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Antioxidant Systems in Poultry Biology: Superoxide Dismutase

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Abstract

Commercial poultry production is associated with various stresses responsible for decreasing productive and reproductive performance of growing chicks, breeders and commercial layers. A growing body of evidence shows that most of stresses in poultry production at the cellular level are associated with oxidative stress. Recently, a concept of the cellular antioxidant defense has been revised with special attention paid to cellular redox status maintenance and cell signaling. In fact, antioxidant systems of the living cell are based on three major levels of defense and superoxide dismutase (SOD) is shown to belong to the first level of the antioxidant defense network. Cellular antioxidant defenses are shown to include several options and vitagene activation in stress conditions is considered as a fundamental adaptive mechanism. The vitagene family includes various genes regulating synthesis of protective molecules including thioredoxins, sirtuins, heat shock proteins and SOD. However, by the time of writing no comprehensive review on the roles and effects of SOD in poultry biology has appeared. Therefore, the aim of this review is a critical analysis of the role of SOD in poultry biology with specific emphasis to its functions as an essential part of the vitagene network. From the analysis of the recent data related to SOD in poultry physiology and adaptation to stresses it is possible to conclude that: a) SOD as important vitagene is the main driving force in cell/body adaptation to various stress conditions. Indeed, in stress conditions additional synthesis of SOD is an adaptive mechanism to decrease ROS formation; b) If the stress is too high SOD activity is decreased and apoptosis is activated; c) there are tissue-specific differences in SOD expression which also depends on the strength of such stress-factors as heat, heavy metals, mycotoxins and other stressors; d) SOD is shown to provide an effective protection against lipid peroxidation in chicken embryonic tissues and semen; e) SOD is shown to be protective in heat and cold stress, toxicity stress as well as in other oxidative-stress related conditions in poultry production; f) there are complex interactions inside the antioxidant network of the cell/body to ensure an effective maintenance of homeostasis in stress conditions. Indeed, in many cases, nutritional antioxidants (vitamin E, selenium, carotenoids, phytochemicals, etc.) in the feed can increase SOD expression; g) nutritional means of SOD upregulation in stress conditions of poultry production and physiological and commercial consequences await further investigation; h) vitagene upregulation in stress conditions is emerging as an effective means for stress management.

Key words:

Antioxidant system; chicken; HSP; poultry; stress; vitagenes

Abbreviations:

AO- antioxidant; ARE- antioxidant response element; CAT – catalase; NOS- nitric oxide synthase; GSH – glutathione; GSH-Px- glutathione peroxidase; GST- glutathione transferase; HSP – heat shock protein; MDA- malondialdehyde; NF-κB- nuclear factor-kappa B; Nrf2- Nuclear factor-erythroid-2-related factor 2; SOD – superoxide dismutase.

Introduction

Commercial poultry production is associated with various stresses responsible for decreasing productive and reproductive performance of growing chicks, breeders and commercial layers. A growing body of evidence shows that most of stresses in poultry production at the cellular level are associated with oxidative stress. Recently, a concept of the cellular antioxidant defense has been revised with special attention paid to cellular redox status maintenance and cell signalling. It has been suggested that the antioxidant defense

network of the living cell is based on three major levels of defense and include several options [1-2]: Decreasing localized oxygen concentration; reducing activity of pro-oxidant enzymes and improving efficiency of electron chain in the mitochondria and decreasing electron leakage leading to superoxide production; preventing chain initiation by scavenging initial radicals due to inducing various transcription factors (e.g., Nrf2, NF-κB and others) with ARE-related synthesis of AO enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), glutathione reductase (GR), glutathione transferase (GST), etc.; binding metal ions (metal-binding proteins) and metal chelating; decomposing peroxides by converting them to non-radical, nontoxic products (Se-GSH-Px); chain breaking by scavenging intermediate radicals such as peroxyl and alkoxyl radicals (vitamins E, C, reduced glutathione (GSH), uric acid, ubiquinol, bilirubin, etc.); repairing and removing damaged molecules (methionine sulfoxide reductase, DNA-repair enzymes, chaperons, etc.) and vitagene activation and synthesis and increased expression of protective molecules (GSH, thioredoxins, SOD, heat shock proteins, sirtuins, etc.). Indeed, elucidating roles of vitagenes in stress resistance of poultry as a background for the development of effective strategies to deal with stresses is an emerging topic of research [1-5]. It is known that adaptive SOD synthesis is under vitagene control. However, by the time of writing no comprehensive review on the roles and effects of SOD in poultry biology has appeared. Therefore, the aim of this review is a critical analysis of the role of SOD in poultry biology with specific emphasis to its functions as an essential part of the vitagene network, responsible for adaptive ability of the cells and whole organism to various stress conditions.

Free radicals and reactive oxygen and nitrogen species

Free radicals are atoms or molecules containing one or more unpaired electrons. Free radicals are highly unstable and reactive and are capable of damaging all types of biologically relevant molecules including DNA, proteins, lipids and carbohydrates. The animal body is under constant attack from free radicals, formed as a natural consequence of the body's normal metabolic activity and as part of the immune system's strategy for destroying invading microorganisms. Collective terms reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been introduced [6] and they include not only the oxygen or nitrogen radicals, but also some non-radical reactive derivatives of oxygen and nitrogen.

