

Oxidative stress in animals: the gentle saver and the silent killer

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Abstract

Due to global pandemic occasioned by SARS-COVID 19 infection, which is accounting for human mortality worldwide, the importance of oxidative stress in managing human and animal health has been heightened. This paper was aimed at elucidating the relevance of oxidative stress as an indicator of animals' well-being and welfare. Oxidative stress is an imbalance between pro-oxidants generation and antioxidant defence in a biological system. The generation of pro-oxidants is inevitable in all living cells (both plant and animal). Pro-oxidants are products of cellular respiration in the mitochondria of all living cells; a process required for the generation of cellular energy (adenosine triphosphate; ATP). The generation of pro-oxidants is important for normal functioning of cells and also in the defense of the body against bacteria and virus by phagocytosis. However, antioxidants are meant to control the rate of pro-oxidant accumulation in biological systems. Over production of pro-oxidant beyond the scavenging ability of antioxidants leads to oxidative stress. Oxidative stress has been implicated as the basis of most degenerative and terminal illnesses in man. It has been reported to influence the level of fertility in human, livestock and poultry population. Oxidative stress management has gained attention as the leading candidate to improve animal health and welfare so as to prevent the development of degenerative diseases such as COVID 19 in humans.

Keywords: Animal welfare, thermal stress, antioxidant, peroxides, spermatozoa

Le stress oxydatif chez les animaux : le sauveur doux et le tueur silencieux



Résumé

En raison de la pandémie mondiale occasionnée par l'infection par le SRAS-COVID 19, qui est à l'origine de la mortalité humaine dans le monde, l'importance du stress oxydatif dans la gestion de la santé humaine et animale a été renforcée. Cet article visait à élucider la pertinence du stress oxydatif comme indicateur du bien-être et du bien-être des animaux. Le stress oxydatif est un déséquilibre entre la génération de pro-oxydants et la défense antioxydante dans un système biologique. La génération de pro-oxydants est inévitable dans toutes les cellules vivantes (végétales et animales). Les pro-oxydants sont des produits de la respiration cellulaire dans les mitochondries de toutes les cellules vivantes ; un processus nécessaire à la génération d'énergie cellulaire (adénosine triphosphate; ATP). La génération de pro-oxydants est importante pour le fonctionnement normal des cellules et également dans la défense de l'organisme contre les bactéries et les virus par phagocytose. Cependant, les antioxydants sont destinés à contrôler le taux d'accumulation de pro-oxydants dans les systèmes biologiques. La surproduction de pro-oxydant au-delà de la capacité de piégeage des antioxydants conduit à un stress oxydatif. Le stress oxydatif a été impliqué comme étant à la base de la plupart des maladies dégénératives et terminales chez l'homme. Il a été signalé qu'il influençait le niveau de fertilité des populations humaines,

animales et avicoles. La gestion du stress oxydatif a attiré l'attention en tant que principal candidat pour améliorer la santé et le bien-être des animaux afin de prévenir le développement de maladies dégénératives telles que le COVID 19 chez l'homme.

Mots clés: Bien-être animal, stress thermique, antioxydant, peroxydes, spermatozoïdes

Introduction

Oxidative stress refers to any situation where there is a serious imbalance between the production of free radicals (FR) or reactive oxygen species (ROS), called the oxidative load, and the antioxidant defense system. Reactive oxygen species can be classified into oxygen-centred radicals and oxygen-centred non-radicals. Oxygen-centred radicals are superoxide anion (AO_2^-), hydroxyl radical (AOH), alkoxyl radical (ROA), and peroxy radical (ROOA). Oxygen-centred non-radicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$) (Eqbal *et al.*, 2011). Superoxide and hydroxyl radicals, along with non-radical oxygen species such as hydrogen peroxide H_2O_2 have the highest biological activity. The ROS are produced in all cells, depending on the intensity of aerobic metabolism, especially in activated neutrophils, monocytes, smooth muscle cells and in endothelial cells (Jimoh, 2016). It is widely accepted that mitochondrial respiration is the primary source of reactive oxygen species (ROS; Turrens, 2003). Most of the oxidants produced by cells occur as a consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system. Oxidative burst from phagocytes; white blood cells as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins antigens are denatured. Xenobiotic metabolism, i.e., detoxification of toxic substance (Percival, 1998). Levels of ROS are determined by production and by the rate of ROS degradation and/or inactivation, an appropriate balance is important for

