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## Preventing free radical damage: The significance of including antioxidants in diet to strengthen immunity

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### Abstract

Free radicals (FRs), also known as reactive oxygen species (ROS), are usually established in the body when adequate oxygen depletion occurs. Oxidative stress and the establishment of FRs in the body are mainly caused by high metabolic activity, the need for rapid growth, inadequate flock management, exposure to viral and bacterial microorganisms, and adverse environmental conditions. Furthermore, FRs can also be produced during the activity of phagocytes when they depend on the action of ROS to kill the engulfed pathogen. FRs have very adverse effects on all cells, particularly the cells of the immune system. They are extremely erratic and reactive molecules that directly harm DNA, cellular proteins, lipids, and carbohydrates within cells. Antioxidants are substances that can eliminate and neutralize FRs within the body and free the body from the oxidative stress that occurs due to the accumulation of FRs. Many vitamins and minerals support the activity and effect of the immune system in fighting against microbes and cancer, which mostly depend on their antioxidant elements to diminish the negative impact of FRs in the body. Examples are vitamin C, vitamin E, superoxide dismutase, selenium, glycine, cofactors of glutathione peroxidase, manganese, essential oils, and phenolic compounds.

**Keywords:** Free radicals, Antioxidants, Oxidative stress, Nutrition, Immune system.

### Introduction

The poultry industry remains one of the most important sources of income for many countries, especially developing ones. Chickens can supply a high-quality and reasonably priced source of animal proteins for human consumption, fulfilling daily protein requirements in compliance with dietary guidelines. Many factors, particularly environmental elements like cold, heat, and overcrowding, can negatively impact the poultry industry. Moreover, other factors, including physiological (internal), technological, and dietary stressors, can influence the production rate, performance, and protein content quality of poultry meat (Akinyemi and Adewole, 2021). Environmental stress continues to be a key danger to the immune system's ability to respond to different diseases, even though several factors influence the immune system's strength and performance in the body. Among the mechanisms impaired by environmental stress are the cytokine-receptor interaction pathway, toll-like receptor signaling pathways, and the production of excessive

reactive oxygen species (ROS) in response to multiple impaired immune pathways (Hofmann *et al.*, 2020). Oxidative stress, one of the main causes of immune dysfunction and a common stressor in poultry, arises from an imbalance between the rate of ROS production and accumulation in cells and interstitial tissues and its detoxification and clearance by the body's available antioxidants.

Fortunately, scientific advancements, particularly in studying free radicals (FRs), ROS, and antioxidants, have improved the immune system and defense mechanisms. These enhancements have been achieved by manipulating the nutritional content of poultry diets to strike a balance (Lobo *et al.*, 2010). This review focuses on the mechanisms of oxidative stress in poultry and highlights the importance of including antioxidants in poultry feed to reduce the detrimental effects of ROS on the different body systems, especially the immune system.

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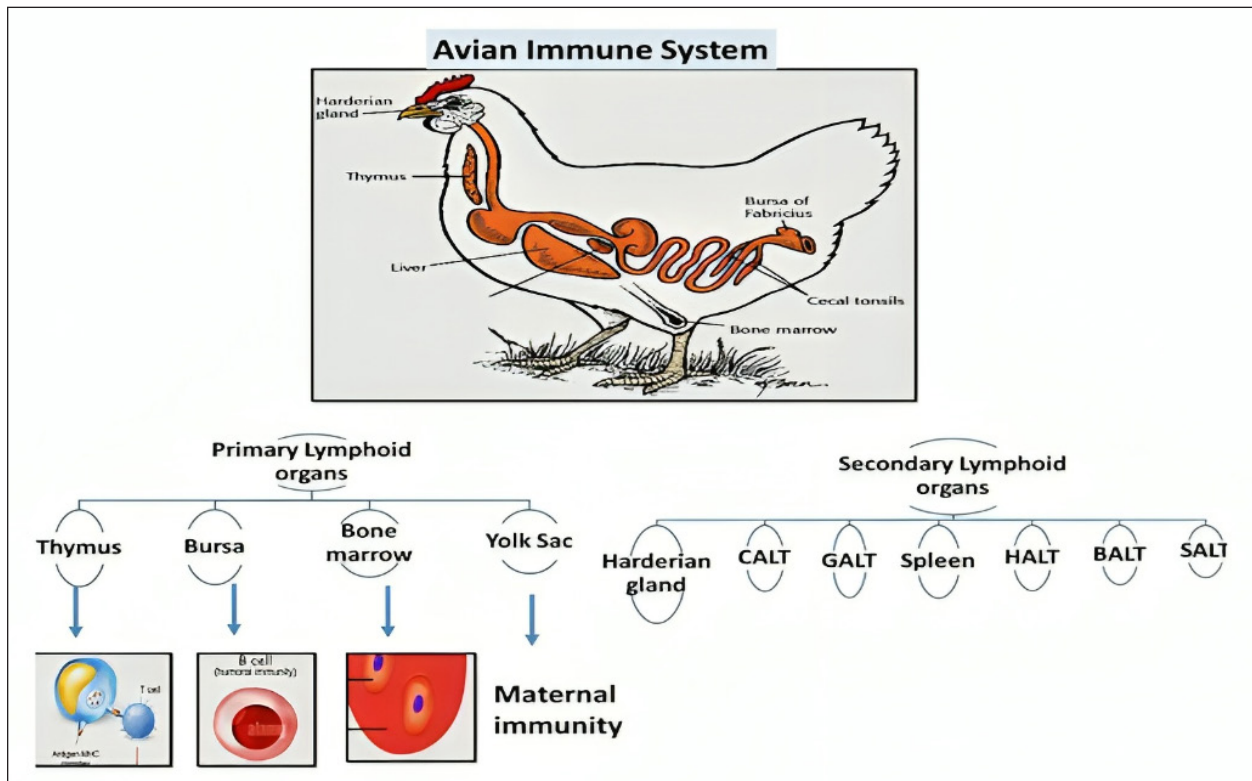
### Avian immune system

The immune system comprises a complicated network of chemicals, cells, and organs. Together, they provide and mount an immune response against many pathogens they encounter. Apart from defense against microbes, it is the responsibility of the immune system to eliminate any developing abnormal cells categorized under various tumor-forming cells. Moreover, homeostasis and scavenging dead cells and debris are among the functions of the immune system (Bonilla and Oettgen, 2010).

