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Chapter

Assessment on Oxidative Stress in Animals: From Experimental Models to Animal Production

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Abstract

Oxygen is a key element involved in a variety of vital physiological reactions in aerobic organisms, including those produced in the electron transport chain, hydroxylation, and oxygenation. Reactive oxygen species and reactive oxygen nitrogen species (ROS/RONS) are naturally formed as by-products from these previously mentioned processes and reactions involving the O₂ molecules. Under healthy conditions, the harmful effects of ROS/RONS in the organisms are controlled by antioxidants, molecules of enzymatic or non-enzymatic nature, able to prevent, retard, or eliminate oxidative damage. Nevertheless, when ROS/RONS production exceeds the antioxidant capacity of one organism, oxidative stress emerges, leading to the apparition of many diseases, some of which can depict significant losses in the field of animal production. Thereby, looking for increasing animal productivity, procedures to mitigate the effects of oxidative stress on living organisms are tested in laboratory animal models, and the obtained results are used to develop strategies that avoid oxidative stress in farm animals either invertebrates (mollusks and crustacean species) or vertebrates (fish, birds, and mammals). In this chapter, oxidative stress will be addressed from the field of animal health and welfare and its impact on animal production, presenting some strategies, studies conducted, and recent perspectives to mitigate the effects of oxidative stress and improve the productivity indicators in farm animals.

Keywords: oxidative stress, environmental stress, livestock handling, anti-stress procedures, animal production

1. Introduction

Our planet was formed as a condensed mass of cosmic debris about 4.5 billion years ago. It had a primitive ocean, atmosphere, climate, and recurrent cataclysmic events. This hostile and hellish environment eventually became more suitable for the first forms of life, but this was a life without oxygen [1]. In the atmosphere, oxygen appeared primarily as a result of solar radiation disruption of water (H₂O) into
hydrogen (H\textsubscript{2}) and oxygen (O\textsubscript{2}) and, much later, by the photosynthetic process. Consequently, anaerobic life forms have to adapt to using oxygen and/or contend with the potential dangers elicited by oxygen and its metabolic by-products.

Oxygen is a key element involved in a variety of vital physiological reactions in aerobic organisms, including those produced in the electron transport chain, hydroxylation, and oxygenation. Only a very small proportion of oxygen used in cells (about 1%) is transformed into reactive oxygen-nitrogen species (ROS) and reactive oxygen and nitrogen species (RONS), which are primarily a result of essential biochemical pathways associated with aerobic metabolism, which takes place in the mitochondria and peroxisomes [2] and includes not only some radicals such as hydroxyl radical (\textit{HO}-), peroxyl (\textit{ROO}-), but also other molecules such as superoxide anions (O\textsubscript{2}-), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), reactive aldehydes (ROCH), and nitric oxide (NO). Intending to mitigate the adverse effects caused by ROS/RONS, organisms use antioxidants defense system composed of endogenous molecules and molecules having nutritional nature, able to retard or inhibit the damage produced by free radicals when present in a lower concentration are concerning them [3]. The cellular antioxidant systems composed of several enzymes, including superoxide dismutases (SOD), catalases (CAT), and glutathione peroxidases (GPx), together with other antioxidant molecules such as thioredoxin 2 (TRX2), glutaredoxin 2 (GRX2), cytochrome c oxidase (complex IV), coenzyme Q, ascorbic acid, tocopherol, vitamin E, and carotene, among others [3–5].

Stress is the response reflex reaction exposed by the incapacity of an organism to face its environment. This condition may lead to unfavorable consequences, ranging from discomfort to death: a concise review of the impact of stress on growth, production, reproduction, and disease [6]. When ROS/RONS production exceeds the antioxidant capacity of one organism, oxidative stress emerges. Oxidative stress may be involved in several pathological conditions, including conditions relevant to animal production and the general welfare of individuals [7]. Farm animals usually suffer from the onslaught of oxidative stress as a result of the unsuitable enclosure and overpopulation of stables, nutritional and health conditions, and their handling according to the production cycle established for each species [8–10].

The aim of this chapter is to present some studies on oxidative stress carried out in experimental living models, making emphasis on how experimental data gained in these research studies can be applied to farm animals through some ways to measure and nutritional strategies to reduce the adverse effects that oxidative stress produces on the health and physiological development of wild and farm animals.

2. Generation of oxidative stress

We can group the stressors in three categories: endogenous and pathogenic illness and external. ROS/RONS production is a key mechanism involved in the damage caused by different diseases and infections provoked by both pathogenic and no-pathogenic organisms.

2.1 Endogenous sources

The ROS/RONS has both enzymatic and non-enzymatic nature. Numerous biochemical processes also generate those types of reactive species including such acting
as intercellular signals with remarkable incidence in specific biological processes and functions [11, 12]. In the cell, mitochondria have the ability to produce ATP through respiration and mitochondrial oxidative phosphorylation, the most important source of ATP [13]. The mammalian cytochrome P450 CYP-dependent ROS/RONS electron transport system and the mitochondrial electron transport chain are the most important source of ROS/RONS. In mitochondria, the formation of such reactive substances by the activity of NADPH oxidase (NOX) enzymes can also be possible, generating superoxide anion (O$_2^-$) from cytoplasmic NADPH [14]. In endoplasmic reticulum, cytochrome P450 (CYP) enzymes play an important role in the activation of oxygen, generating O$_2^{•−}$ while catalyzing NADPH-dependent reactions in mitochondrial respiratory chain. Cytochrome P450 is the main way of xenobiotic detoxification, including drugs, medications, chemical products, alcohol, aromatic substances, pesticides, and other industrial byproducts. Cytochrome P450 houses enzymes catalyzing the oxygenation of organic substrates and the simultaneous reduction of molecular oxygen, and it plays a key role in the reduction of O$_2$, hydroperoxides, arene, N-oxides, azido and azodic compounds, halogen, nitrogenic compounds, hydroxylamines, and other xenobiotics. Some intermediaries of the processes mentioned above ROS/RONS initiate lipid peroxidation and directly damage the DNA and the cellular membranes [15]. Peroxisomes are the important generators of ROS/RONS. The main function of peroxisomes is to break down long fatty acid chains through beta-oxidation and synthesis necessary phospholipids, such as plasminogen, which in vertebrates are critical for correct function of the brain, lung, and heart. Furthermore, this subcellular structure aids certain enzymes with energy metabolism in many eukaryotic cells as well with cholesterol synthesis in animals [16]. The major sources of ROS/RONS in central nervous system are the microglia cells, which support neuronal survival through the secretion of growth factors and anti-inflammatory cytokines. Activated microglia cells produce various pro-inflammatory mediators, nitric oxide, and ROS/RONS; however, their prolonged activation can cause many neurodegenerative disorders, as it was previously exposed, the phagocytosis is an important ROS/RONS generator, and this process is intrinsically linked to pathogenic infections as a part of immune response [17, 18].

In animals, oxidative balance could be broken by different organic, nutritional, and environmental conditions leading to different pathologies including neurodegenerative, cardiovascular, hepatic, renal, metabolic, and autoimmune diseases, and even behavioral and reproductive disorders [11, 19–21]. Farm animals live usually suffer from the onslaught of oxidative stress due to unsuitable enclosure and overpopulation of stables, nutritional and health condition, and their handling according to the production cycle established for each species.

2.2 Exogenous sources

Exogenous conditions can promote the production of ROS/RONS and induce oxidative stress in the organism. Factors such as illness and pathological process, drugs and medicaments, exposure to radiations, chemical by-products of industry, pollution, and adverse environmental and living conditions induce oxidative stress [22]. ROS/RONS such as hydroxyl radicals and superoxide are critical in the apoptosis of infected cells as it has been demonstrated [23]. Pathogenic bacteria can provoke inflammation and destruction of infected cells and tissues. Experimental data suggest that periodontal disease-induced oxidative stress and inflammation is mediated through purine degradation pathway, a major biochemical source for ROS/RONS.
production [24]. The connection between bacterial pathogens and unfolded protein response (UPR) evidences that pathogenic bacteria induce oxidative stress in the endoplasmic reticulum. This suggests this is the route to access and gain nutrients from the host, obviating the need to become internalized or inflict irreversible cell damage [25]. Other example is the case with idiopathic pulmonary fibrosis (IPF), a fatal lung disease of unknown origin characterized by chronic and progressive ROS/RONS interstitial pneumonia, which progressively impairs lung function [26]. Oxidative stress induces hypoxic tissue condition and injuries in different cell and tissues [27].