Superoxide (O_2*-) is the main free radical produced in biological systems during normal respiration in mitochondria and by autoxidation reactions with half-life at 37°C in the range of 1 x 10^{-6} second. Superoxide can inactivate some enzymes due to formation of unstable complexes with transition metals of enzyme prosthetic groups, followed by oxidative self-destruction of the active site [7]. Depending on condition, superoxide can act as an oxidizing or a reducing agent. It is necessary to mention that superoxide, by itself, is not extremely dangerous and does not rapidly cross lipid membrane bilayer [8]. However, superoxide is a precursor of other, more powerful ROS. For example, it reacts with nitric oxide with a formation of peroxynitrite (ONOO-), a strong oxidant, which leads to formation of reactive intermediates due to spontaneous decomposition [9-10]. In fact, ONOO- was shown to damage a wide variety of biomolecules, including proteins (via nitration of tyrosine or tryptophan residues or oxidation of methionine or selenocysteine residues), DNA and lipids [11]. Superoxide can also participate in the production of more powerful radicals by donating an electron, and thereby reducing Cu^{2+} and Fe^{3+} to Fe^{2+} and Cu+, as follows:

$$O_2^- + Fe^{3+}/Cu^{2+} \rightarrow Fe^{2+}/Cu+ + O_2$$

Further reactions of Fe^{2+} and Cu+ with H_2O_2 are a source of the hydroxyl radical (*OH) in the Fenton reaction:

$$H_2O_2 + Fe^{2+}/Cu+ \rightarrow {}^{*}OH + OH- + Fe^{3+}/Cu^{2+}$$

The sum of reaction of superoxide radical with transition metals and transition metals with hydrogen peroxide is known as the Haber-Weiss reaction. It is necessary to underline that superoxide radical is a "double-edged sword". It is beneficial when produced by activated polymorphonuclear leukocytes and other phagocytes as an essential component of their bactericidal activities but in excess it may result in tissue damage associated with inflammation (Figure 1).

Hydroxyl radical is the most reactive species with an estimated half-life of only about 10^{-9} second. It can damage any biological molecule it touches, however, its diffusion capability is restricted to only about two molecular diameters before reacting [12]. Therefore, in most cases damaging effect of hydroxyl radical is restricted to the site of its formation. In general, hydroxyl radical can be generated in human/animal body as a result of radiation exposure from natural sources (radon gas, cosmic radiation) and from man-made sources (electromagnetic radiation and radionuclide contamination). In fact, in many cases hydroxyl radical is a trigger of chain reaction in lipid peroxidation.

Therefore, ROS/RNS are constantly produced in vivo in the course of the physiological metabolism in tissues. It is generally accepted that the electron-transport chain in the mitochondria is responsible for major part of superoxide production in the body [6]. Mitochondrial electron transport system consumes more than 85% of all oxygen used by the cell and, because the efficiency of electron transport is not 100%, about 1-3% of electrons escape from the chain and the univa lent reduction of molecular oxygen results in superoxide anion formation [13-15]. About 10^{12} O_2 molecules processed by each rat cell daily and if the leakage of partially reduced oxygen molecules is about 2%, this will yield about 2 x 10^{10} molecules of ROS per cell per day [16]. An interesting calculation has been made by Halliwell [17], showing that in the human body about 1.72 kg/year of superoxide radical is produced. In stress condition it would be substantially increased. Clearly, these calculations show that free radical production in the body is substantial and many thousand biological molecules can be easily damaged if are not protected.

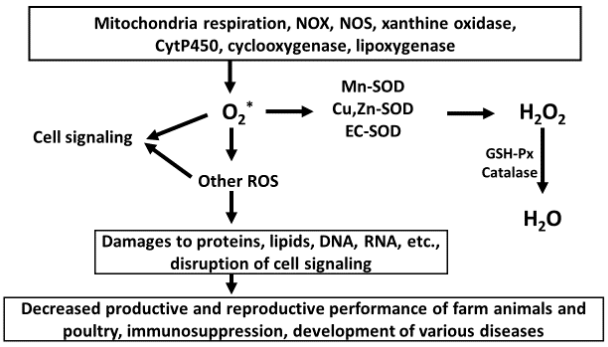

Figure 1: Protective roles of SOD in animal/poultry physiology.
Superoxide (O_2^*) is the main free radical produced in biological systems during normal respiration in mitochondria. In addition to mitochondria, superoxide can be generated by other redox-active enzymes, including xanthine oxidase, cytochrome p450, cyclooxygenase, lipoxygenase, nitric oxide synthase (NOS) and NADPH oxidases (NOXs). Superoxide, by itself, is not extremely dangerous being a signaling molecule. However, superoxide is a precursor of other, more powerful ROS, including peroxynitrite and hydroxyl radical, which can damage all types of biological molecules, including proteins, lipids and nucleic acids. This results in immunosuppression, decreased productive and reproductive performance and development of various diseases. Therefore, three main forms of SOD are responsible for conversion of superoxide into hydrogen peroxide which is further detoxified by GSH-Px and/or Catalase with water formation. This prevents damaging effects of superoxide radical.