The avian physiology and immune system resemble those of other mammals, which all developed from the same reptilian ancestors and have many commonalities (Surai, 2002). The immune system of avian species is primarily composed of primary and secondary lymphoid organs together with lymphatic tissues (Fig. 1). The pivotal role of the immune system in avian species and all other mammals is to maintain homeostasis, in which harmful substances can be distinguished from non-harmful substances and elicit an appropriate response in the event of the entry of a toxic substance. The innate and adaptive immune systems work together to eradicate disease-causing agents, such as pathogenic bacteria and viruses (Bhuiyan et al., 2021).

### Innate immunity

In the avian world, the innate immune system is similar to other mammals, providing the first line of defense. These range from external defenses, such as physical barriers like feathers, skin, and other parts of the bird's body, to chemical barriers, such as nasal discharges, antimicrobial peptides in sweat, and acids in the GIT. Other components of the innate immune system that contribute to the bird's overall protection against invaders include fluctuations in body temperature and acidity levels, particularly at low pH (Luo et al., 2010). The internal defenses of the innate immune system play the most important role in initiating immune responses. While the innate immune system does not respond as specifically as the adaptive immune system, which cannot differentiate between the specificities of pathogens, it does possess a range of receptors designed to recognize common pathogenic structures (Table 1) (Gombart et al., 2020). Another important role of the innate immune system is stimulating and shaping the adaptive immune system. The adaptive immune system cannot target the pathogen without the support of the innate immune system. The functions of the innate immune system include secreting various cytokines and chemokines and presenting antigens to antigen-specific lymphocytes of the adaptive immune system (Ohteki and Koyasu, 2001).



**Fig. 1.** Primary and Secondary lymphoid organs. Enmeshed in the immune complex, mature B and T cells are transferred from primary or lymphoid tissue for the development stage, termed immune movement or immune peripheralization.

**Table 1.** The nonspecific host defense mechanisms.

Barriers	The Mechanism of Action
<b>Anatomical</b>	
Skin	<ul style="list-style-type: none"><li>• The mechanical barrier prevents microorganisms from entering the skin.</li><li>• Microorganisms cannot grow because of the acidic environment (pH 3–5).</li></ul>
Mucous membrane	<ul style="list-style-type: none"><li>• Microorganisms fight for attachment sites with normal flora.</li><li>• Foreign microorganisms are trapped by mucus.</li><li>• Cilia remove microorganisms from the body.</li></ul>
<b>Physiological</b>	
Temperature	<ul style="list-style-type: none"><li>• The body temperature/fever response reduces the production of several harmful bacteria.</li></ul>
Low pH	<ul style="list-style-type: none"><li>• The acidic pH of the stomach destroys most undigested microorganisms.</li></ul>
Chemical mediators	<ul style="list-style-type: none"><li>• Complement lyses microorganisms or aids in phagocytosis.</li><li>• The bacterial cell wall is destroyed by lysozyme.</li><li>• Antiviral defenses of interferon in uninfected cells.</li></ul>
<b>Phagocytic / Endocytic Barriers</b>	
	<ul style="list-style-type: none"><li>• Endocytosis is the process by which various cells internalize and break down foreign macromolecules.</li><li>• Specialized cells (neutrophils, blood monocytes, and tissue macrophages) internalize (phagocytose). Terminate and digest whole organisms.</li></ul>
<b>Inflammatory Barrier</b>	
	<ul style="list-style-type: none"><li>• Damage to tissue and infection generates leaks in the vascular fluid that contain antibacterial serum protein, leading to an influx of phagocytic cells into the affected area.</li></ul>

### **Adaptive immunity**

The adaptive immune system is another part of the bird's immune system, which mainly responds to pathogen invasions and the development of tumor cells via a range of cells called antigen-specific lymphocytes. The response is initiated by the cells of the innate immune system, and lymphocytes respond through adaptation and clonal selection. The immune system's evolution is important in that it can compensate for any missing parts and cells if they exist. When cells of the innate immune system are defective or missing due to any

genetic or acquired defect, the cells of the adaptive immune system can compensate for the mechanisms that are supposed to be provided by the defective cell (Marshall *et al.*, 2018; Martin, 2001).

The adaptive immune system relies on a specific sequence of an antigen's amino acids, available either on the pathogen's surface or internally. T lymphocytes are born in the bone marrow and mature in the thymus in most mammals, including birds. Upon their maturation and subsequent release into circulation, these cells are responsible for performing immune surveillance, a process in which the immune cells look for the entry of any pathogens. Once there is an invasion by a pathogen, there will be clonal selection and subsequent expansion of the antigen-specific T lymphocytes, which usually occurs in a nearby secondary lymphoid organ. This antigen-specific effector T cell wave is responsible for dealing specifically with the pathogens and their elimination from the body. These so-called effector cells use several mechanisms to kill the pathogens, such as the secretion of cytotoxic cytokines that perforate infected cells and later lyse the microbes.

One of the most important advantages of the adaptive immune response over the innate immune system is the development of immunological memory. The memory cells, distributed throughout the bird's body and specifically found in the secondary lymphoid tissues, are responsible for re-initiating the immune response in case of an invasion from the same pathogen for the second time (Bonilla and Oettgen, 2010).

The development of immunological memory by adaptive immune system cells is the basis for vaccination and immunization. Vaccination remains one of the greatest achievements in medicine for saving both humans and animals, and it is the safest method for their protection and overall survival on the planet. The main goal of vaccination is to generate a large pool of antigen-specific antibodies that can neutralize a specific pathogen in the future. In addition, the vaccination process will also lead to the creation of a huge repertoire of antigen-specific memory T and B cells. B cells are the generators of antibodies. They are born in the bone marrow and mature in the bursa of Fabricius, which is only available in avian species. Upon maturation, B cells circulate via blood and lymphatics, and once a vaccine is introduced or the body is invaded with a pathogen, mature B cells, also called naïve B cells, will internalize the antigen and differentiate into plasma cells. Plasma cell maturation also takes time, going through plasmablasts and eventually differentiating into mature plasma cells. Hundreds to thousands of antibodies can be secreted by a fully mature plasma cell per second to generate a huge repertoire (Marshall *et al.*, 2018) (Fig. 2).