3. Role of ROS/RONS in healthy organisms.

The positive roles of ROS/RONS are widely recognized in signal transduction [28, 29], in the induction of endothelial cell migration and proliferation, apoptosis [30], macromolecule oxidation, structural and functional modulation, regulation of gene expression [31–33], mRNA stability, and methylation-mediated DNA epigenetic factors [33, 34]. Phagocytosis and other important aspects of immune defense generate ROS/RONS [35, 36].

Determination of the ROS/RONS sources and the etiology of oxidative stress are important for their implication in human health. The application of specific therapies and clinical treatments to reestablish oxidant/antioxidant balance in the organism is an important part of recovery of health from a convalescent diseased condition. The studies in different animal models definitively contribute to this goal, but the results can be also suitable to extend and apply to other animal species including wild, pets, and farm animal to mitigate the effects of oxidative stress. For farm animals, live in stable conditions and cycles of handles led to the periodical changes in their habitat conditions causing oxidative stress with adverse consequences in the productive and economic indicators of livestock activity [9, 37–39]. The results obtained and the perspectives are encouraging with the consequent improvement of their health, optimal development, and quality of life.

3.1 Cellular models

*Escherichia coli* has largely been used as model microorganism in biological research studies including the generation of ROS/RONS and oxidative stress [40]. Specific protein groups were identified in *E. coli* due their antioxidative properties including molybdenum-binding enzymes and fimbriae assembly proteins for understanding important features of the structural proteome to enable modeling of different stress responses [41]. To mitigate the damage of oxidative stress, different stress response regulons are activated in bacteria, depending on the type of stressor [42].

Yeasts such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are model species for basic studies in cell biology, based on powerful genetic tools that allow gene disruption and phenotypic analyses together with more sophisticated functional screens [43, 44]. Yeast remains single a cell but eukaryotic organism, ant it is evolutionarily more advanced than *E. coli* harboring more complex cellular structures and biological processes. One of the themes in which yeast studies have provided considerable information is the cellular response against oxidative stress and the defense functions involved in such response [45, 46]. Yeast allows study of oxidative stress
and effects in different cell macromolecules, which has been related to a number of human diseases. Although the studies in *E. coli* offer a general spectrum of oxidative stress and how it impacts in cell physiology, the studies in yeast bring the opportunity to see the oxidative stress in eukaryotic cells, where the physiological and molecular processes are more complex.

To elucidate the individual effects of numerous oxidative stress-inducing agents that surround the all living beings and test different strategies to manage the adverse consequence of oxidative stress and restore the oxidant/antioxidant balance, frequently it is necessary to test and elucidate the influence and role of oxidative stress by external agents on general cellular processes. Different unicellular lines can be used also, but when it is necessary to study these effects in specific differentiated cells, these can be taken from animal tissues and organs to carry out transient tests with them [47–50]. Primary cell culture consists of a temporary or transient culture of differentiated cells removed from tissues and plated in appropriate media and provides an opportunity to assess such effects, as well as testing some methods to assess oxidative stress conditions. Some of the most used animal and human culture cells are blood erythrocytes, leucocytes, leukemia cells, hepatocytes, human hepatoma, pheochromocytoma cells, colo-adeno carcinoma cells, among others [51–53]. The tests in primary cell cultures are very convenient because the methodology is simple and well established, and also, the tests are usually fast and low cost. But primary cell cultures are different from those isolated directly from tissue, and originally these cells were parts of organs and tissue in living organisms, in contact with other differentiated cell and constant and active interaction with them. Without such interaction, the isolated cells are very vulnerable to many agents, including those promoting oxidative stress. The mentioned facts imply that often the results obtained in culture cell could differ with tests performed in living animal models and encouraging results in cellular lines became disappointing in when tried *in vivo* [51, 52]. Cellular primary culture and cellular lines are exposed to high oxygen concentrations so the balance between oxidative processes generating RONS and antioxidant cellular defenses is altered in favor of oxidative stress. In addition, the fragile stability of cultured cells is also present in the conservation of original characteristic (genotype and phenotype) due the high mutation rate in cellular lines [51, 54, 55].

4. Experimental model and farm animal

4.1 Invertebrates

Invertebrates have been valuable research models in the discovery of many biological principles owing to the numerous advantages they provide. During the life cycle, many in pathogen-rich environments manage harsh weathers, exposed to a number of chemical compounds, and they are well adapted to both terrestrial and marine ecosystems. Their remarkable ability to successfully face enormous oxidative stress generated in all these circumstances makes them attractive models for research. In addition to mollusks, one of the most recurring invertebrate animal models is *Caenorhabditis elegans*, a free-living nematode that lives in temperate soil environments, and *Drosophila melanogaster*, a widely used model organism, has provided insight into eukaryotic genetics and human disease.
4.1.1 Mollusks

Mollusks (Mollusca) are the second largest phylum of invertebrate animals by the total number of species. The survival of aquatic mollusks depends on their ability to sense and respond appropriately to biotic and abiotic changes in their habitat. The studies and changes in their immune responses can also serve as indicators of changes in ocean environments. Therefore, studies into understanding new factors in their immune systems may aid new biomarker discovery and are of considerable value. In general, these studies were focused on ecotoxicological aspects in mussels such as Mediterranean mussel (Mytilus galloprovincialis), Atlantic ribbed Mussel (Geukensia demissa), Rooved carpet shell Ruditapes decussatus, Blue mussel (Mytilus edulis), Soft Shell Clam (Mya arenaria), Eastern Oyster (Crassostrea virginica), and Atlantic Jackknife Clam (Ensis leei) among others [56–58]. Aquatic and terrestrial mollusks are exposed to metallic polluted water and substrates with contaminants show a reduction of lower rates of reduced glutathione (GSH) when lived in a water or substrate deliberately contaminated with copper and zinc [59]. But at slightly lower levels, effect has been reported in terrestrial mollusks due to the fact that in terrestrial mollusks the rate of ingestion is slower. This behavior induces lipid peroxidation that is well correlated with the reduction in the whole antioxidative enzymes [60]. Not only water and soil pollution induces oxidative stress affecting oxidant/antioxidant balance, abiotic environmental conditions due to the seasonal variation in water temperature, salinity, the rate of dissolved oxygen, the nutritional value and quantity of food available [56, 60], UV radiation, pH, and dissolved O₂ [61]. Bivalve mollusks from freshwater to marine ecosystems are suspension feeders and have been widely studied in ecotoxicology as indicator species for monitoring of environmental perturbations. They have highly efficient cellular internalization system for external compound and micro- and nanoparticles able to modify and disturb vital physiological functions including intracellular digestion and cellular immunity [56, 62].

Mollusk communities are getting endangered by oxidative stress and different mollusks have been used as biological models to study the effect of oxidative stress and the antioxidant mechanisms to alleviate the relating damage. The most important studies on OS were focused on the impact of pollution-induced OS generating physiological and morphological modifications due to carcinogenic and toxic nature of many chemical compounds. The most frequent consequences are neoplasies as blood neoplasm, hematopoietic neoplasm, disseminated neoplasies, hemic neoplasies, leukemia, and proliferate cellular disorder [63]. Many compounds such as polyaromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), and heavy metals (Pb, V, Cr, Ni, Cu, Mn, Zn, Cd) are implicated in the induction of the production of RONS although many specific aspects of the mechanism remain unknown [64]. The alteration in catalases, superoxide dismutase, glutathione peroxidases, glutathione S-transferase, and other enzymes was observed as well as higher rates of reduced glutathione. These alterations of OS indicators were found mainly in gills and in the digestive gland, the most exposed organs, indicating the activation of antioxidant defenses [56].

4.1.2 Shrimps

In the last two decades, there has been an increase in crustacean aquaculture production, reaching approximately 9.3 million tons in 2018. The increase in production output is primarily due to aquaculture intensification, such as improved feed
efficiency, feed formula, and feeding management techniques and technological improvements, with considerable impact on protein efficiency and nitrogen retention. Intensive aquaculture is associated with several stressing factors, including increased stress resulting from high stocking density, which can cause high mortality. To face these stress conditions, white shrimp (Litopenaeus vannamei) and shrimp in general display an innate immune system that is characterized by defense mechanisms based on cellular and humoral reactions that are mainly related to the hemolymph. Hemocytes have been identified as immune defense components that exhibit high phagocytic activity [65]. The modulation of immunological parameters in response to adverse conditions is an important indicator of shrimp health status. High stocking density conditions require the adaptation of shrimp to lower rate of dissolved oxygen levels, reduced space, and increased interactions with other organisms, which lead to increased energy consumption for the maintenance of physiological conditions. These facts have direct effect on cellular hemocyte composition [66, 67].