Three levels of antioxidant defense

During evolution living organisms have developed specific antioxidant protective mechanisms to deal with ROS and RNS [6]. Therefore it is only the presence of natural antioxidants in living organisms which enable them to survive in an oxygen-rich environment [13]. These mechanisms are described by the general term "antioxidant system". It is diverse and responsible for the protection of cells from the actions of free radicals. This system includes [18-20]:

natural fat-soluble antioxidants (vitamins A, E, carotenoids, ubiquinones, etc.);

water-soluble antioxidants (ascorbic acid, uric acid, taurine, carnitine, etc.);

antioxidant enzymes: GSH-Px, CAT and SOD;

thiol redox system consisting of the glutathione system (glutathione/glutathione reductase/glutaredoxin/glutathione peroxidase and a thioredoxin system (thioredoxin/thioredoxin peroxidase/thioredoxin reductase).

The protective antioxidant compounds are located in organelles, subcellular compartments or the extracellular space enabling maximum cellular protection to occur. Thus antioxidant systems of the living cell include three major levels of defense [18-21].

The first level of defense is responsible for prevention of free radical formation by removing precursors of free radicals or by inactivating catalysts and consists of three antioxidant enzymes namely SOD, GSH-Px and CAT plus metal-binding proteins. Since the superoxide radical is the main free radical produced in physiological conditions in the cell [13] SOD (EC 1.15.1.1) is considered to be the main element of the first level of antioxidant defense in the cell [18]. This enzyme dismutates the superoxide radical in the following reaction:

$2O_2^* + 2H+ \text{ SOD} \rightarrow H_2O_2 + O_2$

The hydrogen peroxide formed by SOD action can be detoxified by GSH-Px or CAT which reduce it to water as follows:

$H_2O_2 + 2GSH + \text{GSH-Px} \rightarrow GSSG + 2H_2O$

Catalase

$2H_2O_2 \rightarrow 2H_2O + O_2$

Transition metal ions also accelerate the decomposition of lipid hydroperoxides into cytotoxic products such as aldehydes, alkoxyl radicals and peroxyl radicals. Therefore, metal-binding proteins (transferrin, lactoferrin, haptoglobin, hemopexin, metallothionenin, ceruloplasmin, ferritin, albumin, myoglobin, etc.) also belong to the first level of defense. It seems likely that carnitine with its regulating functions on the mitochondria free radical production [1] can also be part of the first level of antioxidant defense.

Unfortunately, this first level of antioxidant defense in the cell is not sufficient to completely prevent free radical formation and some radicals do escape through the preventive first level of antioxidant safety screen initiating lipid peroxidation and causing damage to DNA and proteins. Therefore, the second level of defense consists of chain-breaking antioxidants - vitamin E, ubiquinol, carotenoids, vitamin A, ascorbic acid, uric acid and some other antioxidants. Glutathione and thioredoxin systems also have a substantial role in the second level of antioxidant defense. Chain-breaking antioxidants inhibit peroxidation by keeping the chain length of the propagation reaction as small as possible. Therefore, they prevent the propagation step of lipid peroxidation by scavenging peroxyl radical intermediates in the chain reaction:

$LOO^* + Toc \rightarrow Toc^* + LOOH$

(LOO^* is lipid peroxyl radical; Toc - tocopherol, Toc^* - tocopheroxyl radical, LOOH – lipid hydroperoxide).

However, even the second level of antioxidant defense in the cell is not able to prevent damaging effects of ROS and RNS on lipids, proteins and DNA. In this case, the third level of defense is based on systems that eliminate damaged molecules or repair them. This level of antioxidant defense includes lipolytic (lipases), proteolytic (peptidases or proteases) and other enzymes (DNA repair enzymes,

methionine sulphoxide reductase, ligases, nucleases, polymerases, proteinases, phospholipases and various transferases) and chaperones, including HSPs.

Antioxidant-prooxidant balance in the body and oxidative stress

In the body/cell a delicate critical balance exists between antioxidant defense and repair systems and free radical generation [18-20]. In physiological conditions the right and left parts of the so-called "balances" are in equilibrium i.e. free radical generation is neutralised by the antioxidant system and some free radicals and products of their metaboilism participate in cell signaling and transcription factor activation. Exogenous factors are among the most important elements, which increase an efficiency of the antioxidant system of the organism. Natural and synthetic antioxidants in the feed as well as optimal levels of Mn, Cu, Zn and Se help maintaining the efficient levels of endogenous antioxidants in the tissues. Optimal diet composition allows the antioxidants of the food to be efficiently absorbed and metabolised. Optimal temperature, humidity and other environmental conditions are also required for the effective protection against free radical production. The prevention of different diseases by antibiotics and other drugs is an integral part of the efficient antioxidant defense as well.

Different stress conditions are associated with overproduction of free radicals and cause oxidative stress i.e. a disturbance in the prooxidant-antioxidant balance leading to potential tissue damage [22]. Stress conditions can be generally divided into three main categories [19]. The most important part is nutritional stress conditions including high dietary levels of PUFAs, deficiencies of vitamin E, Se, Zn or Mn, Fe-overload, hypervitaminosis A and presence of different mycotoxins and other toxic compounds in the feed. A second group of stress factors includes environmental conditions: increased temperature or humidity, hyperoxia, radiation etc. Internal stress factors include various bacterial or viral diseases as well as allergy. All the above-mentioned conditions stimulate free radical generation in the mitochondria.