### **Poultry production stressors**

A wide range of external factors, such as environmental factors, can cause stress for the avian species and others. In addition, internal factors can also cause stress. The stress level can vary based on the type of stressor the

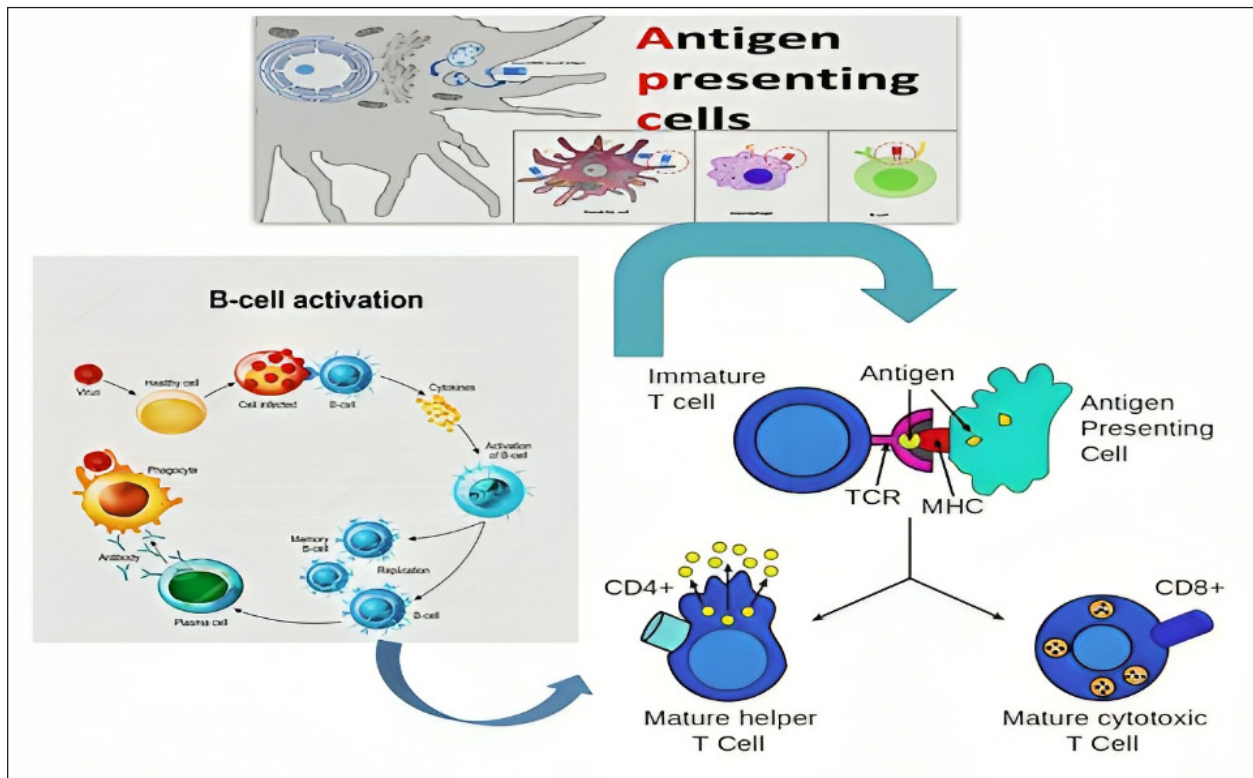


Fig. 2. Adaptive immune system response, showing the flow from antigen to APC, MHC II, CD4<sup>+</sup> T cells, B cells, antibodies, macrophages, and cytotoxic CD8<sup>+</sup> T cells.

bird encounters. Homeostasis, maintaining a steady balance of internal mechanisms and cell numbers, can be restored in cases of moderate stress through the function of the hypothalamic-pituitary-adrenal gland. Neurological involvement and the immune system can also contribute to the restoration of the effects caused by moderate stress. However, intense stress is difficult to restore and can result in a complex cascade of metabolic modifications, such as metabolic shifts and increased energy mobilization. The long-term effects of intense stress can lead to other complications and primarily result in reduced poultry and livestock production, the business's primary goal of production management (Bureau *et al.*, 2009).

Although nutritional stress that leads to various metabolic diseases such as muscular dystrophy, encephalomalacia, and exudative diathesis is rare in commercial poultry production, mainly due to providing well-controlled and balanced nutrition, livestock management remains an important factor to be investigated and monitored (Surai, 2002).

Due to the genetic selection of specific strains of broilers, layers, and turkeys that can provide a rapid growth rate, improved feed conversion, domestication, and a high egg production rate, these birds can be exposed to oxidative stress, which eventually leads to a marked reduction in production and reproduction capacity.

A sharp or moderate reduction curve in production ability in these livestock management practices is an important factor contributing to substantial economic losses (Soleimani *et al.*, 2011).

Environmental, technological, internal, and nutritional stresses are considered among the four most important types of stresses that can affect poultry production. These various stress types can impact the bird at a molecular/cellular level and then have effects at a physiological level, ultimately influencing the production capability and performance of commercial livestock, especially in terms of reproductive capacity (Surai *et al.*, 2018). According to several research studies (P. F. Surai and Fisinin, 2016; Peter F. Surai *et al.*, 2018), oxidative stress is the main type of commercial stress influencing poultry production during livestock management (Table 2).

#### Free radicals

FRs are defined as electrons that are not paired. Many of these radicals are highly reactive and unstable; they can serve as reducing or oxidizing agents by taking an electron from another molecule or giving it to them (Cheeseman and Slater, 1993; Lobo *et al.*, 2010).