Fatty acids are essential for the constitution of the body and maintenance of vital metabolic activities. The n-3 highly unsaturated fatty acids (n3-HUFAs), such as EPA and DHA, are important components of the phospholipids of the cell membrane, determining their fluidity, and they are involved in other functions, such as the reproductive and immune functions of aquatic organisms [68]. Crustaceans have difficulty in converting polyunsaturated fatty acids into highly unsaturated fatty acids (HUFAs) through the elongation of carbon chains and the desaturation of fatty acids [69]. Moreover, it has been determined that high levels of n3-HUFAs can be easily oxidized, leading to oxidative stress [70, 71]. Determination of the optimum amount of fatty acids required in shrimp diet is very important. A decrease in growth and an increase in mortality at dietary lipid levels greater than 10% have been reported previously [72]. Additionally, it has been observed that high lipid levels in feed can affect the lipid composition of shrimp tissues, resulting in high concentrations of lipids in the digestive gland and muscles [73]. Increases in respiratory burst capacity, total hemocyte count (THC), and CAT and GPx activities are well correlated with increasing dietary lipid levels, suggesting that the optimum lipid supplementation level is between 10% and 12% [74].

The best performance with respect to zootechnical and immunological parameters was observed in shrimp when they were fed with high-carbohydrate diets, and the highest growth rate and hemocyte number obtained in shrimp fed diets with approximately 40% carbohydrate under different stress conditions [75]. Providing shrimps with diets rich in nutrients (e.g., proteins, lipids, carbohydrates, vitamins, minerals, and additives) is a suitable approach for improving performance of animals, faster development, and increases in productivity as well as more resistance against oxidative stress [76]. Due to its economic importance, the different dietary carbohydrate/protein and lipid/protein ratios on the growth performance, protein efficiency, nitrogen retention, and immunological and oxidative stress status of white shrimp are cultured under an intensive system [77].

4.2 Vertebrates

4.2.1 Fishes

Fish, like all living organisms, suffer from the consequences of oxidative stress caused by the generation of reactive ROS/RONS species induced by different factors such as diseases and the processes associated with them, capture, transport, and
handling of fish, hypoxia, environmental temperature, inadequate salinity of the waters, both by chemical nature of dissolved salts and per their concentration, malnutrition, pollution by different types of contaminants. The sea and freshwaters are prone to contamination by flowing into both surface and underground water reservoirs, through rain runoff and natural or artificial drainage lines to rivers, lakes, and seas. Among these contaminants, we can highlight those that induce the generation of ROS/RONS at levels that overload the antioxidant defense of the organism altering the normal morphological and physiological functioning of the organs and tissues [78].

The first important indicators to test pollution and environmental affectations in water-inducing oxidative stress are the deterioration of DO level (dissolved oxygen in water), pH, temperature, and other basic parameters of aquatic environment [79]. Described alterations led significant affectations on fish morphology, growth rates, and reproductive functions with adverse combinational and cumulative affectation in quantity and quality of fish production.

Zebrafish is a valuable and one of most versatile experimental animal models particularly in Ref. to fish biological and genetic studies due its small size, rapid development, and genetic and biochemical homology with higher vertebrates. It has been used for drug screening, determination of bioactivity, toxicity, and collateral side effects of novel drug candidates, but it is also a useful experimental model to study oxidative stress-linked disorders [80]. Zebrafish larvae were used to evaluate the antioxidant activity of 15 commercially available flavonoids against UV-induced phototoxicity, along with the computational quantitative structure–activity relationships (QSAR) method to investigate the correlations between the observed biological activities and the physicochemical properties of the different compounds. Among these compounds, chrysin and morin showed higher ROS-scavenging rates (99 and 101%, respectively) and lower toxicity (LD50 > 100 ppm) [81]. Flavonoids are the most abundant polyphenols in our diet and the most studied ones. They are characterized by a common benzo-γ-pyrene moiety, variously substituted with hydroxyl and methoxyl groups. Based on their chemical structures, flavonoids may be divided into six major subclasses: flavonols, flavones, flavanones, flavanols, anthocyanins, and isoflavones [82–84].

The studies on oxidative stress carried on this model help to understand the effect of oxidative stress in ecological and economic fields applied to both wild and aquaculture species. About 60 million people around the world are involved in the primary sector of capture fisheries and aquaculture, and this economic field involved in capture, fish handling, processing, and selling, making this activity an important source of human food and animal feed [85, 86]. Industrial fishing is highly developed sector but capture fishing pressure faces growing environmental problems and other operative adverse factors, as overexploitation of marine and freshwaters species, climate change, and toxic pollution. Recent data from the Food and Agriculture Organization of the United Nations (FAO) indicate that the share of marine fish stocks within biologically sustainable levels is declining [87]. Worldwide, the most important fish species used in fish aquaculture are carp (Cyprinus carpio and Cyprinus ssp), salmon (Salmo ssp), tilapia (Oreochromis spp. y Tilapia sp), catfish (Silurus ssp), and trout (Oncorhynchus) [87].

Different taxa and different species of the same taxa of fishes exhibit differential tolerance to oxidative stress [88]. The oxidative stress can affect reproduction, fecundity, and genetic degeneration in progeny, leading to the extinction of some of the fish species [89]. Some chemicals such as duroquinone-inducing oxidative stress affect the spermatozoa of common carp (C. carpio) and reproductive damages were also
Inadequate aquatic environment greatly affects the growth and development of fish affecting food chain and entire trophic communities, causing significant losses to traditional fishing production and that is carried out in a semi-intensive and intensive way in aquaculture farms [89].

4.2.2 Poultry

Poultry in commercial settings is exposed to a range of stressors. A growing body of information clearly indicates that excess ROS/RONS production is the major detrimental consequence stressor inducing oxidative stress in poultry production [90]. In addition, other environmental stressors such as NH\textsubscript{3} pathogenic infections are considered also as risk factors for oxidative stress in all animals that are raised under the intensive mode of livestock production [91]. Farm chickens live in a pro-oxidative environment, due to the high population densities, fluctuations in temperature, and high levels of ammonia due to bird droppings and handling of animals during the entire productive cycle, affecting poultry health and production parameters. The genetic selection on fast growth rates and complexion, selected under commercial criteria with lean and large breast, makes farm chickens and other farm birds more susceptible to oxidative stress [92]. The economic impact of oxidative stress farm bird industry is enormous. Poultry is one of the most and fastest-growing animal production sectors and has a substantial contribution to food security due to the accessibility and nutritional value of its products, particularly meat and eggs, for the majority of the world’s population. This livestock sector exhibits one of highest rate of growth and technological innovation and improvement and is listed among the largest world agricultural commodities. The poultry sector, which has a market value of $ 310.7 billion in 2020, is expected to grow to $ 322.55 billion in 2021 and record at compound annual growth rate (CAGR) of 3.8%. The market is expected to hit $ 422.97 billion in 2025 [93].

The main source of RONS in chicken muscles is the leakage of electrons from the respiratory chain in mitochondria during the reduction of molecular oxygen to water process observed in all aerobic organisms, as in other studied animal models [14]. Among animal source foods, poultry meat has also been recognized as a material highly sensitive to oxidative processes owing to the high unsaturation degree of the muscle lipids and the susceptibility of meat to undergo oxidative reactions involves many other endogenous (i.e., antioxidant enzymes) and external factors including environmental conditions during the period of growth and fattening, for animals destined for meat; and of growth and productive period of eggs. According to the literature reports, heat and diet are the most remarkable means of oxidative stress in domestic birds. These adverse factors may lead to biological damage, serious health disorders, lower growth rates, and, hence, economic losses [92]. Supplementation of feed using antioxidant dietary has been proposed to alleviate the oxidative stress of poultry produced in hot climates although low temperature also decreases the level of antioxidant activity lowering the antioxidant defenses in the animal in different tissues and organs [94, 95].

