344.

BRZEZINKA – SLEBODZINSKA, E. (2003). Species differences in the susceptibility of erythrocytes exposed to free radicals *in vivo*. *Vet. Res. Comm.* 27: 211-217.

BUCKHAM SPORER, K.R., WEBER, P.S.D., BURTON, J.L., EARLEY, B. and CROWE, M.A. (2008). Transportation of young beef bulls alters circulating physiological parameters that may be effective biomakers of stress. *J. Anim. Sci.* 86: 1325-1334.

BUETTNER, G.B. (1993). The pecking order of free radicals and antioxidants: Lipid peroxidation, alpha-tocopherol and ascorbate. *J. Biochem. Biophys.* 300: 535 - 543.

BUCKINGHAM, J.C., COWELL, A.M., GILLIES, G.E., HERBISON, A.E and STEEL, J.H. (1993). The neuron endocrine System: *Anatomy, Physiology and Responses to stress.* In Stress, Stress Hormones and Immune System. Edited by: Buckingham J.C., Gillies, G. E., Cowell, A.M., John Wiley and Sons, Ltd. Chichester, 9 - 47.

CHANDRA, B.S. and DAS, N. (2001). The handling and short-haul road transportation of spent buffaloes in

relation to bruising and animal welfare. *Trop. Anim. Hlth. Produc.* 33: 155 - 163.

CHAUDIERE, J. and FERRRI-ILIOU, R. (1999). Intracellular antioxidants: From chemical to biochemical mechanisms. *Food Chem. Toxicol.* 37: 949 - 962.

CHEESEMAN, K.H. and SLATERr, T.F. (1993). An introduction to free radical biochemistry. In: CHEESEMAN, K. H. and SLATER, T. F. (Ed.). *Free Radicals in Medicine*. London (UK): Churchill Livinstone. Pp. 481 - 493.

CHRISTOPHERSON, R.J. and KENNEDY, P.M. (1983). Effects of the thermal environment on digestion in ruminants. *Canadian J. Anim. Sci.* 63: 447 - 455.

CURTIS, E.S. (2003).The effects of environmental stress on Animal performance. *Stress management Manual.*

CULLOTA, V.C., YANG, M., O'HALLORAN, T.V. (2006). Activation of superoxide dismutase: putting the metal to the pedal. *Biochem. Biophys. Acta.* 1763: 747 – 758.

DARGEL, R. (1991). Lipid peroxidation—a common pathogenic mechanism? *Exp. Toxicol. Pathol.* 44: 169 – 81.

DAVIES, K. (1995). Oxidative stress: The paradox of aerobic life. *Biochem. Soc. Symp.* **6**: 1-31.

DEMIR, S., YILMAZ, M., KOSEGLU, M., AKALIN, N., ASLAN, D. and AYDIN, A. (2003). Role of free radicals in peptic ulcer and gastritis. *Turkey J. Gastroenterol.* 14(1): 39-43.

DEKKERS, J.C., VAN DOORNEN, L.J.P. and KEMPER HAN, C.G. (1996). The role of antioxidant vitamins and enzymes in the prevention of exercise–induced muscle damage. *Sport Med.* 21: 213 - 238.

DEVASAGAYAM, T.P.A., TILAK, J.C., BOLOOR, K.K., SANE, K.S., GHASKADBI, S.S., LELE, R.D. (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects". *J. Assoc. Physicians India.* 52: 796.

DOROEVIC, N.Z., BARBIC, G.M., MARKOVIC, S.D., OGNJANOVIC, B.K., ŠTAJN, A.S., ŽIKIC, R.V., SAICIC, Z.S. (2008). Oxidative stress and changes in antioxidative defense system in erythrocytes of preeclampsia in women. *Reprod. Toxicol.* 25: 213 - 18.

DROOGE, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev.* 82(1): 47 - 95.

EINARSSON, S., BRANDT, Y., LUNDEHEIM, N. and MADEJ, A. (2008). Stress and its influence on reproduction in pigs. A review. *Acta Vet. Scandinav.* 50: 47 – 50.

EPP, O., LADENSTEIN, R, WENDEL, A. (1983). The refined structure of the selenoenzyme glutathione peroxidase at 0.2-nm resolution. *Eur. J. Biochem.* 133(1): 51–69.