Living cells permanently balance the process of formation and inactivation of ROS and as a result ROS level remains low but still above zero. Adverse environmental conditions initiate attempts of organisms to resist the environment that became more aggressive [23]. Cells can usually tolerate mild oxidative stress by additional synthesis of various antioxidants (glutathione, antioxidant enzymes, etc.) in an attempt to restore antioxidant/oxidant balance. At the same time, energy expenditures increased and respiration activated leading to the increased yield of ROS [23]. However these adaptive mechanisms have limited ability. Once the free radical production exceeds the ability of antioxidant system to neutralise them, oxidative stress develops and causes damage to unsaturated lipids in cell membranes, amino acids in proteins and nucleotides in DNA and as a result, membrane and cell integrity is disrupted. Membrane damage is associated with a decreased efficiency of absorption of different nutrients and leads to an imbalance of vitamins, amino acids, inorganic elements and other nutrients in the organism. All these events result in decreased productive and reproductive performances of animals. Immunity incompetence and unfavourable changes in the cardio-vascular system, brain and neurones and muscle system due to increased lipid peroxidation make the situation even worse [20].

As it was shown above all antioxidants in the body are working as a "team" responsible for antioxidant defense and we call this team the antioxidant system. In this team one member helps another one working efficiently. Therefore if relationships in this team are effective, which happens only in the case of balanced diet and sufficient provision of dietary antioxidant nutrients, then even low doses of such antioxidants as vitamin E could be effective. On the other hand when this team is subjected to high stress conditions, free radical production is increased dramatically. During these times, without external help it is difficult to prevent damage to major organs and systems. This 'external help' is dietary supplementation with increased concentrations of natural antioxidants. For nutritionist or feed formulator it is a great challenge to understand when the internal antioxidant team in the body requires help, how much of this help to provide and what the economic return will be. Again, it is necessary to remember about essentiality of keeping right balance between free radical production and antioxidant defense. Indeed, ROS and RNS have another more attractive face participating in a regulation of varieties of physiological functions.

Therefore, the antioxidant defense mechanisms include several options [1-36 19-20]:

Decreasing localized oxygen concentration;

Reducing activity of pro-oxidant enzymes and improve efficiency of electron chain in the mitochondria decreasing superoxide production (carnitine);

Redox-signaling with induction of various transcription factors (e.g. Nrf2 and NF-kB) and gene expression with ARE-related synthesis of AO enzymes (SOD, GSH-Px, catalase, GR, GST, etc.) and other important protective molecules;

Vita-gene activation and synthesis and increased expression of protective molecules (HSP, thioredoxins, sirtuins, SOD, etc.);

Binding metal ions (metal-binding proteins) and metal chelating;

Decomposing peroxides by converting them to non-radical, nontoxic products (Se-GSH-Px);

Chain breaking by scavenging intermediate radicals such as peroxyl and alkoxyl radicals (vitamins E, C, GSH, uric acid, carnitine, ubiquinol, bilirubin, etc.);

Antioxidant (vitamin E) recycling mechanisms (vitamins B1,B2, Se, ascorbic acid);

Repairing and removing damaged molecules (Msr, DNA-repair enzymes, proteasomes, HSP and other chaperons, etc.);

Apoptosis activation and removal terminally damaged cells and restriction of mutagenesis.

It is important to mention once more that ROS are no longer viewed as just toxic by-products of mitochondrial respiration, but are now appreciated for their role in regulating various cellular signaling pathways [24]. Indeed, the adaptation to stressful conditions of our life is mediated via vitagene network in the body.

Superoxide Dismutase in biological systems

SOD was discovered by McCord and Fridovich in 1969 as an enzymatic activity in preparations of carbonic anhydrase or myoglobin that inhibited the aerobic reduction of cytochrome C by xanthine oxidase [25]. Therefore, haemocuprein, which was discovered much earlier, became Cu, Zn-SOD [26]. This discovery opened new era in free radical research. At present, three distinct isoforms of SOD have been identified in mammals, and their genomic structure, cDNA, and proteins have been described [27]. The fourth form of the enzyme Fe-SOD was isolated from various bacteria but not found in animal. Furthermore, a novel type of nickel-containing SOD was purified to apparent homogeneity from the cytosolic fractions of Streptomyces sp. [28]. The biosynthesis of SODs, in most biological systems, is well controlled. In fact, exposure to increased pO_2, increased intracellular fluxes of O_2-, metal ions perturbation, and exposures to several environmental oxidants have been shown to influence the rate of SOD synthesis in both prokaryotic and eukaryotic organisms [29]. A range of transcriptional factors, including NF-κB, AP-1, AP-2, and Sp1, as well as CCAAT-enhancer-binding protein (C/EBP), have been shown to regulate the constitutive or inducible expression levels of all three SODs [30]. Furthermore, it seems likely that in addition to transcriptional control, epigenetic regulation and posttranscriptional modifications are responsible for a regulation of the SOD functional activity [30]. Comparative characteristics of SOD1, SOD2 and SOD3 are summarised in Table 1[30-31].