Oxidative stress can affect important organs and tissues, including liver, kidney, reproductive organs, and immune function. The liver plays a crucial in avian species, compared with the majority of most mammalians, including rodents, ruminants, swines, equines. Liver is the most important organ for “de novo” lipogenesis in birds and about 90% of fatty acids that are synthesized in liver, where the rest, the minor proportion, is contributed from adipose tissue. This fact is even more significant in
commercial poultry due to the fact that dietary fat content is relatively low [96]. Fatty acids synthesized by the liver are transformed, by the biochemical route of low-density lipoproteins (VLDL), which act as an energy source to other tissues for immediate use or storage. The critical role of avian liver in lipid metabolism is also highlighted during egg production, which demands a shift of hepatic lipids to the yolk to nourish the embryo [96, 97]. RONS can oxidize and damage cellular proteins and lipids, but are normally kept in check by the liver’s robust system of antioxidants and antioxidative enzymes that quickly neutralize excess RONS to maintain the redox balance [90, 98]. It has been observed that in the liver cells the mitochondrial function and other complex activity in low-feed efficient broilers. This fact is associated with higher oxidative stress and differential protein expression [99].

The levels of essential microelements in poultry feed must be high enough to satisfy the birds’ requirements and, at the same time, low enough to ensure the safety of both animal feed and meat and eggs for human nutrition. The essential role of vanadium (V), chromium (Cr), and nickel (Ni) in poultry nutrition is still under investigation, while their toxicity was well established a long time ago. Vanadium plays a role in lipid metabolism and its deficiency in feed can be associated with decreased levels of blood and bone iron, which can result in abnormal bone development. A range of experiments indicated the crucial role of trivalent Cr (Cr³⁺) in the maintenance and regulation of blood glucose levels (via the glucose tolerance factor – GTF). Dietary Cr³⁺ improves insulin effectiveness by enhancing its binding to receptors and the sensitivity of the target cells [100]. In chickens, insulin promotes high rates of protein synthesis and the better amino acid transport and lower rates of protein degradation. This fact increases the weight of pectoral muscles and the meat of these broilers contained less fat and cholesterol causes disorders of carbohydrate and protein metabolism, reductions in insulin sensitivity in the peripheral tissues, and decreases in growth rate [100]. The dietary addition of Cr propionate improved feed efficiency and decreased mortality [101].

The essential function of Ni has been established in rats, poultry, swine, goats, and sheep. Ni plays a role either as a structural component in specific metalloenzymes (urease, hydrogenase). This element acts also as a cofactor in facilitating intestinal absorption of ferric ions. Experiments have suggested its importance in cardiovascular pathology; that is, elevated serum Ni concentrations are observed in patients with acute myocardial infarction and stroke [102]. Ni ions have a high affinity for proteins and amino acids and cause protein oxidation in cells. Its binding to chromatin in somatic cells can result in oxidative and structural damage to cell proteins. Currently, eight enzymes containing Ni have been identified [103].

Selenium is an essential micronutrient and well antioxidant naturally found in soil, water, and some foods. Selenium compounds in trace quantities are indispensable for proper physiological functioning of vertebrate organisms. The beneficial effects of selenium are related to the selenoproteins, playing relevant role in the many physiological functions, including endocrine, muscular, cardiovascular, nervous, reproductive, antioxidative, and immune functions [10, 104, 105]. Selenium compounds improve immune responses modulating the production of certain cytokines secreted by cells of the immune system and enhancing the resistance of the immune cells to the oxidative stress. Selenium supplementation had inhibitory effects on tumor necrosis factor alpha (αTNF) levels in heat-stressed broiler chicks, but the details are not completely elucidated and are in continuous investigation [10, 106]. These mentioned microelements in inorganic formulations are suitable for consumption in all animals as dietary supplement, but in trace well-established amounts, above which they are
harmful to the health causing severe intoxication and inducing oxidative stress. The damage by these stressors has been observed, with particularly severity, in liver and kidneys and gonads, suggesting that these organs are greatly vulnerable to metal intoxication with the logical affectation of hepatic, renal, and reproductive functions [55, 107]. Environmental problems also affect the farm animals in intensive husbandry, and ammonia (NH$_3$), a severe air pollutant, is an important factor for the formation of secondary particles in the heavy haze pollution [108, 109]. It is known that high concentrations of atmospheric ammonia induce alterations in the hepatic proteome of broilers [91, 110].

4.2.3 Rodents

Rodents are mammals of the order Rodentia, which are characterized by a single pair of continuously growing incisors in each of the upper and lower jaws. About 40% of all mammal species are rodents. Difference has been extensively served as experimental models: guinea pigs (Cavia porcellus), mice (Mus musculus), rats (Rattus norvegicus domestica) syrian hamster (Mesocricetus auratus), or rabbits (Oryctolagus cuniculus). It has been firmly established that rats, mice, and humans each have approximately 30,000 genes of which approximately 95% are shared by all three species [111]. Other advantages for using rodents as animal model for scientific research are relatively small and require little space or resources to maintain, have short gestation times and large numbers of offspring, and have quite rapid development to adulthood and relatively short life cycle spans. Mice have a gestation period of about 3 weeks, a fourth week period of weaned, and reach sexual maturity by 5–6 weeks of age. This short life cycle allows large numbers of mice to be generated for studies fairly quickly. However, in many areas of research rats are preferred, including cardiovascular research, behavioral studies, and toxicology.

The rabbit (O. cuniculus) is phylogenetically closer to primates than other rodents [112] and is large enough to permit non-lethal monitoring of physiological changes. The rabbit is also standard laboratory animal in biomedical research, and transgenic rabbits are used as animal models for a variety of human genetic and infection diseases. It is routine, the use of rabbits includes antibody production, development of novel surgical techniques, physiological clinical methods, and toxicological studies for the testing of new drugs and development of new medical treatments. There are a great number of research studies where mice and rabbits, including genetically transformed animals, were used in many important topics, including lipoprotein, atherosclerosis, cardiovascular research, and hypertrophic cardiomyopathy. Some of these mutants haven conceived to constitutively develop oxidative stress, which in some cases lead to the status for the spontaneous development of tumors. One of the most remarkable cases is the mutants where gene encoding for well-known tumor suppressor p53 is knocked out. In humans around 50% of cancer studied cases have shown this suppressor gene mutated [113]. The p53 gene is induced and expressed in response to different type of stresses, including oxidative stress. This is an example when genomic stability and integrity are endangered via DNA damage under oxidative stress. Many genes, acting in basic functions as a transcription factor p53 gene, are targets of oxidative stress. There many other genes encoding for regulatory proteins or functional RNA, regulated either positively or negatively, are targets for oxidative stress attack. These target genes are usually involved in various cellular processes converging into genome stability maintenance such as cell cycle arrest, senescence,
apoptosis, and DNA repair, suggesting a possible reason to explain the tendency in those mutants to develop cancerous tumors [114].

Mouse is the most widely used model in biochemical research, and mice provide ideal animal models for biomedical research and comparative medicine studies because they have many similarities to humans in terms of anatomy and physiology. Oxidative stress mutant mouse models were either specifically designed to assess the oxidative role of candidate molecule or shown to be oxidative models “by chance.” The models genetically modified mutant mice are transgenic animals where a deregulation of redox status has been modified by genetic transformation [115, 116]. One of these mutant mouse models lacks the transcription factor Nrf2, which is very important in the regulation of expression of phase II-antioxidant enzymes genes. Each mutant has a knock-out of one of the components of the cellular antioxidative defense disabling an adequate full response to oxidative stress. Some mutants also are susceptible to neoplastic tumors induced by increased ROS/RONS level. This applies to superoxide dismutase 1 (SOD1) and 2 (SOD2), and such animals develop liver cancer and are conducive to the development of cancerous tumors. The case for peroxiredoxin 1 (PRDX1)-deficient animals that die from malignant cancers after surviving hemolytic anemia, and for OGG1-deficient mice in which a defect in the elimination of oxidative-damaged DNA facilitates lung tumor development [117]. Studies in mice and other rodents can be preliminary steps not only to applied successfully assayed prophylactics, drugs, and treatments to human health but also to wild and farmer mammalian animals. Some rodents are not only experimental models, but at the same time they are animals of economic interest and the basis of an important food industry, that is, the case of rabbits, for example. This is an important fact that allows extending obtained results on the causes and effects of oxidative stress not only under animal husbandry conditions but also under production conditions, providing a robust basis for applying appropriate strategies in other farm animals such as pigs and cattle.

4.2.4 Swines

Swines (Sus domesticus) are an important species in livestock production. Total global pork exports for 2021 are expected to reach 11.8 million tons, mainly with China as a leader, followed by the EU and United States, and the global pork meat market size is constant growth and the projection by 2027 is expected to reach US $257,874.5 million [118]. Many breeds of pig exist, with different colors, shapes, and sizes. According to The Livestock Conservancy, as of 2016, three breeds of pig are critically rare (having a global population of fewer than 2000 [119].