GERMAN, J.B. (1999). Food processing and lipid oxidation. *Adv. Exp. Med. Biology*. 459: 23 –50.

GREENWALD, R.A. and MOY, W.W. (1980). Effect of oxygen derived radicals on hyaluronic acid. *Arthritis Rheuma.* 23: 455 – 463.

GUTTERIDGE, J.M., HALLIWELL, B. (1988). Oxygen radicals and tissue injury. *FASEB J.* 13: 9 - 19.

HERRERA, E. and BARBAS, C. (2001). Vitamin E: Action, metabolism and perspectives. *J. Physiol. Biochem.* 57(2): 43–56.

HALLIWELL, B. and GUTTERIDGE, J.M.C. (1985). The chemistry of oxygen and other oxygen derived species. *In: Free Radicals in Biology and Medicine*, New York; Oxford University Press, Pp. 20 – 64.

HALLIWELL, B. and GUTTERIDGE, J.M.C. (1989). Lipid peroxidation a radical

chain reaction. *In: Free Radicals in Biology and Medicine*, 2nd Edition, Oxford University Press, New York, 704 pp.

HIDIROGLON, M. and LESSARD, J.R. (1970). Some effects of fluctuating low ambient temperature on beef cattle. *Can. J. Anim. Sci.* 51: 111 - 117.

HYMAN, M., PIZZORNO, J. and WEIL, A. (2005). A rational approach to antioxidant therapy and vitamin E. *Altern. Ther. Hlth.* Med. 11: 14 – 17.

IGONO, M.O., MOLOKWU, E.C.I. and ALIU, Y.O. (1982). Body temperature responses of Savanna Brown goat to the harmattan and hot-dry season. *Int. J. Biometeorol.* 26: 225 – 230.

IMLAY, J.A. and LINN, S. (1986). DNA damage and oxygen radical toxicity. *Sci.* 2140: 1302 – 1309.

JACOB, R.A. (1996). Three eras of vitamin C discovery. *Sub-cellular Biochem.* 25: 1–16.

KERR, L.D., INOUE, J. and VERM, I.M. (1992). Signal transduction: The nuclear target. *Current Opinion in Cell Biol.* 4: 496 - 01.

KHANSARI, D.N., MURGO, A. and FAITH, R.E. (1990). Effects of stress on the immune system. *Immunol. Today 11: 170.*

KNIGHT, J.A. (1998). Free radicals: Their history and current status in aging and disease". *Ann. Clinical Lab. Sci.* 28(6): 331–46.

KOCHAVER, I. (1993). Basic principles in photomedicine and photochemistry. New York: Dekkers.

KOLLANJIPPAN, K., MANOHARAN, S., KAYALVIZHI, M. (2002). Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. *Clin. Chimica Acta.* 326: 143 – 49.

LARKINS, N.J. (1999). Free radical biology and pathology. *J. Equine Vet. Sc.* 19: 84 - 89.

LANGSDORF, L.J. and ZYDNEY, A.L. (1993). Effect of uremia on the membrane transport characteristics of red blood cells. *Blood*, 81: 820 - 827.

LEE, C.N. (1993). Environmental stress effects on bovine reproduction. *Vet. Clinic North Amer. Food Anim. Prac.* 9(2): 263-273.

LEENWEMBURGH, C. and HEINECKE, J.W. (2001). Oxidative stress and antioxidants in exercise. *Current Med. Chem.* **8**: 829 – 838.

LECAPENTIER, Y. (2007). Physiological role of free radicals in skeletal muscles. *J. Appl. Physiol.* **103**: 1917 - 1918.

LINSTER, C.L. and VAN SCHAFTINGEN, E. (2007). Vitamin C. *FEBS J.* 274(1): 1 – 22.

MADEJ, A., MWANZA, A., KINDAHL, H. and EINARSSON, S. (2005). Effect of ACTH and CRH on plasma levels of cortisol and prostaglandin $F_{2\alpha}$ metabolites in cycling gilts and castrated boars. *Acta Vet. Scandinavica*, 46: 249-259.

MACHIN, L.J. and BENDICH, A. (1987). Free radical tissue damage: Protective role of antioxidant nutrients. *Fed. Amer. Soc. Exp. Biol. J.* 1: 441 - 445.