Table 1: Biochemical properties of mammalian superoxide dismutase (Adapted from [30-31]).

Enzymes	CuZn-SOD	Mn-SOD	EC-SOD
Gene designation (human/ mouse)	SOD1/Sod1	SOD2/Sod2	SOD3/Sod3
Chromosome location man/ mouse)	HAS21/MMU16	HAS6/MMU17	HAS4/MMU5
Disease caused by enzyme defects	Amyotrophic lateral sclerosis(ALS)	None	None
Metal co-factor(s)	Cu^{2+} - catalytically active Zn^{2+} - maintains enzyme stability	Mn^{2+} - catalytically active	Cu^{2+} - catalytically active Zn^{2+} - maintains enzyme stability
Active form	Dimer	Tetramer	Tetramer
Molecular Mass, kDa	88	32	135
Subcellular locations	Cytosol, intermembrane space of mitochondria, nucleus	Mitochondria matrix	Extracellular matrix and circulation
Tissue distribution (from high to low)	Liver, kidney, brain, heart	Heart, brain, skeletal muscle	Blood vessels, lung, kidney, uterus
Post-translational modification	Nitration, phosphorylation, glutathiolation, glycation	Acetylation, nitration, phosphorylation	Glycosylation
Inducibility	Not inducible	Inducible	Induced by antioxidants and regulated through NRF

SOD1, or Cu, Zn-SOD, was the first enzyme of this family to be characterised and is a copper and zinc-containing homodimer that is found almost exclusively in intracellular cytoplasmic spaces. It exists as a 32 kDa homodimer and is present in the cytoplasm and nucleus of every cell type examined [27]. The chromosomal localization and characteristics of the sod1 gene have been identified in rodents, bovines, and humans and the human sod1 gene is shown to be localized on chromosome 21q22. Furthermore, sod1 gene consists of five exons interrupted by four introns, which is significantly similar in different species in terms of the size of exons, particularly the coding regions [30]. The sequence and structure of Cu, Zn-SOD is highly conserved from prokaryotes to eukaryote and mammalian SOD1 is highly expressed in the liver and kidney [32]. Enzymatic activity of SOD1 depends on the presence of the Cu and Zn. While copper is needed for SOD1 catalytic activity, Zn participates in proper protein folding and stability. Over 100 mutations in the human gene SOD1 are described to lead to some inherited diseases, but their mechanisms remain unclear [33].

The second member of the family (SOD2) has manganese (Mn) as a cofactor and therefore called Mn-SOD. SOD2 is shown to have a unique genetic organization and little similarity with SOD1 and SOD3 [30]. The primary structure of SOD2 genes is shown to be highly conserved and it shares more than 90% sequence homology in the coding region in mouse, rat, bovine and human and the human sod2 is located

on chromosome 6q25.3 [30]. It was shown to be a 96 kDa homotetramer and located exclusively in the mitochondrial matrix, a prime site of superoxide radical production [6]. Therefore the expression of Mn-SOD is considered to be essential for the survival of all aerobic organisms from bacteria to humans and it participates in the development of cellular resistance to oxygen radical-mediated toxicity [34]. Indeed, Mn-SOD is shown to play a critical role in the defense against oxidant-induced injury and apoptosis in various cells. In fact, Mn-SOD is inducible enzyme and its activity is affected by cytokines and oxidative stress. Therefore, Mn-SOD has been shown to play a major role in promoting cellular differentiation and in protecting against hyperoxia-induced pulmonary toxicity [34] being a crucial determinant of redox status of the cell. Furthermore, Mn-SOD influences the activity of transcription factors (such as HIF-1α, AP-1, NF-κB and p53) and affects DNA stability [35]. A critical role of Mn-SOD under physiological and pathological conditions has recently been reviewed in details and the following findings of Mn-SOD confirm the critical role of Mn-SOD in the survival of aerobic life [36-39]:

Escherichia coli and yeasts lacking the Mn-SOD gene are highly sensitive to oxidative stress;

Mn-SOD gene knockout mice can only survive few days after birth, with pathological findings of many various diseases due to mitochondrial disorder, suggesting a critical role of the enzyme;

Cells transfected with Mn-SOD cDNAs have increased resistance to various free radical-generating toxicants (paraquat, tumor necrosis factor, doxorubicin, mitomycin C, irradiation, ischemic reperfusion, smoking, etc.);

Human Mn-SOD gene transgenic mice show reduced severity of free radical-induced pulmonary damage and adriamycin-induced myocardial damage.