As other farm animal, pigs are exposed to various types of stressors during their life cycle: dietary, social, environmental stress, but also metabolic stress through high performance in intensive livestock farm. Some stages are especially critical and can induce ROS/RONS and induce oxidative stress. In the weaning phase, piglets often show growth depression and are vulnerable more susceptible to diseases, a phenomenon known as post-weaning stress syndrome [120]. Pigs suffer oxidative stress during late pregnancy, high lactation, and weaning. Weaning is one of the most stressful stages in piglets that results in intestinal, immunological, and behavioral changes and animals became more vulnerable to different disease. During this period, pigs are subjected to a number of stressors, including abrupt separation from the sow, transportation and handling stress, a different food source, social hierarchy stress, co-mingling with pigs from other litters, a different physical environment (room, building, farm, water supply, etc.), increased exposure to pathogens, and dietary or
environmental antigens. The piglet must adapt to all of these stressors rapidly to be productive and efficient. If the incidence of the different stressors in this stage is too great for the pig, it can lead to poor performance and increased mortality due the oxidative stress. When the piglet is weaned, their young organisms must be abruptly adapted from highly digestible and palatable liquid milk from its mother that is equally spaced throughout the day to a solid dry diet that is less digestible and palatable. As a consequence, feed intake is usually reduced initially after weaning and the piglet becomes malnourished with reduced transient growth rate [121]. In addition, we must point out the trauma that the definitive separation from their mothers causes to young pigs, which also stresses them. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine a well as modification in absorptive, secretory, and barrier functions of the intestine. In the moment piglet stops feeding on breast milk, begins a period of adaptation to many changes stressing their routine normal behavior: the separation of mother and the change in their routine feed. This is a period of variable duration, a period of reduced feed intake. It is considered that by the end of the first post-weaning week, metabolizable energy intake is reduced to about 60–70% of pre-weaning milk intake, and it takes about 2 weeks to complete recovery to the pre-weaning energy intake values [121]. Low-feed intake predisposes the pig to intestinal barrier dysfunction, which is often accompanied by intestinal inflammation and negatively affects villus height and crypt depth.

Environmental problems also affect the farm animals in intensive husbandry, and ammonia (NH$_3$), a severe air pollutant, is an important factor for the formation of secondary particles in the heavy haze pollution [108, 109]. However, there were a few studies on the effects of NH$_3$ on pigs but for sure the high-density feeding in livestock houses caused air pollution, and the main by gas pollutant is NH$_3$ resulting from microbial decomposition of nitrogen-containing organic matter. Some studies have indicated that oxidative stress is one of the toxicity mechanisms of NH$_3$ [122]. When pigs were exposed to 100 ppm, NH$_3$ during 6 days the pigs reduced food intake and lost weight and increases up to 100 and 150 ppm provoke acute inflammatory reaction. The effect of ammonia is observed in tracheal epithelium, and this organ is an important barrier between internal organ and tissues and the environment. Ammonia can cause dramatic changes when inhaled, including smooth muscle hyperplasia, pulmonary fibrosis, basal layer thickening, cell composition changes, and inflammatory cell infiltration, loss of cilia or the production of more mucus covered on the basal layer of tracheal cilia. The increased mucus secretion can lead to the development of chronic respiratory diseases including chronic obstructive pulmonary disease (COPD) and asthma [123]. NH$_3$ exposure leads to loss of cilia or the production of more mucus covered on the basal layer of tracheal tissue as revealed by microscopy observation [122]. The epithelial tissue of trachea forms a cellular barrier limiting the internal organism with the environment. The attack to this barrier by external irritant and harmful substances, particularly gases, combining with other stressor and pathological conditions, alters the function of respiratory structure inducing dramatic chance and muscle hyperplasia, pulmonary fibrosis, damages to the respiratory tissues, inflammation, increase of secretion (mucus), and obstructive respiratory diseases and asthma. All these pathological conditions are accompanied by oxidative stress. Oxidative stress indexes such as superoxide dismutase (SOD), reduced glutathione (GSH), reduced glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) in the tracheal tissue allow the inhalation of ammonia with the damage-producing oxidative stress. The activities of SOD and GSH-Px and the level of GSH in the trachea tissue

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were significantly decreased compared with the control group, when animals were exposed. On the contrary, MDA content in the trachea tissues of pigs exposed to NH$_3$ was significantly higher than that in the control group [122]. Transportation of animals is important economic activity in many of the livestock production but is an important stressor affecting many of the farm animals but the transport of animal is also an important stressor that can induce the generation of ROS/RONS and drive the affecting organisms to oxidative stress provoked morbidity, mortality, carcass trim loss, and undesirable meat characteristics [124]. Due to the relevance of this topic within the cattle sector, we will deal with it in the section corresponding to cows.

4.2.5 Cows

Cattle (*Bos taurus*) are large domesticated bovines and the most widespread species of the genus *Bos* [125]. In dairy cows, oxidative stress has a negative impact on immune and reproductive functions: increased mastitis frequency and higher somatic cell counts in milk, decreased fertility, increased embryo mortality, post-partum retained placenta, and early calving. Animal metabolic and physiological traits respond quite differently to different oxidative stress factors and management strategies, but the specific cellular mechanisms of action are not often described, although our observation points out that in general, the mechanism is the same as already was described but particularities derived from intrinsic physiology of each animal species and the characteristics of handling with them during the entire productive cycle. Some oxidative stress factors considered and described are linked with the microbial pathogenic cells inducing mastitis, metritis, and degenerative diseases. In dairy cows, feeding management is based on the use of different concentrates and forage ratios that certainly have effect on some expressed genes associated with oxidative stress pathways. In cows, microbial infections are one of the causes of oxidative stress. Microbial activities cause driver of degenerating oxidative stress diseases. Mastitis and metritis are some of the diseases that have been correlated with other pathogens as: *Streptococcus dysgalactiae*, *Streptococcus uberis*, *E. coli*, and *Klebsiella, non-aureus Staphylococci* (NAS), *Staphylococcus aureus*, and *Enterococcus spp*. have been well correlated with mastitis occurrence and with oxidative Stress [126]. Mastitis is an intra-mammary infection driven by host-pathogen interactions that cause severe economic losses, including decrease in milk production and quality, premature culling, lower conception rates, and treatment costs in dairy cattle. Therefore, host-microbial interactions have been studied, aiming to optimize the lactating performance of cows [127] and depending on the pathogenic microbes’ primary reservoir and mode of transmission. Mastitis has been categorized into contagious and environmental forms. Some detrimental microbes such as *S. dysgalactiae*, *S. uberis*, *E. coli*, and *Klebsiella, non-aureus Staphylococci* (NAS), *S. aureus*, and *Enterococcus spp*. have been well correlated with mastitis occurrence, but unfortunately, the mechanisms underpinning host-microbial interactions inducing mastitis are unclear [128], but probably it is related to a drop in the immune defenses of the animal and what favors the opportunistic infection of these pathogens. The pathogenic invasion mainly occurs from environmental microbes through the mammary teat canal opening in the direction of the tissues, and then to the epithelium cells of the duct, causing inflammation and development of granulation tissues that finally appear as polyoid swelling. Pathogens in the epithelium that remain undestroyed by neutrophils will cause edema, leading to vacuolization and desquamated condition of epithelial acini. The pathogenic bacteria undergo a rapid multiplication, leading to reductions in healthy milk secretory tissue,
scanty milk with blood traces as well as gangrene and thrombosis damaging the udder tissue, which can potentially lead to toxemia and death in acute cases. Mastitis milk is source of zoonosis-like tuberculosis, brucellosis, and gastroenteritis. A number of previous studies analyzed the microbiome of mastitis cows compared to healthy ones, indicating a lower microbial diversity in mastitis bovine milk, quantified by a lower Shannon Diversity Index, also called the Shannon-Wiener Index, which is a method to evaluate the diversity of species in a community. The correlation analysis between milk metabolites of the intra-mammary infected cows and their microbial populations has been described [127]. In the case of Metritis, a uterine wall inflammation also is associated by an increasing in *Fusobacterium, Bacteroides*, and *Porphyromonas* has been associated with metritis [128, 129], while a reduction in the same bacteria has been associated with the antibiotic treatment of this disease. From published data, we can say that both diseases and associated microflora induce oxidative stress.