MATES, J.M., PEREZ-GONIEZ, C. and HUNEZ DE CASTRO, I. (1999). Antioxidant enzymes. *Clin. Biochem.* 32(8): 595 – 603.

MATTILL, H.A. (1947). Antioxidants. *Ann. Rev. Biochem.* 16: 177 – 92.

MCCORD, J.M. and FRIDOVICH, I. (1969). Superoxide dismutase. Anenzymic function for erythrocuprein (thermocuprein). *J. Biol. Chem.* 244: 6049 – 6055.

MCCORD, J.M. and FRIDOVICH, I. (1988). Superoxide dismutase. The first twenty years (1968 – 1998). *Free Rad. Biol. Med.* 5(5-6): 363 -369.

MEERSON, F.Z. (1986). The general mechanism and role of stress responses

in adaptation. Major stages in adaptation process. In: *Physiology of Adaptation Processes*. Nauka Publishing House. Moscow, Pp. 77 – 123 (In Russian).

MEISTER, A. (1994). Glutathione-ascorbic acid antioxidant system in animals. *The J. Biol. Chem.* 269(13): 9397–400.

MOBERG, G.P. (1993). (A Review) Developing management strategies to reduce stress in pigs: A new approach utilizing the biological cost of stress. Manipulating Pig Production IV. Australiasiain Pig Science Association, Attword, E.S. Balterham. 116-126.

MSTL, E. and PALME, R (2002). Hormones as indicator of stress. *Domestic Anim. Endocrinol.* 23: 67 - 74.

NISA, M.U., SARWAR, M., BILAL, Q. and FEROZ, M.A. (1999) (Review) Effect of temperature stress on nutrient utilization and different physiological functions of ruminant animals. *Int.. J. Agric. Biol.* 3: 174-178.

OLADELE, S.B., AYO, J.O., OGUNDIPE, S.O and ESIEVO, K.A.N. (2003). Seasonal and species variations in erythrocytes osmotic fragility of indigenous poultry species in Zaria, Northern Guinea Savannah zone of Nigeria. *Bull. Anim. Hlth. Produc. Afr.* 51: 204 - 214.

PADAYATTY, S.J., KATZ, A., WANG, Y.E., P., KWON, O., LEE, J., CHEN, S., CORPE, C. (2003). Vitamion C as an antioxidant: Evaluation of its role in disease prevention. *J. Amer. Coll. Nutr.* 22 (1): 18–35.

PACKER, L., WEBER, S.U., RIMBACH, G. (2001). Molecular aspects of alpha-tocotrienol antioxidant action and cell signaling. *The J. Nutr.* 131(2): 369S–73S.

PANDEY, K.T and RIZVI, S.I. (2009). Protective effect of resveratrol on markers of oxidative stress in human erythrocytes subjected to *In vitro* oxidative insults. Phytotherapy Res. DOI: 10.1002/ptr.2853.

POWERS, S.K. and JACKSON, M.J. (2008). Exercise-induced oxidative stress: Cellular mechcanisms and impact on muscle force production. *Physiol. Rev.* 88: 1243 - 1276.

RAN, Q, LIANG, H. and IKENO, Y. (2007). "Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis". *J. Gerontol. A Biol. Sci. Med. Sci.* 62(9): 932–42.

RAZDAN, P. (2003). Stress and early pregnancy in sows. Effects on endocrinology, ova transport and embryo development. In Ph.D thesis, Veterinarie 153, Acta Universitasis Agriculture Sueciae, Swedish University of Agricultural Sciences Uppsala.

REITER, R.J. PAREDES, S.D., MANCHESTER, L.C. and TAN, D.X. (2009). "Reducing oxidative/nitrosative stress: A newly-discovered genre for melatonin". *Critical Revi. Biochem. Mol. Biol.* 44(4): 175–200.

RITTER, M.J., ELLIS, M., BERTELSEN, C.R., BOWMAN, R., BRINKMANN, J., DEDECKER, J.M., KEFFABER, K.K., MURPHY, C.M., PETTERSON, B.A., SCHLIPF, J. M. and WOLTER, B.F. (2007). Effects of distance moved during loading and floor space on the trailer during transport on losses of market weight pigs on arrival at the packing plant. *J. Anim. Sci.* 85: 3454 - 3461.