ROS are radicals centered around oxygen, forming a subset of reactive nitrogen species (RNS). They all emerge due to regular cellular metabolism (Phaniendra *et al.*, 2015). ROS and RNS have been categorized as

**Table 2.** Main stressors in poultry production.

Stressors	
Technological stressors	Placement of chick
	The stocking density is increasing.
	Grading group formation, weighing transfer to breeder houses, or catch.
	Egg storage for too long, egg transportation, insufficient egg storage conditions, and inappropriate incubation schedules
Environmental stressors	Insufficient temperature
	Increased dust and insufficient ventilation
	Insufficient lighting
Nutritional stressors	Mycotoxins
	Oxidized fat
	Toxic metals (lead, cadmium, mercury, etc.)
	Imbalance of minerals (Se, Zn, Mn, Cu, etc.) and other nutrients
	Water of poor quality
	Use of coccidiostats and other drugs through feed or water
Internal stressors	Vaccinations
	Microbial or Viral Challenges
	Gut diseases bacteriosis
	Pipping and hatching

advantageous and detrimental due to their positive and adverse impacts on biological systems (Di Meo *et al.*, 2016). Positive outcomes are observed when ROS/RNS levels are within low to moderate concentrations, yet detrimental effects arise at higher levels, surpassing the overall antioxidant capacity required to regulate them (Agarwal *et al.*, 2005).

FRs are generated through three distinct mechanisms: (a) via the hemolytic rupture of a normal molecule's covalent bond, (b) by extracting an electron from an ordinary molecule, and (c) by introducing an electron into a regular molecule (Chen *et al.*, 2021). FRs can target various molecules within the body. The detrimental impacts of ROS/RNS leading to biological harm are termed oxidative stress and nitrosative stress, respectively (Lobo *et al.*, 2010; Phaniendra *et al.*, 2015). FRs can cause cellular damage and disrupt homeostasis by attacking essential macromolecules. Excessive ROS can impair the proper functioning of cellular lipids, proteins, or DNA. Redox homeostasis is maintained through "redox regulation," establishing a balanced equilibrium between FRs' beneficial and detrimental effects within a living organism (Birben *et al.*, 2012).

### **Oxidizing agents in poultry**

Although genetics play an important role in poultry production, the environment and nutrition should be optimal for maximum growth and health statuses (Kachungwa Lugata *et al.*, 2022). Stressors are agents capable of producing stress at any time (Rostagno, 2020). Stress initiates the defense system and increases energy and nutritional demands, especially amino acids, trace elements, and vitamins. Oxidative stress results from ROS overproduction or insufficient antioxidant defense systems (Halliwell, B. and Gutteridge, J.M. (2015). Various environmental, nutritional, and biological stressors can impact poultry production, reducing reproductive and growth performances and compromising the general health of the birds (Surai *et al.*, 2019).

Many metals, such as calcium, magnesium, potassium, sodium, and iron, are essential animal micronutrients. However, they may be toxic at high concentrations by increasing ROS formation (Koivula and Eeva, 2010). Nonoptimal temperature, lighting (Huth and Archer, 2015), and ventilation impact the overall growth rate and are considered environmental stressors for poultry. Moreover, nutritional stressors of poultry include mycotoxins, oxidized fats, low water quality, and toxic metals such as lead, cadmium, and mercury (Guo *et al.*, 2019). Also, microbial and viral infections and disturbances in gut microbiomes are some of the internal stressors that negatively affect the growth performance of poultry.

### **Endogenous sources of ROS**

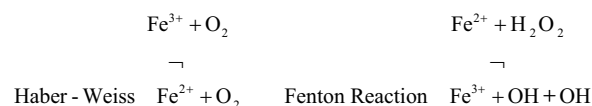
ROS are generated through the ordinary metabolic processes of cells, originating from molecular oxygen. ROS can be categorized into two types: FRs and nonradicals. FRs are molecules possessing one or more unpaired electrons that confer reactivity upon the molecule. Nonradical forms emerge when two FRs exchange their unpaired electrons. The superoxide anion ( $O_2^-$ ), hydroxyl radical ( $\cdot HO$ ), and hydrogen peroxide ( $H_2O_2$ ) are the three most physiologically significant ROS (Table 3) (Phaniendra *et al.*, 2015). The superoxide anion is formed by adding a single electron to molecular oxygen (Miller *et al.*, 1990). The fundamental components include nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase, xanthine oxidase, and the mitochondrial electron transport system. The mitochondria, responsible for ATP synthesis, act as the primary source of the superoxide anion. Typically, electrons move through the mitochondrial electron transport chain to convert oxygen into water. However, a small fraction, roughly 1%–3%, escapes, generating superoxide. NAD(P)H oxidase is found in polymorphonuclear leukocytes, monocytes, and macrophages. These cells create a surge of superoxide during phagocytosis, contributing to bactericidal action. Superoxide dismutases (SODs) are pivotal in converting superoxide to hydrogen

**Table 3.** Major endogenous sources of ROS.

Oxidant	Formula	Reaction Equation
Superoxide anion	$O_2^-$	$NADPG + 2O_2 \rightarrow NADP^+ + 2O_2^- + H^+$ $2O_2^- + H \rightarrow O_2 + H_2O_2$
Hydrogen peroxide	$H_2O_2$	$Hypoxanthine + H_2O + O_2 \rightarrow xanthine + H_2O_2$ $Xanthene + H_2O + O_2 \rightarrow uric\ acid + H_2O_2$
Hydroxyl radical	$\bullet OH$	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$
Hypochlorous acid	$HOCl$	$H_2O_2 + Cl^- \rightarrow HOCl + H_2O$
Peroxyl radical	$ROO\bullet$	$R\bullet + O_2 \rightarrow ROO\bullet$
Hydroperoxyl radical	$HOO\bullet$	$O_2^- + H_2O \rightarrow HOO\bullet + OH^-$

peroxide. The plasma membrane facilitates the easy diffusion of hydrogen peroxide.

Moreover, xanthine oxidase, amino acid oxidase, and NAD(P)H oxidase contribute to hydrogen peroxide production (Dupuy *et al.*, 1991). Peroxisomes utilize molecular oxygen for metabolic processes. In the presence of transition metals such as  $Fe^{2+}$  or  $Cu^{2+}$ ,  $H_2O_2$  can participate in a sequence of reactions known as the Haber-Weiss and Fenton reactions, ultimately leading to the creation of  $OH^-$  (Fenton, 1894).