The increases in ROS/RONS species can provoke lipid peroxidation and other cell disorders including the activation of apoptosis so it is necessary to maintain a robust antioxidant defense to maintain the functional status of the organisms. Dairy cows need an adequate intake of coarse fiber to ensure proper rumination, saliva processing, and rumen buffering; it is recommended that at least 40% of the feed particles containing dietary ingredients in total mixed rations (TMR) for dairy cows be larger than 8 mm [130]. However, commercial cow lactation diets are typically high in concentrate to maximize nutrient intake and milk production efficiency, resulting in diets with low or moderate physically effective neutral detergent fiber (PeNDF) [131]. However, high-concentrate diets are likely to result in metabolic and systemic dysfunction, leading to a rise in the concentration of ruminal volatile fatty acids and a corresponding decrease in ruminal pH [132]. Low pH causes a lysis of rumen microbes and release of endotoxins lipopolysaccharide (LPS) from Gram-negative bacteria, and increases the permeability of the rumen barrier in cows but also in other ruminants. LPS in the rumen fluid is absorbed mostly by the rumen wall, then travels through the ruminal veins to the liver via the portal vein [133], and subsequently reaches the mammary gland, triggering inflammatory responses that result in decreased production. In some tested dairy cattle fed with high concentrate, the oxidant and antioxidant biomarkers such as LPS concentration in the rumen fluid, hepatic vein plasma, portal vein plasma, and jugular vein plasma were higher than in cattle fed with low concentrate [126].

Malondialdehyde (MDA) is a well-accepted and used biomarker to determinate lipid peroxidation and SOD activities. MDA is increased in high concentrate feed formulations for cow, and it is accompanied with a reduction of total antioxidant capacity (T-AOC), GPx, and CAT activity. Regarding the composition and structure of milk metabolites, blood metabolites, hormones, and enzymes, a substantial difference was observed in high concentrate compared to low-concentrate cattle. It was reported that albumin and paraoxonase concentrations are inversely related to oxidative stress due to their contribution to the protection of low-density lipoprotein and high-density lipoprotein against lipid peroxidation, along with protein carbonyl and lactoperoxidase. Increased LPS levels in portal and hepatic veins further damage hepatocytes and impair liver function, shown by enhanced TNF receptor-associated factor 6 (TRAF6), p-NF-B, p38 MAPK, IL-1, and serum amyloid A (SAA) levels in the liver [134]. LPS increases the concentration of LPS-binding protein (LBP), serum amyloid A (SAA), and haptoglobin (HP) in peripheral blood during SARA [135]. The high concentrations of LPS can induce the production of ROS/RONS by Kupffer cells and neutrophils. Kupffer cells, also known as stellate macrophages and Kupffer-
Browicz cells, are specialized cells localized in the liver within the lumen of the liver sinusoids and are adhesive to their endothelial cells, which make up the blood vessel walls. Inadequate regulation of ROS accumulation within metabolically active tissues results in oxidative stress. Isoprostanes (IsoP) are also important biomarkers of lipid peroxidation damage because they form when ROS oxidizes arachidonic acid. Indeed, IsoP was found in the blood and milk of dairy cows during periods of high oxidative stress, such as the peripartum and mastitis periods [136].

Handling of cows also derived in oxidative stress increasing the susceptibility to different diseases with increased incidence and severity of disorders during the transition period (around 3-week prepartum late pregnancy through 3-week post-partum). Transition period is characterized by dramatic physiological and immunological changes from stages of prepartum gestation stage to lactation. It is known that during late pregnancy, glucose and amino acid requirements increase in order to support fetal development. Transition cows may display an overt systemic inflammatory response around the time of calving even without signs of microbial infections or other pathologies [137]. Low-grade chronic inflammation in transition cows has adverse effect and can aggravate metabolic stress due the increasing lipolysis and, in general, affecting hepatic functions. Pro-inflammatory cytokine TNF-α was observed in serum of cows with fatty livers, and its concentration is well correlated with biomarkers of inflammation and the increment with a concomitant impairment of health and milk production [138]. It has been suggested that inflammatory-associated pathways are involved in adaptations to lactation. Many health disorders of dairy cows could be explained by the abrupt changes in nutrient requirements taking place in the lapse of calving, when the stressors lead the organism to oxidative stress. To mitigate the damage by oxidative stress through the maintenance of an adequate level of endogenous non-enzymatic antioxidants (BSA, glutathione) as well as enzymatic ones (superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx)) and exogenous antioxidant (vitamins, betaglucane, carotenoids, and flavonoids) according to the necessity, so it is important to know the requirements of the organism [139].

Transportation of animals is an essential component of livestock production and presents both economic and animal welfare concerns. Economic costs associated with transport are very high and negative data such as including morbidity, mortality, and carcass trim loss, and lower meat quality [39]. Transportation of cattle can contribute to the development of oxidative stress in several ways. Psychological stress and food deprivation stimulate fatty acid and amino acid mobilization for use as metabolic fuel, which increases mitochondrial ROS/ROSN production. During the transportation, the cows do not take food. This food deprivation and water deprivation provoke physical exertion in the animal that can stimulate an inflammatory response, which results in ROS/RONS production by phagocytic immune cells and as a by-product of eicosanoid biosynthesis. This costly condition, transportation stress, is a predisposing factor for bovine respiratory disease (BRD). In case of beef, animals may be transported several times during their lives: from birthplace to an auction market, stocker or backgrounding handling, feedlot, and finally to a processing facility implying great economic loss. Usually, when cattle have arrived to final destination, the privation of food and water continued until the animals are weighted. Public and consumers have increased their concern in how animals are raised and handled before slaughter, and the effects of transit on animal well-being is focus of interest [39].

As other organisms with highly developed central nervous systems, oxidative stress can be caused by psychological stressing factors derived from handling, fatigue,
and potential for injury during the transport operation. The joined actions of different types of stressors activate several biological pathways known to lead to the development of oxidative stress. If some bioindicators of oxidative stress are compared between the calves that are not transported with those that are transported considerable distances, we find great differences between both groups. Lipid peroxidation was 218% greater, and antioxidant capacity was 186% lesser in leukocytes isolated from transported calves [140]. The same tendencies were found when cells were incubated with antioxidants (α-tocopherol or ascorbic acid) at concentrations greater than 0.1 mg/mL [141] as well as increased lipid peroxidation (177%) and decreased antioxidant status (11%) in the serum of beef calves after transit relative to pretransit values. The post-transit serum malondialdehyde (MDA) concentrations were 43% greater in calves, which later died of acute BRD, and calves that experienced ≥3 episodes of BRD had twofold greater MDA concentrations after transit than healthy calves [142]. It was observed greater linoleoyl tyrosine oxidation products before and after transit in blood samples from calves that eventually had pulmonary adhesions at slaughter, providing evidence that oxidative status may be a contributing factor in the development of BRD. ROS/RONS species produced by immune cells to eliminate pathogenic bacteria are able to damage and even kill the pulmonary cells, provoking inflammation in this damaged tissue resulting in respiratory dysfunction. These data indicate that transit-induced oxidative damage can impair immune-cell and respiratory function, which ultimately increases morbidity and mortality. Oxidative stress induced by transportation accomplished by increasing of respiratory rates because the effort to repair, degrade, or replace damaged biomolecules as well as deployment of antioxidant defense requires a great amount of energy. Indeed, a negative relationship between oxidative stress and feed efficiency in livestock has been observed and described [143]. During the trip, while trucks or cargo trains are moving, cows usually tend not to lie down, but when travel is very long, the animals will trait to lie down if adequate space is available.

5. General assessment to preventive strategies

Strategies based on dietary antioxidants may alleviate the impact of other sources of oxidative stress in farm animals and inhibit the negative influence of this stress on livestock production. These strategies generally involve reducing the concentration of polyunsaturated lipids in diets as well as supplementation with α-tocopherol (around 200 mg tocopherol/kg feed) and ascorbate (up to 1000 mg ascorbate/kg feed) alone or in combination with other elements with antioxidant potential, such as selenium, magnesium, and zinc in poultry [144–146]. The vitamin E, α-tocopherol, is an effective inhibitor of lipid peroxidation and well scavenger of free radicals such as peroxyl and alkoxyl and lipid radicals by transferring hydrogen, resulting in non-radical molecules. In addition to increasing the concentration of dietary antioxidants in tissues, these strategies, including dietary fat modification, may also support antioxidant protection by promoting the concentration and activity of endogenous antioxidant enzymes such as glutathione peroxidase (GSHPx). Selenium yeast may be, according to some recent reports, a promising dietary strategy to improve the oxidative stability in farm animals [90, 147]. In broiler chickens, dietary organic selenium improves antioxidant capacity and enhances growth performance in broiler chickens and the supplementation with algae-based selenium yeast in addition to improving not only the antioxidant defense capacity in live broilers, but also allowing to preserve the
quality of the meat after slaughter by increasing lipid and protein oxidative stability of meat through promotion of antioxidant enzyme activity [148].