SAHIN, K., SAHIN, N., ONDERCI, M., YARALIOGU, S. and KUCUK, O. (2001). Protective role of supplemental vitamin E on lipid peroxidation, vitamins E, A and some mineral concentrations of broilers reared under heat stress. *Vet. Mex, (Czechslosvakia),* 46: 140 - 144.

SEILER, A., SCHNEIDER, M., FORSTER,

H., ROTH, STEPHAN, WIRTH, E. K., CULMSEE, C., PLESNILA, N. and KREMMER, E. (2008). Glutathione Peroxidase 4 Senses and Translates Oxidative Stress into 12/15-Lipoxygenase Dependent- and AIF-Mediated Cell Death. *Cell Metabol.* (3): 237–48.

SELYE, H. (1975). Stress. *J. Human Stress.* 1(2): 37-44.

SIDHU, P., GARG, M.L., DHAWAN, D.K., (2004). Protective effects of zinc on oxidative stress enzymes in liver of protein deficient rats. *Nutrición Hospitalaria*, **XIX(6)**: 341 - 47.

SIES, H. (1985) Oxidative stress. London: Academic.

SIES, H. (1997). Oxidative stress: Oxidants and antioxidants. *Exp. Physiol.* 82(2): 291–5.

SMIRNOFF, N. and WHEELER, G. L. (2000). Ascorbic Acid in Plants: Biosynthesis and Function. *Critical Rev. Biochem.Mol. Biol.* 35(4): 291–314.

STALDMAN, E.R. (1986). Oxidation of protein by mixed-function oxidation system: Implication in protein turnover, ageing and neutrophil function. *Trends in Biochem. Sci.* 11: 11 – 12.

TAN, D.X., MANCHESTER, L.C., TERRON, M.P., FLORES, L.J. and REITER, R.J. (2007). One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.* 42(1): 28–42.

TKACZYK, J. and VIZEK, M. (2007). Oxidative stress in the lung tissue - sources of reactive oxygen species and antioxidant defence. *Prague Med. Report* 107(2): 105 - 114.

TRABER, M.G. and ATKINSON, J. (2007). Vitamin E , antioxidant and nothing more. *Free Rad. Biol. Med.* 43(1): 4–15.

VATHANA, S., KANG, K., LOAN, C.P., THINGGARD, G., KABASA, J.D. and TER MEULEN, U. (2002). Effect of vitamin C supplementation on performance of broiler chickens in Cambodia. Proc. Int. Conf. Agric. Res. Dev. Witzenhausen: Deutcher Tropentag.

VELLUCI, S.V. (1997). The autonomic and behavioural response to stress. In Stress, Stress Hormones and the Immune system. Edited by Buckingham, J.C., Gillies, G. E., Cowell, A.M., John weily and Sons, Ltd. Chichester, 48-70.

VERTUANI, S., ANGUSTI, A. and MANFREDINI, S. (2004). "The Antioxidants and Pro-Antioxidants Network: An Overview". *Current Pharmac. Des.* 10(14): 1677–94.

VOSLAROVA, E., JANACKOVA, B., VITULA, F., KOZAK, A. and VECEREK, V. (2007). Effects of transport distance and the season of the year on death among hens and roosters in transport to poultry processing plants in Czech Republic in the period from 1997 to 2004. *Vet. Med.* 52(6):262 - 266.

WALTER, M.W. (1969). Length of the rainy season in Nigeria. *Nig. Geog. J.* 10: 123 – 128.

WANG, X., QUINN, P.J. (1999). "Vitamin E and its function in membranes". *Prog. Lipid Res.* 38(4): 309–36.

WELLS, W.W., XU, DIAN, P. Y., YANFENG, R. and PAMELA, A. (1990). Mammalian thioltranseferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. *The J. Biol. Chem.* 265(26): 15361–4.

WILCOX, D.E, SCHNK, A.D., FELDMAN, B.M. and XU, Y. (2001). Oxidation of zinc binding cysteine residues in transcription factor proteins. *Antioxidant Redox Signal.* 3:549 – 564.

WU, D. and CEDERBAUM, A.I. (2003). Alcohol, oxidative stress and free

radical damage. *Alcohol Res. Hlth*. 27(4): 277 – 284.

YU, B.P. (1994). Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.*