$O_2^-$  can also produce  $OH^-$  when it interacts with  $H_2O_2$  (Haber and Weiss, 1934) The hydroxyl radical, the most reactive type of ROS, can damage proteins, carbohydrates, lipids, and DNA. Furthermore, by extracting an electron from polyunsaturated fatty acids, it can initiate lipid peroxidation (Phaniendra *et al.*, 2015). Granulocytic enzymes, specifically eosinophil peroxidase and myeloperoxidase (MPO), further heighten the reactivity of  $H_2O_2$ . When activated neutrophils are present, MPO absorbs  $H_2O_2$ . In the presence of chloride ions,  $H_2O_2$  transforms into hypochlorous acid (HOCl). HOCl possesses potent oxidative properties and plays a pivotal role in eradicating pathogens in the airways (Klebanoff, 2005). However, HOCl can also interact with DNA, leading to DNA-protein interactions, pyrimidine oxidation products, and adding chloride to DNA bases (Kulcharyk and Heinecke, 2001). During processes such as halogenation, nitration, and the formation of protein cross-links through tyrosyl radicals, eosinophil peroxidase, and MPO contribute to oxidative stress. Furthermore, these enzymes induce oxidative stress by modifying proteins through halogenation, nitration, and forming protein cross-links via tyrosyl radicals (Davies, 2011).

Peroxyl radicals ( $ROO\bullet$ ) constitute another class of oxygen-derived FRs. The hydroperoxyl radical ( $HOO\bullet$ ) is particularly significant, as it participates in

fatty acid peroxidation. By extracting a hydrogen atom from a side-chain methylene carbon, FRs initiate a chain reaction in the process of lipid peroxidation. The peroxyl radical arises when the lipid radical encounters oxygen, converting polyunsaturated fatty acids into lipid hydroperoxides and initiating a chain reaction. These lipid hydroperoxides are highly unstable and rapidly degrade into aldehydes (e.g., 4-hydroxy-2,3-nonenal) and malondialdehydes (MDA). Another category of products stemming from lipid peroxidation are isoprostanes, formed when arachidonic acid undergoes peroxidation. Research has shown that elevated levels of isoprostanes in both asthmatic plasma and breath condensate the integrity of cell membranes is disrupted by lipid peroxidation, which results in membrane reorganization (Wood *et al.*, 2000).

Standardized tests can easily measure hydrogen peroxide, superoxide radical, oxidized glutathione (GSSG), MDAs, isoprostanes, carbonyls, and nitrotyrosine, acting as biomarkers in plasma, blood, or bronchoalveolar lavage samples (Birben *et al.*, 2012). Antioxidants can directly counteract ROS, indirectly enhance the body's defenses against oxidation, or inhibit ROS production. These compounds include monohydroxy/polyhydroxy phenols (Mann *et al.*, 2018).

Guttering and Halliwell have classified antioxidants into three distinct groups: primary antioxidants, crucial for preventing the production of oxidants; secondary antioxidants, recognized for their ability to scavenge ROS; and tertiary antioxidants, responsible for mending damaged molecules through dietary or sequential antioxidants (Singh *et al.*, 2003). Moreover, they can be further categorized into various groups based on their properties, origins, and mechanisms of action (Fig. 3). Antioxidants have two types classified as enzymatic or nonenzymatic antioxidants. RH, ROH, ROOH, and a relatively unreactive phenoxyl radical are formed when antioxidants (AH) react with R, RO, and ROO (RH). Antioxidants provide electrons for FRs, allowing them to be neutralized (Table 4). When cells are exposed to oxidative stress, a defense system is activated, promoting the production and regulation

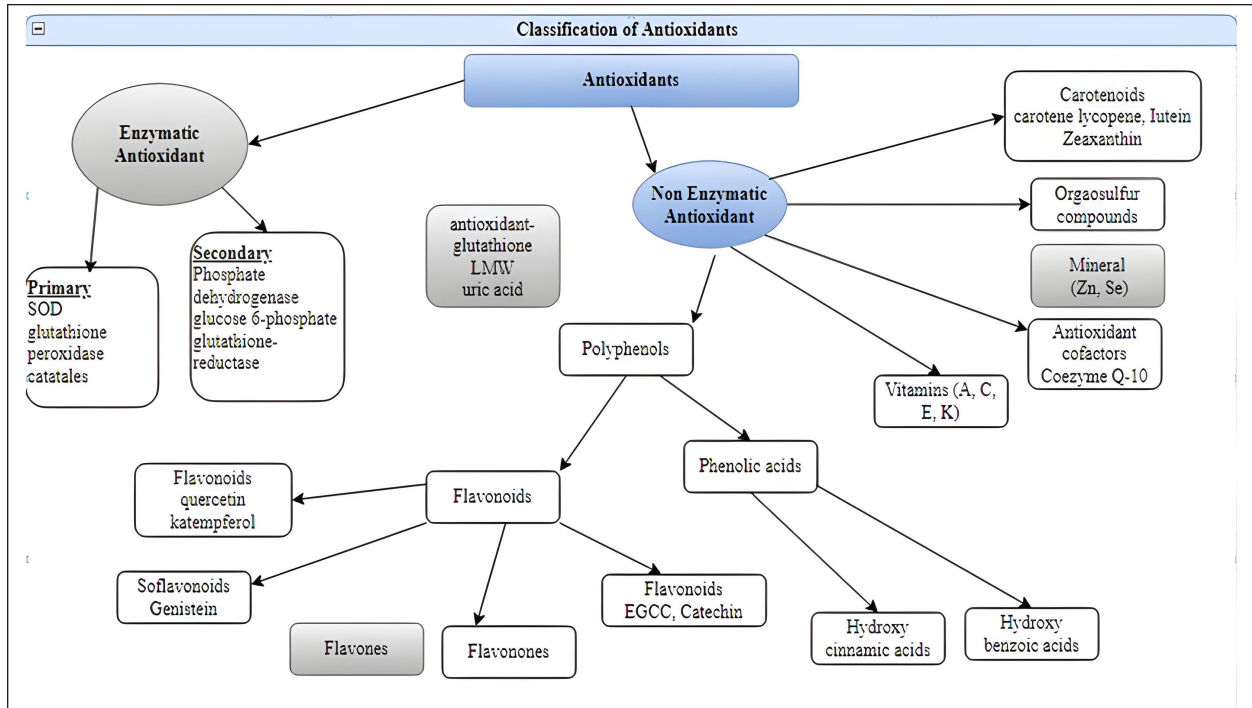


Fig. 3. Enzymatic and nonenzymatic classification of antioxidants.