To establish the composition of antioxidant diet, it is necessary to define the specific nutritional requirements of the species, taking into consideration not only basic feeding parameters but also all the other feeding parameters would make essential micronutrients act as anti- or pro-oxidants. Molecular mechanisms triggered after feeding high-concentrate inducing sub-acute ruminal acidosis (SARA), a digestive disorder also known as chronic or subclinical acidosis, in dairy cows [149]. This is a relevant health problem in most dairy herds. This is digestive disorder characterized by changes in oxidative stress parameters, including the genomic signaling pathways in different organs and tissues: mammary gland, liver, hind-gut, and uterus and in epithelial tissue. Cows with SARA often develop complications or other diseases and associate physiologically with immunosuppression, inflammation, and oxidative stress. The prevention of SARA includes the establishment of feeding and management guidelines seeking to minimize rumen acidic load including probiotics, fiber, and vitamins as well as yeast-derived supplements [150]. In our experience, this nutritional strategy improves overall animal performance in all aspects of their life and in productive parameters not only in cattle but also in other ruminants. A simple practical method for assessing the performances in dairy cows is the so-called body condition score (BCS). BCS helps to identify animals at increased risk of metabolic diseases and oxidative stress [151, 152]. By managing the BCS we can establish the relationships between animal health, reproductive function, milk production, and overall development of the cows. This index is associated with key hepatic enzymes associated with animal metabolism and related biomarkers, including oxidative stress biomarkers in liver tissue and plasma. Cows with high BCS show higher plasma concentrations of fatty acids compared with the other ones with normal BCS although concentration of reactive oxygen metabolites found in both groups was similar. High BCS cows showed lower overall concentrations of β-carotene and tocopherol, explaining the lower indicator (ferric reducing ability of plasma) of antioxidant capacity and showed lower hepatic protein abundance of the 1-carbon metabolism enzymes cystathionine-β-synthase, betaine-homocysteine methyltransferase, methionine adenosyltransferase 1 A, glutathione metabolism-related enzymes, and glutathione S-transferase α4 and GPx3. The published data suggest that the BCS values are well correlated with milk yield, immune response, and synthesis of antioxidant [126, 153, 154]. In deterioration of physical environment during the dry season and in the first time in body condition postpartum has been related to higher probabilities of metabolic and infection diseases [20, 155, 156]. The activity of specific additives is to efficiently manage the effect of oxidative stress derived from the determination of every specific functional element in the diet and determination of the optimized quantity and proportion in the feed formulation. Among such additives, we have amino acids, vitamins, microelements, prebiotics, and probiotics, and all of them must be continuously evaluated and valorized in animal nutrition, especially when natural antioxidant defenses have been breached. Little information exists on optimum inclusion levels and synergic effects of rumen protected amino acids on their oxidative status. Increasing antioxidants activity has a beneficial effect on animal health and can decrease the incidence rate of metabolic disorder diseases such as ketosis [157]. Other additives are analogues that can be used to improve antioxidant defense of the organism like N-carbamylglutamate (NCG). NCG is a metabolically stable analogue of N-acetylcysteine synthase that produces endogenous arginine, and it is proven that can stimulate and improve the immune function and oxidative status in suckling lambs.
[74]. Dietary supplementation with L-arginine (Arg) and N-carbamylglutamate (NCG) on intrauterine growth-retarded (IUGR) suckling lambs. Methionine supplementation coupled with choline enhanced gene expression of TLR2 and L-selectin, a part of pathogen recognition mechanisms [31]. Cells incubated without choline had high mRNA abundances encoding IL1B, IL6, IL10, myeloperoxidase (MPO), glutathione reductase (GSR), GSS, cystathionine gamma-lyase (CTH), and cysteine sulfinic acid decarboxylase (CSAD), suggesting higher inflammation and oxidative stress [158, 159].

6. Practical examples

Our group carried out a series of experiments using a mixture of hepatoprotectors and antioxidant compounds (HPAC) in the stressed mice by intramuscular via for 1 month. HPAC mixture is an injectable aqueous solution composed of salts (sodium acetate, potassium chloride, calcium chloride, and magnesium sulfate), amino acids (phenylalanine, leucine, isoleucine, valine, tryptophan, arginine, cysteine, histidine, lysine, methionine, and threonine), vitamins (nicotinamide, pyridoxine, cyanocobalamin, riboflavin, pantothen, ascorbic acid, and tocopherol), and polysaccharides (dextrlose, yeast β-glucan soluble fraction). Stressed mice were obtained according to the protocol described in the previous section and the same biomarkers were measured as well. The results showed that the mixture made up of hepatoprotectors and antioxidants at optimized proportions could prevent an oxidative stress-mediated damage in liver, kidney, and immunological functions. The obtained results clearly indicated the direct relationship between the excess of free radicals found in immune-depressed animals and their susceptibility to disease [159]. The results also showed interrelationship between important physiological functions and the oxidant/antioxidant balance in the organism. Non-treated control group presented alteration in oxidative status. In another research, a high-producer β-glucans strain of S. cerevisiae was

![Figure 1](image-url)

Figure 1. Dynamic of weight gain week on BalC mice subjected to induced environmental stress and treated with HPAC. The treatments correspond to: N-not treated and not stressed (not stressed), NS-not treated and stressed, T-treated but not stressed, TS-treated and stressed. The best response to induced stress and the best weight recovery was observed in the group that received HPAC-treatment at days 1, 3, 6, and 9 of the experimental trial. The values shown in Tables 1 and 2 correspond to this experiment [10].
selected from our culture collection to evaluate its ability to assimilate selenium by growing it in YPD (Yeast Extract, Peptone, Dextrose) medium supplemented with inorganic sodium selenite. This strain was also used as a host to express the murine lactoferrin gene under the control of the promoter of the \textit{S. cerevisiae} glyceraldehyde-3-phosphate dehydrogenase (GPD) gene. The yeast strain was cultivated to obtain biomass made up of high β-glucans levels, the incorporated selenium and recombinant murine lactoferrin. This biomass was harvested and dried to obtain probiotic supplements T1 and T2. The amount of bioselenium and murine lactoferrin were determined in the resulting product and used to feed BALB/c mice during 30 days (Figure 1). Several parameters served to monitor evaluate the immune stimulatory effect and the physiological state of the animals during the test. Measurements were carried out at 0, 15th, and 30th days. The results showed the composite supplement improves the physiological and immunological conditions of the tested animals compared to the control group (Tables 1 and 2). The results obtained pave the way for developing food supplements with similar characteristics for economically important species [10].

<table>
<thead>
<tr>
<th>Organic System</th>
<th>Test</th>
<th>T</th>
<th>Day 1</th>
<th>s</th>
<th>Day 15</th>
<th>s</th>
<th>Day 30</th>
<th>s</th>
<th>Observed Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic</td>
<td>Alanine Transaminase U/dL</td>
<td>ND</td>
<td>26.41</td>
<td>1.57</td>
<td>26.45</td>
<td>2.74</td>
<td>27.93</td>
<td>2.15</td>
<td>Without significant changes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDT</td>
<td>25.37</td>
<td>1.56</td>
<td>42.5</td>
<td>1.65</td>
<td>54.76</td>
<td>1.98</td>
<td>Increment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>26.18</td>
<td>2.01</td>
<td>67.72</td>
<td>2.92</td>
<td>37.94</td>
<td>2.26</td>
<td>Increment, but recovering the normal values</td>
</tr>
<tr>
<td>Aspartate Transaminase U/L</td>
<td>ND</td>
<td>65.38</td>
<td>1.95</td>
<td>66.41</td>
<td>2.05</td>
<td>67.42</td>
<td>2.58</td>
<td>Without significant changes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDT</td>
<td>63.14</td>
<td>1.65</td>
<td>86.76</td>
<td>1.43</td>
<td>89.83</td>
<td>1.74</td>
<td>Increment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>64.78</td>
<td>2.81</td>
<td>83.42</td>
<td>2.27</td>
<td>65.72</td>
<td>2.36</td>
<td>Increment, but recovering the normal values</td>
</tr>
<tr>
<td>Alkaline Phosphatase U/L</td>
<td>ND</td>
<td>39.32</td>
<td>2.21</td>
<td>40.15</td>
<td>2.05</td>
<td>39.54</td>
<td>2.75</td>
<td>Without significant changes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDT</td>
<td>38.87</td>
<td>1.95</td>
<td>156.89</td>
<td>2.05</td>
<td>162.54</td>
<td>2.62</td>
<td>Increment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T</td>
<td>40.07</td>
<td>2.32</td>
<td>142.95</td>
<td>2.15</td>
<td>56.56</td>
<td>1.59</td>
<td>Increment, but recovering the normal values</td>
</tr>
<tr>
<td>Malondialdehyde MDA Nmol/mg.pr</td>
<td>ND</td>
<td>3.56</td>
<td>1.47</td>
<td>3.51</td>
<td>1.48</td>
<td>3.53</td>
<td>1.54</td>
<td>Without significant changes</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>NDT</td>
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<td>1.23</td>
<td>7.05</td>
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<tr>
<td></td>
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<td>T</td>
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<td>1.06</td>
<td>6.52</td>
<td>1.34</td>
<td>6.43</td>
<td>1.24</td>
<td>Increment, but recovering the normal values</td>
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### Table 1.
Blood biochemical tests performed in peripheral blood of BALB/c mice to evaluate hepatic, renal and serum oxidative status.