maintaining cellular homeostasis (Covarrubias *et al.*, 2008). A major threat to homeostasis and therefore to the integrity of aerobic organisms arises from chemical species possessing one or more unpaired electrons in their outer orbital, called free radicals (Halliwell and Gutteridge, 1997). Oxygen free radicals can develop during several steps of normal metabolic events. Although low levels of ROS are essential in many biochemical processes, accumulation of ROS may damage biological macromolecules i.e. lipids, proteins, carbohydrates and DNA (Kumar *et al.*, 2011). External factors such as heat, trauma, ultrasound, infections, radiations, toxins etc. can lead to increased free radicals and other ROS resulting to oxidative stress. Cells can tolerate moderate oxidative loads by increasing gene expression to up-regulate their reductive defense systems and restore the oxidant/antioxidant balance. But when this increased synthesis cannot be achieved due to damage to enzymes, or substrate limitations, or when the oxidative load is overwhelming, an imbalance persists and the result is oxidative stress (Halliwell, 2005). Levels of ROS produced within the mitochondria are reported to increase with age. This is expressed in Jimoh *et al.* (2021a) that pubertal rabbits possess the best antioxidant stability matured and adult age groups. Consequently, oxidative damage to mitochondria would also appear to increase with age, this explains the vulnerability of the aged patients of COVID 19. It has been suggested that mitochondrial dysfunction is related to damage caused by ROS produced as a consequence of increased oxidative stress and insufficient antioxidant defenses (Shigenaga *et al.*, 1994). This damage results in a decrease in

energy production by some of the cell's mitochondria. Mitochondrial function is supported by a broad spectrum of nutritional modulators including oxidants and antioxidant. Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of "leaky gut" syndrome; and exposure to drugs or toxins such as smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body's oxidant load (Percival, 1998). The ultimate effects of ROS accumulations include mutations, metabolic dysfunction and cell ageing. They in turn are a cause of development of inflammatory processes, oncogenesis and impaired organ functioning (Maggi-Capeyron *et al.*, 2002). Oxidative stress is considered to play a pivotal role in the pathogenesis of aging and several degenerative diseases, such as atherosclerosis, cardiovascular disease, type 2-diabetes and cancer (Storz, 2005).

Antioxidant defense

Antioxidants are believed to play a very important role in the body defense system against ROS (Vivek and Surendra, 2006). Antioxidant is a chemical that delays the start or slows the rate of lipid oxidation reaction. It inhibits the formation of free radical and hence contributes to the stabilization of the lipid. Naturally, there is a dynamic balance between the amount of free-radicals generated in the body and antioxidants to quench and/or scavenge them and protect the body against their deleterious effects. The body develops a defense mechanism to protect it from the accumulations of free radical and reactive oxygen species. These protective mechanisms are either to scavenge or detoxify ROS, block their production, or sequester transition metals that are the source of free radicals. They include enzymatic and non-enzymatic antioxidant

defenses produced in the body, namely, endogenous (Sies, 1999) and others exogenously supplied with the diet (Yao *et al.*, 2004). The enzymatic antioxidants include superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT). SOD catalyzes the dismutation of superoxide anion radical to peroxide H_2O_2 and molecular oxygen. Gpx, GR and catalase converts hydrogen peroxide to water and oxygen. Non-enzymatic antioxidants, such as glutathione, tocopherols, retinols, and ascorbate, play an important role in scavenging ROS. Exogenously, natural antioxidants present in the diet increase the resistance toward oxidative damages and they may have a substantial impact on human health (Boskou, 2006). Antioxidant compounds play an important role in our body due to favorable effects on human health. Consumption of food containing phytochemical with potential antioxidant properties can reduce the risk of disease (Temple, 2000). The antioxidant system involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals. These components include Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid. Antioxidant enzymes e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions. Metal binding proteins such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions (Dauqanet *et al.*, 2011). However, the amounts of these protective antioxidant principles present under the normal physiological conditions are sufficient only to cope with the

physiological rate of free-radical generation (Ashok and Sushil, 2005), but during high physiological demands and stress, the level of ROS generated outweighs the antioxidant system. Such high physiological demands include diseases, lactation, heat stress and gestation, external factors such as heat, trauma, ultrasound, infections, radiations, toxins etc. can lead to increased free radicals above the antioxidant defense. In a study designed to evaluate the effect of African mistletoe on serum oxidative status of pullets due to dearth of information on effects of this plant on welfare of laying pullets. It revealed that mistletoe inclusion in laying pullets diet enhance antioxidant profile and inhibits oxidative stress (Jimoh *et al.*, 2018a). Jimoh *et al.* (2018b) reported that more emphasis should be paid to management of oxidative stress at early lay, because birds have higher antioxidant defense as laying progresses. Due to scanty information on oxidative stress markers in Nigerian indigenous goat and sheep population, a study was carried to document the reference range of oxidative stress markers in West African dwarf (WAD) goat and sheep which is necessary for health assessment of small ruminants in Nigeria. Ihejirika *et al.* (2017) revealed that that the West African dwarf bucks have better antioxidant activity and are less susceptible to lipid peroxidation than does. Jimoh *et al.* (2018c) reported that WAD rams had better antioxidant defense mechanism compared to bucks. Higher oxidative stability in rams and buck will prolong cell life and protect against tissue and organ damage, which are essential for good semen output (Jimoh *et al.*, 2018c). Similarly, Jack *et al.* (2016) reported that WAD rams have better antioxidant activity than ewes. In a study to document the baseline range of oxidative stress markers and requirements for antioxidant defense at different physiological stages of West African dwarf does. Jimoh *et al.* (2019)