Table 4. Antioxidant defensive agents.

ROS scavengers	ROS Protective Enzymes	Sequestration of Transition Metal Ions which form ROS
Glutathione	Superoxide	Transferrin
Uric acid	Catalase	Ferritin
Ascorbic acid	Glutathione peroxidase	Metallothionein
Albumin	Glutathione reductase	Ceruloplasmin

of numerous antioxidant enzymes to shield cells from damage caused by FRs (Pham-Huy Lien Ai *et al.*, 2008).

**Antioxidants**

The body’s antioxidant systems can tolerate mild oxidative stress in poultry. However, oxidative stress can be reduced by reducing exposure to oxidizing agents, increasing endogenous and exogenous antioxidant levels, and stabilizing mitochondrial energy production efficiency.

**Enzymatic antioxidants**

The direct enzymatic antioxidant system and the indirect enzymatic antioxidant system serve as crucial defenses against ROS. Enzymatic antioxidants include catalase, SOD, glutathione peroxidase, glutathione reductase, and other relevant enzymes (Ighodaro and Akinloye, 2018).

There are two categories of enzyme antioxidants: primary and secondary defenses. Glutathione peroxidase plays a role in reducing peroxides by

contributing two electrons, forming selenols, and also neutralizing peroxides. On the other hand, catalase converts hydrogen peroxide into water and molecular oxygen, utilizing them as substrates. Additionally, SOD converts superoxide anions into hydrogen peroxide, which subsequently acts as a substrate for catalase (Rahman, 2007).

**Nonenzymatic antioxidants**

Different classes of nonenzymatic antioxidants are present, encompassing vitamins (A, C, E, and K), enzyme cofactors (Q10), minerals (Zn and Se), organosulfur compounds (like allium and allium sulfur), nitrogen compounds (such as uric acid), peptides (like glutathione), and polyphenols (which include flavonoids and phenolic acid)(Mann *et al.*, 2018; Tan *et al.*, 2018).

**Endogenous antioxidants**

Endogenous antioxidants can be categorized into primary antioxidants, which deactivate ROS by converting them into intermediate forms. Primary

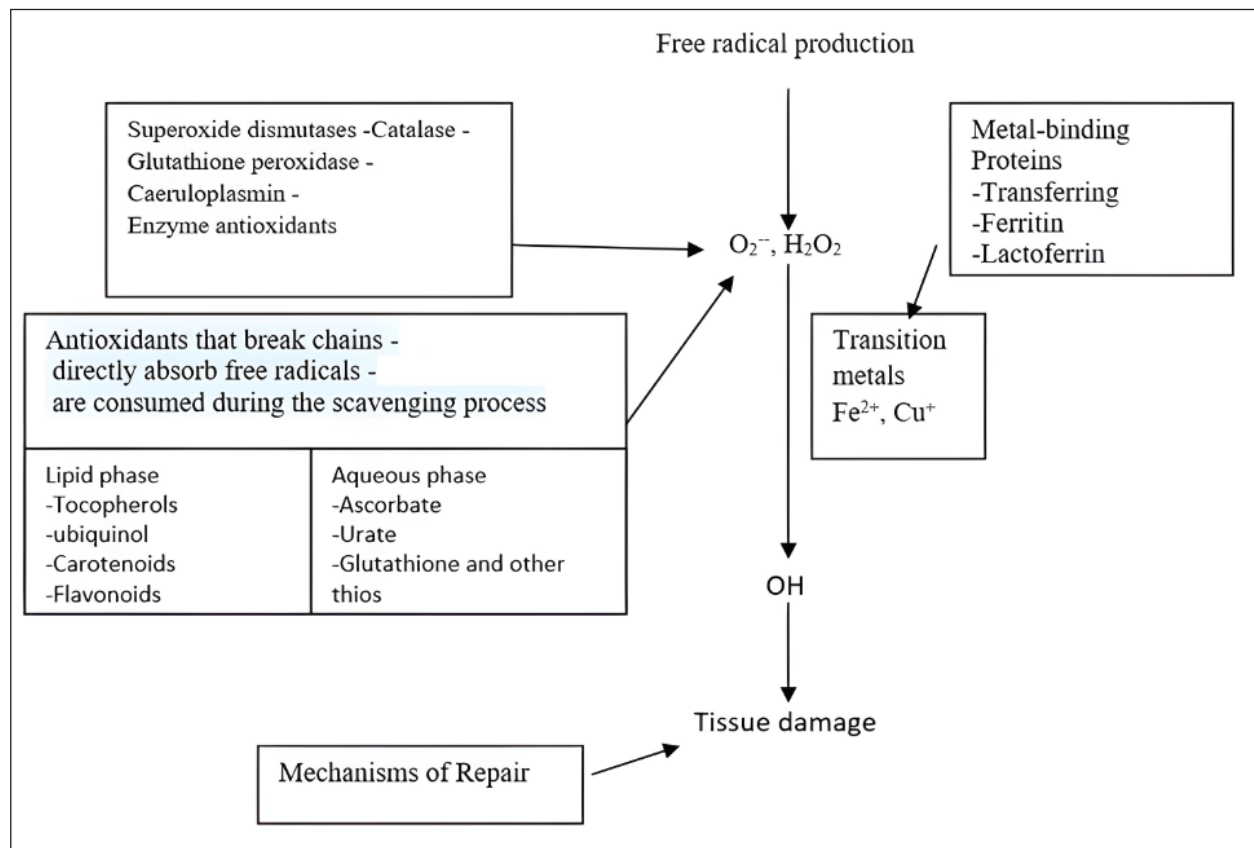
antioxidant enzymes include SOD, catalase, and glutathione peroxidase. These antioxidants are found in water-soluble forms like ascorbate, glutathione, and uric acid, as well as lipid-soluble forms such as tocopherols, ubiquinols, and carotenoids (Shu, 1998). Secondary antioxidant enzymes directly eliminate ROS. They modulate their functions by reducing peroxide levels and providing a constant supply of NADPH (nicotinamide adenine dinucleotide phosphate) and glutathione to primary antioxidant enzymes. Notable secondary antioxidants comprise glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione-s-transferase, and ubiquinone. Moreover, the activity of antioxidant enzymes is enhanced by the presence of iron, copper, zinc, manganese, and selenium (Gale, 2001; Vertuani *et al.*, 2004).