In 1982, a third SOD isozyme was discovered by Marklund and co-workers and called extracellular superoxide dismutase (EC-SOD), due to its exclusive extracellular location. EC-SOD is a glycoprotein with a molecular weight of 135,000 kDa and high affinity for heparin [40]. However, there are some species-specific variations in molecular weight. The human EC-SOD gene has been mapped to chromosome 4q21 and consists of three exons and two introns [41]. The full-length mouse EC-SOD cDNA is shown to be 82% identical to that of rat and 60% identical to the human EC-SOD [30]. EC-SOD is the only antioxidant enzyme that scavenges superoxide specifically in the extracellular space. EC-SOD is present in various organisms as a tetramer or, less commonly, as a dimer and contains one copper and one zinc atom per subunit, which are required for enzymatic activity [42]. The expression pattern of EC-SOD is highly restricted to the specific cell type and tissues where its activity can exceed that of Cu,Zn-SOD or Mn- SOD. As a copper-containing enzyme, the activity of EC-SOD is regulated by copper availability [41]. EC-SOD is comparatively resistant to high temperatures, extreme pH, and high urea concentrations; it can be inhibited by various agents including azide and cyanide and inactivated by diethyldithiocarbamate and hydrogen peroxide. Oxidative stress and post-translational modification of EC-SOD are shown to cause loss of EC-SOD activity [30].

SOD in avian biology

Chicken SOD

Chicken SOD was described and purified in early 1970. Indeed, in chicken liver two types of SOD were identified, one of which was localized in the mitochondria while the other was found in the cytosol [43]. The cytosol SOD was inhibited by cyanide, whereas the mitochondrial enzyme was not. Later this feature was used to distinguish between two forms of enzymes during assays. The cytosol SOD was purified to homogeneity with apparent molecular weight in presence of mercaptoethanol to be 30,600 Da and to contain copper and zinc, being similar to the other Cu, Zn-SOD which have been isolated from diverse eukaryotes. In fact, purified cytosol SOD from chicken liver contained 0.30% copper and 0.25% zinc. This corresponds to 0.9 atom of copper and 0.8 atom of zinc per subunit. It was also shown that this chicken liver Cu, Zn-SOD had a tendency to polymerize [43]. In contrast, the mitochondrial SOD was found in chicken liver to be a manganoprotein which has a molecular weight of 80,000 Da. It is composed of four subunits of equal size, which are not covalently joined. It contains 2.3 atoms of manganese per molecule and is strikingly similar to the SOD previously isolated from bacteria. This supports the theory that mitochondria have evolved from aerobic prokaryotes. In fact, Mn-SOD was first isolated from the chicken liver [43]. The Mn-SOD was found primarily in the mitochondrial matrix space whereas the Cu,Zn-SOD, previously isolated from the cytosol, was found in the intermembrane space [44].

Cu, Zn-SOD was purified from chicken liver with a subunit Mr of 16900 [45]. Low dietary copper was associated with a decrease in SOD activity and when the 10-day-old deficient chicks were injected with 0.5 mol of CuSO4 intraperitoneally, SOD activity in aorta was restored to control levels in about 8 h. Indeed, dietary copper regulates SOD activity in the tissues of young developing animals. The authors also suggested that a copper deficiency suppresses Cu, Zn-SOD activity without inhibiting synthesis or accumulation of the Cu, Zn-SOD protein in this tissue [45]. Interestingly, molecular properties (amino acid composition, molecular mass and subunit composition) of the chicken enzyme was shown to be similar to those of a bovine erythrocyte Cu, Zn SOD [46]. Purified chicken liver Cu, Zn-SOD was confirmed to contain two subunits having Cu and Zn elements with a molecular weight of 16000+/-500 for each subunit [47]. The optimum pH of purified Cu, Zn-SOD was determined to be 8.9. The enzyme was found to have fair thermal stability up to 45^{o}C at pH 7.4 over a 1-h incubation period. The SOD enzyme was not inhibited by DTT and beta-mercaptoethanol, but inhibited by CN(-) and H_2O_2 [47]. SOD purified from chicken heart has a molecular weight 31.0 +/- 1.0 kDa and is composed of two equally sized subunits each having 1.1 +/- 0.03 and 0.97 +/- 0.02 atoms of Cu and Zn elements, respectively [48]. The Mn-SOD cDNA in chicken heart was

shown to be 1108 bp in length. The molecular weight of the mature peptide was 22 kDa. A comparison of the deduced amino acid sequence with those of the human, rat, C. elegans and D. melanogaster showed that the amino acid homology rates were 82.4%, 84.7%, 62.4%, and 59.3%, respectively [49]. SOD activity in avian tissues depends on many different factors including genetics, nutrition and various stress-related factors. For example, SOD activity in the Jungle Fowl feather melanocytes was shown to be 2- and 4-fold higher than that in Barred Plymouth Rock and White Leghorn tissue respectively [50]. Indeed, understanding the molecular mechanisms of the regulation of SOD gene expression and the factors involved in tissue- and cell-specific expression of the SOD genes are of great importance for a developing novel strategies for preventing negative consequences of various stresses in poultry production.

SOD in chicken embryo

Chick embryo tissues contain a high proportion of highly polyunsaturated fatty acids in the lipid fraction [51] and therefore need antioxidant defense [18]. The antioxidant system of the newly hatched chick includes the antioxidant enzymes SOD, GSH-Px, catalase [52], fat-soluble antioxidants vitamin E and carotenoids [53], water-soluble antioxidants ascorbic acid [53] and glutathione [52] as well as selenium [54-57]. Vitamin E [58], carotenoids [59-64] and selenium [54-57] are transferred from feed into egg and further to embryonic tissues. Glutathione and antioxidant enzymes GSH-Px, SOD and catalase are also expressed in the embryonic tissues at various stages of their development [52, 65]. Our results indicate that there are tissue-specific features in antioxidant defense strategy during embryonic development of the chicken and SOD plays a crucial role as an integral part of the antioxidant network.