revealed that West African dwarf does in third trimester and lactation displayed oxidative stress due to intense metabolism during the physiological states. It is recommended that antioxidant fortification/supplementation should be employed during late gestation and lactation as management practice to combat the oxidative stress observed in West African dwarf goats (Jimoh *et al.*, 2019).

Heat stress and oxidative stress

Heat stress occurs when the core body temperature of a given specie exceeds its specified range for normal activity resulting from a total heat load (internal heat production and heat gained from environment), exceeding the capacity for heat dissipation. Heat stress is one of the wide varieties of factors which cause oxidative stress *in-vivo* during summer and/or in tropics. Altan *et al.* (2003) have demonstrated that heat stress increased lipid peroxidation which was associated with production of large number of free radicals which are capable of initiating peroxidation of polyunsaturated fatty acids. Jimoh (2019) documented oxidative indicators in rabbit breeds in Nigeria, due to paucity of reports on thermal comfort conditions which could serve as the baseline for interpretations, diagnosis, and evaluating environmental contributions to oxidative stress in the tropics. It revealed that the four breeds of rabbit (British spot, Chinchilla, New Zealand white and Fauve de Bourgogne) have similar oxidative status at thermal comfort. However, British Spot has low superoxide dismutase scavenging ability and could pose danger to lipid peroxidation at high metabolic or physiological conditions.

In a study conducted by Jimoh *et al.* (2017) to assess the oxidative stress indicators of four exotic breeds of rabbit at peak of heat stress in Ibadan, Nigeria, (revealed that British Spot breed of rabbit had better adaptable to heat stress in Nigeria, because

British spot had best oxidative stability among the four breeds of rabbit. This implies that British spot rabbits can be better managed in the prevailing heat stress condition of Nigeria. The revelation of Jimoh (2019) at thermal comfort that British spot has low superoxide dismutase scavenging ability, and Jimoh *et al.* (2017) that British Spot breed of rabbit had better adaptable to heat stress, shows that British spot rabbits can regulate their antioxidant defense to meet oxidative load. In a study to investigate the protective effect of soursop juice against heat stress-induced oxidative stress in rabbit bucks by Jimoh *et al.* (2018d). It revealed that soursop juice reduced serum lipid peroxidation and enhanced antioxidant activity in heat stressed rabbit over 8 weeks. Chronic oral administration of soursop juice proved beneficial in promoting good health of heat-stressed bucks.

Oxidative stress and spermatozoa function

Spermatozoa are unique in structure and chemical composition and are characterised by high proportions of polyunsaturated fatty acids (PUFAs) in the phospholipid fraction of their membranes (Balogun *et al.*, 2017). However, high level of PUFAs increases the susceptibility of cells to free radical attack and lipid peroxidation (De Lamirande *et al.*, 1997), which degrades membrane structure, sperm metabolism and DNA integrity (Balogun *et al.*, 2016). Uncontrolled production of reactive oxygen species that exceeds the antioxidant capacity of the seminal plasma leads to oxidative stress, which is harmful to spermatozoa. Although high concentrations of ROS cause sperm pathology (ATP depletion, leading to insufficient axonemal phosphorylation, lipid peroxidation and loss of motility and viability), low and controlled concentrations of ROS play an important role in sperm physiology. Mild and even undetectable oxidative stress for extensive periods could alter the

condensation state of sperm DNA, an effect that would not be visible before fertilization (De Lamirande *et al.*, 1997).

In a study to evaluate semen characteristics and seminal oxidative status of breeds of rabbit during the peak of heat stress in Ibadan, Nigeria. It was revealed that chronic heat stress induces oxidative stress in sperm cells of rabbit bucks. At peak of thermal discomfort in Nigeria, heat stress adversely affects semen biochemicals which deleteriously influence semen quality. New Zealand white bucks had better semen quality and British Spot bucks had better oxidative stability among the breeds, and are reliable breeders for genetic improvement for an uninterrupted breeding cycle for commercial enterprise (Jimoh and Ewuola, 2018). In a similar study, which was aimed to determine and document the semen characteristics as well as the oxidative status of semen ejaculates produced by rabbit bucks at their peak of thermal comfort. New Zealand White had the best oxidative status across the four breeds, and this enhanced its semen quality parameters. However, British spot bucks had comparable high semen quality as New Zealand White bucks, and seminal biochemical parameters in the breeds were within a similar range (Jimoh and Ewuola, 2019). Vegetables and fruits maintain life due to their high contents of medicinal compounds essential for health (El-Sheshtawy *et al.*, 2017). These natural extracts and infusions are used in semen extenders for preserving animal sperms (Sansone *et al.*, 2000). Their strong antioxidant capacity protects spermatozoa from oxidative damage. Coconut water and watermelon are characterised by high contents of antioxidants as expressed by the phytohormones, sugar, vitamins, electrolytes and amino acids (Yong *et al.*, 2009). Jimoh (2020) evaluate the potential of coconut water as diluent for rabbit semen, it utilise the antioxidant