**Exogenous antioxidants**

Exogenous antioxidants are present in various herbs, spices, foods, vitamins, plants, and other sources. These agents with antioxidant properties have gained significant importance in both the clinical and research realms, as they can be utilized to treat various pathological conditions (Lourenço *et al.*, 2019). A multitude of polyphenolic compounds, including flavonoids, isoflavones, flavones, isocatechins, anthocyanins, coumarins, lignans, catechins, and epicatechins, as well as phenolic acids like hydrocinnamic acid, hydrobenzoic acid, gallic acid, ellagic acid, and others, have emerged as crucial phytochemical antioxidants. Clinical and preclinical trials are currently in progress to explore the bioactive characteristics of these compounds (Farag *et al.*, 2020).

**Table 5.** Antioxidant enzymes that scavenge ROS.

Antioxidants produced by enzymes	Catalyzed Reaction
Superoxide dismutase (SOD)	$O_2^- + O_2 + 2H \rightarrow 2H_2O_2 + O_2$
Catalase (CAT)	$H_2O_2 \rightarrow H_2O + 1/2O_2$
Glutathione peroxidase (GPX)	$H_2O_2 \rightarrow H_2O + GSSG$
Glutathione reductase (GR)	$GSSG + NAD(P)H \rightarrow 2GSH + NAD(P)^+$



**Fig. 4.** A schematic diagram of antioxidant defense mechanisms.



Plant-derived remedies hold medicinal value and are pivotal in research due to their rich content of phytochemicals such as terpenoids, glycosides, alkaloids, polyphenolics, and steroids (Fuhrman *et al.*, 2004; Wu *et al.*, 1999). Antioxidant enzymes are synthesized from dietary minerals, proteins, and amino acids and play an important part in the body's defense mechanism. GSH, creatine, and uric acid are notable direct scavengers of reactive metabolites (Rassaf *et al.*, 2002). Natural antioxidants like polyphenols, flavonoids, and tannins assist in alleviating lipid peroxidation by donating electrons to intermediate radicals generated during periods of oxidative stress or tissue damage (Sharifi-Rad *et al.*, 2020).

#### **Antioxidant defense mechanism**

The endogenous antioxidant defense systems effectively eliminate FRs generated through various activities. These include scavenging FRs, breaking down peroxides, and interacting with pro-oxidant metal ions. (Table 5; Fig. 4).

Antioxidants are classified into three categories:

Primary antioxidants obstruct the generation of oxidants by averting the formation of FRs. These include glutathione peroxidase, catalase, selenoprotein, transferrin, ferritin, lactoferrin, and carotenoids (Fig. 3). Secondary antioxidants serve as scavengers for ROS. They disrupt chain reactions and reduce chain initiation, functioning as radical scavengers (Fig. 3).

Tertiary antioxidants participate in the restoration of oxidized molecules. This process is facilitated by specific proteolytic enzymes, DNA enzymes, and similar agents, with the support of dietary or subsequent antioxidants (Nimse and Pal, 2015).

#### **Mechanism of action of antioxidants**

Antioxidants utilize a range of mechanisms to counteract FRs. These encompass catalytic systems that either neutralize or divert ROS (Fig. 5). Another aspect of antioxidant function involves AH reacting with R, RO, and ROO, leading to the creation of RH, ROH, ROOH, and a relatively unreactive phenoxyl radical (RH, ROH, ROOH) (RH). Through the donation of electrons, antioxidants possess the ability to neutralize FRs (Kurutas, 2016) (Fig. 6).

#### **Mode of action of antioxidants**

Antioxidants that are produced chemically through synthesis are not found in nature. Synthetic antioxidants are categorized into many groups based on how they function. Primary synthetic antioxidants operate by hindering the formation of FRs during the oxidation process. Conversely, secondary antioxidants function by breaking down hydroperoxides, which result from lipid oxidation, into forms that are steadier and more intricate (Kurutas, 2016). It is important to mention that while synthetic antioxidants can have positive effects, they also have the potential to exert detrimental biological effects on molecules.

#### **Phytochemicals**

Flavones, flavonoids, isoflavones, gallic acid, anthocyanins, coumarins, catechins, lignans, isocatechins, and esculin are some of the phytochemicals found in medicinal plants (Panche *et al.*, 2016). These plants have antioxidant properties, as phytochemicals are abundant in various sources, including grapes, berries, tea, herbs, and nutmeg. Many medicinal plants also contain valuable compounds like gallic acids. Furthermore, plants such as *Terminalia chebula*, *Terminalia bellerica*, *Terminalia muelleri*, *Phyllanthus*