In the embryonic liver, SOD specific activity was maximal at day 11 but decreased sharply by day 15 and remained relatively constant thereafter. By contrast, the specific activity of SOD in the brain from day 15 onwards was approximately 2 times higher than that in the liver. In the YSM SOD specific activity increased gradually between days 10 and 15 and then decreased gradually between day 15 and hatching [52]. The specific activities of SOD in kidney, lung, heart and skeletal muscle all showed a gradual decrease between day 15 and hatching. As can be seen from Table 2, the tissues displayed a considerable variation in the Mn-SOD activity, with the heart having the highest value and lung the lowest [65]. By contrast, the lung was characterized by high Cu,Zn-SOD activity; in the heart, activity of Cu,Zn-SOD was comparable to the other tissues. Based on the total SOD activity the tissues could be placed in the following descending order: heart > muscle > YSM > kidney > lung > liver. Mn-SOD is the main enzymatic form in the liver and heart comprising 73.2 and 68% of the total SOD activity respectively. In great contrast, in the lung, YSM and thigh muscle, SOD is exclusively represented by Cu,Zn-SOD comprising 98.5, 98.3 and 84.7% of the total SOD activity respectively. In various parts of the brain (cerebrum, cerebellum, brain stem and optic lobes) of the newly hatched chick the Cu,Zn-SOD activity is also almost 2-fold higher than that of Mn-SOD [65]. Notably, in the kidney both SOD forms are equally represented. Furthermore, the tissues differed markedly in the GSH-Px activities. In all the tissues, Se-dependent GSH-Px was the main enzymatic form, comprising from 65% (lung) up to 90% (heart) of the total enzyme activity. The liver and kidney displayed the highest total GSH-Px activity and the muscle the lowest. As in the case of GSH-Px, catalase activity was also maximal in the liver and kidney.

Table 2: Antioxidant Enzyme Activities in the Tissues of a Newly Hatched Chick (Adapted from [65].

Tissue	Mn-SOD, U/mg protein	Cu-Zn-SOD, U/mg protein	Se- GSH-Px, mU/mg protein	Non-Se-GSH-Px, mU/mg protein	Catalase, U/mg protein
Liver	3.81^{a}	1.46^{a}	177.0^{a}	114.6^{a}	35.8^{a}
Kidney	2.98^{b}	3.15^{b}	159.8^{a}	58.6^{b}	29.5^{a}
Heart	5.79^{c}	2.73^{b}	99.0^{b}	11.6^{c}	5.8^{b}
Lung	0.09^{d}	5.79^{c}	99.8^{b}	53.0^{b}	6.0^{b}
Thigh Muscle	1.06^{d}	6.07^{c}	45.8^{c}	12.6^{c}	3.2^{c}
YSM	0.12^{e}	6.97^{c}	102.6^{b}	37.7^{d}	15.2^{d}

Note: Values are mean + SEM (n = 5). Numbers with different superscripts are significantly different with respect to the column.

SOD in avian semen

Despite the importance of SOD in the protection of cells against lipid peroxidation, its activity in avian semen has received only limited attention. A comparison of SOD activity in sperm from various species including boar, rabbit, stallion, donkey, ram, bull, man and chicken indicated that donkey sperm had the highest and fowl the lowest SOD activity [66]. Furthermore, turkey spermatozoa were found to contain even less SOD activity than fowl spermatozoa [67]. Our data indicate that in seminal plasma of 5 avian species, KCN inhibited 100% of SOD activity, an observation reflecting the presence of only Cu, Zn-SOD [68]. In the seminal plasma, the highest SOD activity was recorded in turkey and guinea fowl while the lowest activity was found in duck. Overall, avian species classified in accordance with decreasing SOD activity (expressed per mg seminal plasma protein) can be placed in the following order: guinea fowl>chicken>goose> duck>turkey. Similarly, in seminal plasma, the activity of GSH-Px was two times greater in the ganders than in chickens, whereas SOD activity was lower than in chickens [69]. In contrast, the SOD activity in spermatozoa, from pre-cited species is classified in an opposite order to that observed in seminal plasma (goose>duck>chicken=guinea fowl>turkey [68]). In chicken semen, the SOD activity significantly increased in cryopreserved seminal plasma with simultaneous decrease of its activity in cells [69]. In sperm both forms of SOD are expressed with significant species-specific differences. For example in goose, Cu,Zn-SOD appears twice higher than Mn-SOD and an opposite distribution between different forms of SOD was recorded in guinea fowl where Mn-SOD was more

than two-fold higher compared to Cu,Zn-SOD [68]. In chicken, about 67% of total SOD activity was detected in spermatozoa as compared to 33% in seminal plasma [70]. The biological meaning and physiological consequences of such species-specific differences in SOD activity and distribution remain to be established. Notably, in laying hens, SOD activity in the utero-vaginal junction was shown to be increased compared to other regions of the lower oviduct (vagina, uterus; [71-72]).