<table>
<thead>
<tr>
<th>Organic System</th>
<th>Test</th>
<th>T</th>
<th>Day 1</th>
<th>s Day 15</th>
<th>s Day 30</th>
<th>s Day 30</th>
<th>Observed Tendency</th>
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<td>1.67</td>
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</tr>
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<td>0.03</td>
<td>0.73</td>
<td>0.03</td>
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<tr>
<td>Serum Oxidative Status</td>
<td>Serum Albumin mg/dl</td>
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<td>0.01</td>
<td>2.65</td>
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<td>3.15</td>
<td>0.12</td>
<td>Increment, but recovering the normal values</td>
</tr>
<tr>
<td>Serum Glutathione Peroxidase * (GSH-Px) nmol/ml</td>
<td>ND</td>
<td>28.5</td>
<td>1.09</td>
<td>28.18</td>
<td>1.34</td>
<td>29.31</td>
<td>1.81</td>
</tr>
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<td></td>
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<td>1.43</td>
<td>29.87</td>
<td>1.28</td>
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<td>1.21</td>
<td>28.89</td>
<td>1.21</td>
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<td>27.7</td>
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<td>28.73</td>
<td>1.68</td>
<td>Increment, but recovering the normal values</td>
</tr>
<tr>
<td>Serum Total Antioxidant Capacity nmol/L</td>
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<td>T</td>
<td>0.97</td>
<td>0.04</td>
<td>1.42</td>
<td>0.06</td>
<td>1.08</td>
</tr>
</tbody>
</table>

ND: No supplemented diet no stressed; NDT: no supplemented diet stressed; T: Supplemented diet stressed. Biomarkers in hepatic, renal and serum oxidative status show some increments in individuals fed with a supplemented diet, but at the level of the entire organism no retard was observed in their development, weight gain or behavior; compared with individuals that were fed with normal diet (Figure 1) [10].
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Test</th>
<th>T</th>
<th>Day 1</th>
<th>s</th>
<th>Day 15</th>
<th>s</th>
<th>Day 30</th>
<th>s</th>
<th>Observed Tendency</th>
</tr>
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<tbody>
<tr>
<td>Leucocytes Cells x 10^3/μL</td>
<td>Lymphocytes Cells x 10^3/μL</td>
<td>ND</td>
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<td>12.55</td>
<td>0.73</td>
<td>12.29</td>
<td>1.05</td>
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<td></td>
<td></td>
<td>T</td>
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<td>0.71</td>
<td>16.31</td>
<td>0.87</td>
<td>18.55</td>
<td>0.32</td>
<td>Increment</td>
</tr>
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<td>Neutrophils Cells x 10^3/μL</td>
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<td>0.43</td>
<td>7.45</td>
<td>1.20</td>
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<td>0.61</td>
<td>8.86</td>
<td>1.07</td>
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<td>0.74</td>
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<td>0.15</td>
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</tr>
<tr>
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<td>0.69</td>
<td>0.13</td>
<td>0.81</td>
<td>0.12</td>
<td>0.91</td>
<td>0.12</td>
<td>Increment</td>
</tr>
<tr>
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<td>T</td>
<td>0.79</td>
<td>0.16</td>
<td>0.89</td>
<td>0.16</td>
<td>0.91</td>
<td>0.14</td>
<td>Increment</td>
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<tr>
<td>Phagocytosis assay (%)</td>
<td>Phagocytosis in Monocytes (%)</td>
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<td>36.09</td>
<td>1.52</td>
<td>38.55</td>
<td>1.29</td>
<td>37.49</td>
<td>1.32</td>
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<td>1.71</td>
<td>57.21</td>
<td>1.53</td>
<td>70.63</td>
<td>1.51</td>
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<td>67.34</td>
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<td>78.36</td>
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<tr>
<td>Cytokines pg/L</td>
<td>Interferon gamma (IFN-γ) pg/L</td>
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<td>187.95</td>
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<td>Interlukin 2 (IL2) pg/L</td>
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<td>200.52</td>
<td>0.13</td>
<td>231.49</td>
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<tr>
<td></td>
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<td>221.31</td>
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<td>Interlukin 12 (IL2) pg/L</td>
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<td>1.05</td>
<td>327.31</td>
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<td>339.67</td>
<td>1.25</td>
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</tr>
<tr>
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<tr>
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<td>315.86</td>
<td>1.32</td>
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<td>1.47</td>
<td>532.48</td>
<td>1.37</td>
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<td>296.45</td>
<td>1.31</td>
<td>326.68</td>
<td>1.09</td>
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7. Concluding remarks

The role of oxidative stress on biological systems is important but controversial, it is important in organic functions particularly in signal transduction. Oxygen participates in an important way in the energy cycle of living beings. It is essential for cellular respiration in aerobic organisms but many of living process generate reactive oxygen species and reactive oxygen and nitrogen species (ROS/RONS) that in excess can drive the organism to oxidative stress, a consequence of the disturbance of the balance between pro-oxidants and antioxidants, with a shift in favor of the ROS/RONS and significantly damaging the physiological functions of the organism. The aerobic organisms should keep the oxidant/antioxidant balance using different antioxidant mechanisms. The cellular antioxidant defenses include the use of free amino acids, vitamins, proteins, polysaccharides, sugars, and certain ions as antioxidant compounds as well as enzymes such as superoxide dismutase and (SOD), glutathione peroxidase, and catalases.

A group of stressors contributes to a greater generation of ROS/RONS that can be caused by the intracellular and organic metabolic processes themselves, and factors of infection by pathogens and environmental conditions. Oxidative stress is a permanent research target due to the importance it generates for human health, and many investigations materialize in different study models from bacteria, simple eukaryotes such as yeast, cell lines, first culture cells, animals, and plants. Using accepted models that exist for different diseases, we can see how the oxidative condition of the organism influences each of these diseases. Oxidative stress also affects the condition of all living organisms and significantly affects all living organisms, including those that are of economic interest, particularly under the conditions imposed by intensive animal production, so the stress is generated due to overpopulation in farms and management in the different steps of the production cycle. The incidence of oxidative stress in animals, both experimental models and farm production animals, was analyzed in some species of interest. To prevent and improve the damage caused by the digestive process, the use of active antioxidants in food is recommended, as well as improvements in living conditions.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Test</th>
<th>T</th>
<th>Day 1</th>
<th>s</th>
<th>Day 15</th>
<th>s</th>
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<td>Increment</td>
<td></td>
</tr>
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<td></td>
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<td>1.28</td>
<td>351.2</td>
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<td>425.82</td>
<td>1.46</td>
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<td>321.43</td>
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<td>389.65</td>
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<td>423.67</td>
<td>1.25</td>
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<td>1.49</td>
<td>537.69</td>
<td>1.51</td>
<td>754.31</td>
<td>0.65</td>
<td>Increment</td>
<td></td>
</tr>
</tbody>
</table>

ND: No supplemented diet no stressed; NDT: no supplemented diet stressed; T: Supplemented diet stressed. The treated individuals are in a state of alert that does not represent damage to the vital functions but shows that increments in immune functions are observed in both individuals fed with supplemented diet and stressed as well as in individuals fed with no supplemented diet and stressed the values in immune functions were lower than in those fed with supplemented diet (Figure 1) [10].

Table 2. Analysis of immunological status leucocyte blood cells counts and cytokine production in BALB/c mice.
Importance of Oxidative Stress and Antioxidant System in Health and Disease

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