potential and fructose content of the diluent to maintain good quality semen. Similarly, Balogun and Jimoh (2017) and Balogun *et al.* (2017) reported that fortification of egg yolk citrate extender with aqueous extracts of fresh and dry garlic enhances the fertility and hatchability of pullet eggs. It was attributed to the antioxidant properties of garlic (Balogun *et al.*, 2016). Jimoh and Ayedun (2020) demonstrated the antioxidant enrichment of watermelon confers watermelon juice is a potent rabbit semen diluent. Similarly, reports (Jimoh *et al.*, 2020; Jimoh *et al.*, 2021b; Jimoh *et al.*, 2021c) have demonstrated that pineapple, watermelon and citrus juices are potent antioxidant sources in poultry semen that maintains spermatozoa oxidative stability and promote semen kinetics for artificial insemination. In an *in vivo* study, to elucidate the reproductive response of rabbit bucks fed herbal supplements namely; *Moringa oleifera*, *Phyllanthus amarus* and *Viscum album* for 84 days (Jimoh *et al.*, 2021d). The study revealed that the inclusion of the three herbal supplements in rabbit diets enriched semen antioxidant profile and reduced its lipid peroxidation, this indicates that the herbal supplements regulated spermatozoa oxidative stability. Similarly, it also showed that for breeding programmes, the inclusion of *Moringa oleifera* supplements in bucks' diets leads to enhance serum luteinising hormone and reproductive organ weights, while *Viscum album* supplements in bucks' diets encourages daily sperm production, testicular sperm reserves, testosterone, and spermatozoa kinetics, and *Phyllanthus amarus* supplementation in bucks' diet enhances weight gain, serum follicle stimulating hormone and extra-gonadal sperm reserves (Jimoh *et al.*, 2021d).

Oxidative stress and the management of infectious diseases

Infectious diseases such as SARS-COVID

19 like most viral infection has no cure, but treatment of patients relies on management of its associated opportunistic infections. The primary defence of animals (immunity) are compromised before they fall ill to diseases. That means the lymphatic (white blood cells, lymph nodes, spleen etc) would have mobilised to site of infection and fail to terminate the foreign bodies. As explained in the earlier sections, phagocytosis is required to destroy viruses by engulfing the foreign body and emptying pro-oxidants (free radicals and ROS) into the surrounding of the virus thereby destroying it. This will significantly reduce the viral load and such infections would be healed. However, invulnerable groups such as aged, gravid (pregnant), lactating (nursing), sickle cell anaemia, diabetic and hypertensive patients; they have compromised immunity due to oxidative stress (high prooxidants tendencies thereby reducing the antioxidant generation). Report of Jimoh *et al.* (2019) claimed that lactation and pregnancy are the most vulnerable reproductive group of females to oxidative stress and recommends antioxidant fortifications to mitigate the associated risks factors. In such vulnerable groups two mechanism are involved; (1) once their lymphatic system mobilise pro-oxidants to counteract foreign bodies, they are more or less poisonous to the system due to low antioxidant power to scavenge the abundant oxidants, (2) their lymphatic cells are fragile due to accumulations of pro-oxidants without scavenging/low scavenging by antioxidants thus abundance of pro-oxidants and reduced immunity. These scenario suggests that the only alternative in the absence of vaccines are antioxidants supplements. As it has been widely attested to cure the associated illness and reduce the viral load and patients' tests negative. It is imperative for all to take solace in fruits, vegetables, herbs and spices, vitamin supplements which have

been confirmed by previous authors to enhance antioxidant and cellular immunity in the body.

Conclusion

The study identified oxidative status as vital tool in management of animal welfare and wellbeing. It is imperative to prevent oxidative stress in animals to reduce the risk factors of degenerative diseases and terminal ailment. Herbs, spices, fruits and vegetables are important supplements and staple food to boost the antioxidant defense of animals. The potentials of antioxidant rich foods to enhance fertility in human, poultry and livestock species (due to impending decrease fertility rate occasioned by external factors such as heat, trauma, ultrasound, infections, radiations, toxins, smoke, pollution, pesticides, and insecticides) has been established.

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