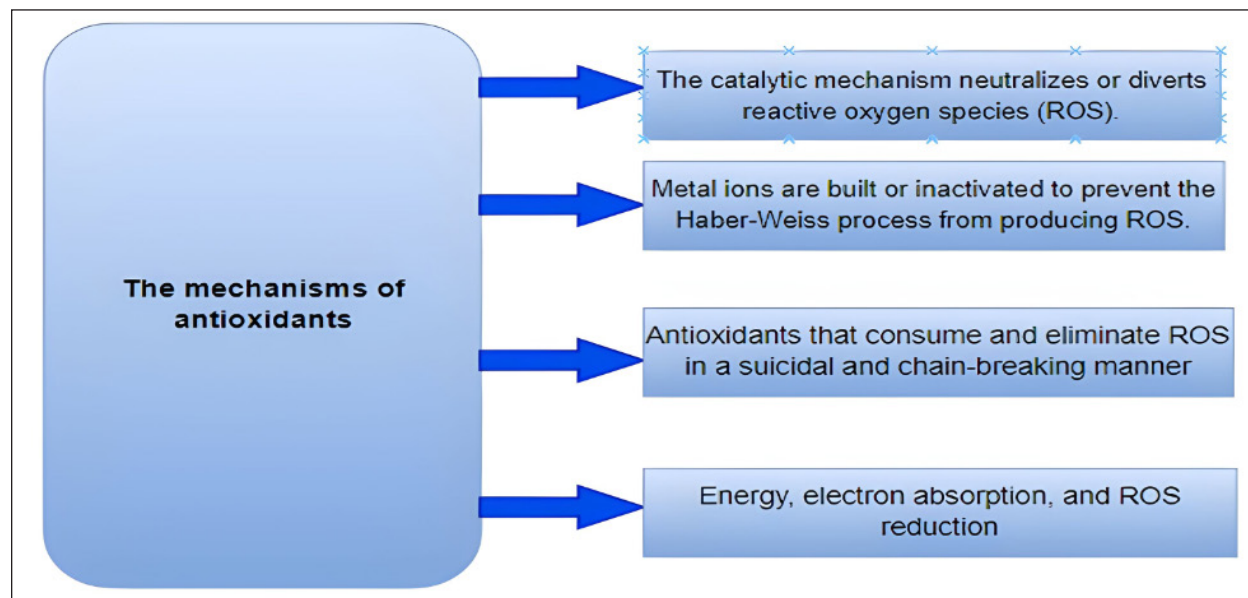
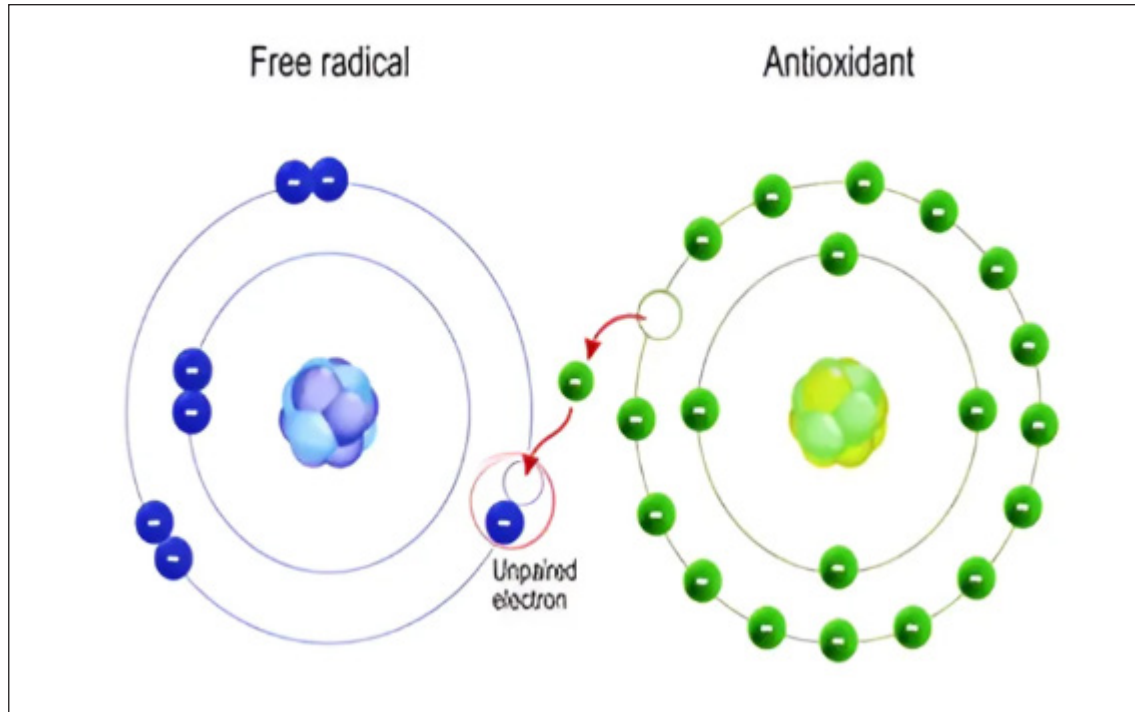


Fig. 5. Various mechanisms of antioxidants against FRs.



**Fig. 6.** Antioxidant mechanism of action. R (alkyl radical), RO (alkoxy radical), ROO (alkylperoxy radical), AH (antioxidants), A (nonradical products), ROOH (alkyl hydroperoxidase).

*emblica*, *Hemidesmus indicus*, *Cichorium indicum*, *Withania somnifera*, *Ocimum sanctum*, *Mangifera indica*, and *Punica granatum* have been identified to possess antioxidant capabilities (Das *et al.*, 2020).

### Conclusion

Various stressors impact poultry's health and poultry enterprises' productivity, from hatching to slaughter. Oxidative stress is considered one of the most important stressors in poultry production. Excess ROS production can negatively affect the immune system and overall health status of birds. The most important ROS include superoxide anion, hydroxyl radical, and hydrogen peroxide ( $H_2O_2$ ), while the principal antioxidant enzyme systems are SOD, glutathione peroxidase, and catalase. The common antioxidant agents in poultry's diet include vitamins C and E, selenium, glycine, manganese, essential oils, and phenolic compounds.

### Ethical approval

The College of Veterinary Medicine Research Committee, University of Sulaimani, Kurdistan Regional Government, Kurdistan, Iraq, approved the study (no. 142: on 20/January/2024).

### Conflict of interest

The authors declare that there is no conflict of interest.

### Authors' contributions

All authors were involved in the planning and hypothesizing of this review article. All the authors were involved in the study's data collection and

literature review. All were involved in drafting and writing the manuscript. RNM was responsible for the final revision and proofing.

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