Dietary modulation of SOD

Mn and Cu in the diet

Mn-SOD is shown to be highly expressed in various organs containing a large number of mitochondria such as the heart, liver, and kidneys. Indeed, in comparison to other tissues, the heart has the highest steady state mRNA Mn-SOD expression level in chickens [73]. It has been proven that Mn availability is a regulating factor of Mn-SOD activity. For example, in primary cultured broiler myocardial cells Mn-SOD mRNA, Mn-SOD protein, and Mn-SOD activity were induced by manganese in dose- and time-dependent manner. Manganese regulates Mn-SOD expression not only at transcriptional level but also at translational and/or posttranslational levels [74]. In both heart and kidney, Mn-SOD activity was significantly depressed by decreased dietary manganese; greatest reduction occurred in the heart [75]. Decreased heart Mn-SOD and Cu,Zn-SOD activities, resulting from dietary Mn and Cu deficiencies, were both associated with increased peroxidation [76]. It seems likely that Mn-SOD activity is very sensitive to dietary Mn levels in commercial corn-soybean meal diets. In fact, Mn deficiency in growing chickens caused the reductions of Mn concentrations of the liver and heart as well as Mn-SOD activity of the heart [77]. In chickens, dietary Mn contents required to reach the plateau of Mn concentrations of the liver, pancreas, kidney, heart, spleen and muscle and to obtain the maximum Mn-SOD activity of heart were calculated to be 110, 111, 141, 123, 109, 99 and 121ppm respectively. Interestingly, Mn-SOD of liver and pancreas were not affected. Therefore, for broilers fed the basal corn-soybean meal diet, 120ppm Mn was suggested as the required level [78] which corresponds to the presently recommended levels of Mn supplementation. Chickens fed a Mn-deficient diet from hatching had significantly lower levels of Mn-SOD activity in liver than did controls. However, activity of the Cu,Zn-SOD in the liver was higher in Mn-deficient chickens than in controls [79]. The activity of both forms of SOD reached normal levels when a Mn-supplemented (1,000 ppm) diet was fed to deficient chickens, but the activity of the manganese enzyme was not affected by feeding the supplemented diet to manganese sufficient chickens. It was shown that heart Mn-SOD activity and heart Mn-SOD mRNA levels increased linearly as dietary Mn levels increased, confirming that dietary Mn significantly affected heart Mn-SOD gene transcription [80]. Furthermore, birds fed supplemental Mn had lower MDA content in leg muscle and greater Mn-SOD activities and Mn-SOD mRNA level in breast or leg muscle than those fed the control diet [81]. Compared with control chickens fed on a diet without Mn supplementation, chickens fed Mn-supplemented diets had higher Mn concentrations, Mn-SOD mRNA levels, Mn-SOD protein concentrations, and Mn-SOD activities within heart tissue [82-83]. Therefore, dietary Mn can activate Mn-SOD gene expression at both the transcriptional and translational levels [82]. However, Mn excess can be toxic for birds. In fact the activities of SOD and GSH-Px in chicken serum and immune organs (spleen, thymus, and bursa of Fabricius; [84]) and testes [85] were decreased due to Mn dietary excess.

It seems likely that dietary Cu is involved in regulation of the SOD activity and in the case of low Cu levels in the basic diet, it is possible to upregulate Cu,Zn-SOD in chickens by dietary Cu supplementation. For example, in the basal low-Cu group, Cu, Zn-SOD activity decreased in the liver, RBC and heart to 14, 25, and 61%, respectively, of control activities after 6 weeks' depletion [86]. On the other hand, Cu,Zn-SOD activity in chicken erythrocytes from the Cu- and vitamin C-supplemented birds was increased by 39 and 20% respectively [87]. Similarly, in the Cu-supplemented chickens, Cu,Zn-SOD activity in the liver, erythrocyte, kidney and heart significantly increased by 75, 40, 12, 12% respectively. Furthermore, Mn-SOD activity in the heart, liver, kidney and brain of the vitamin C –supplemented chickens was increased. In addition, in the heart of Cu-supplemented chickens Mn-SOD was found to be increased by approximately 15%, while in liver tissue of the Cu-supplemented group it was reduced by 19% [88]. However, in an earlier study, hepatic Mn-SOD and Cu,Zn-SOD were not influenced by dietary Cu level or source or LPS in broiler chicks [89] probably reflecting differences in the background Cu levels.

Vitamins, carnitine and amino acids

Dietary vitamin A excess was shown to decrease SOD activity in the chicken liver and brain [90]. Similarly, increased vitamin E supplementation (40-60 mg/kg) or CCl4 injection decreased the activity of SOD in the chicken blood [91]. However, in a recent study a higher vitamin E level (60 vs 30 mg/kg) significantly increased alpha-tocopherol concentrations and SOD activity in serum of laying hens [92]. Initially, L-Carnitine dietary supplementation was shown to increase blood SOD activity in chickens [93]. Furthermore, when chicken fed corn-soybean diets supplemented with different doses of lipoic acid SOD activity in serum (300 mg/kg), liver (100, 200 and 300 mg/kg) and leg muscle (200 or 300 mg/kg) was significantly increased [94]. It was shown that increased Lipoic acid (LA) or acetyl-l-carnitine (ALC) resulted in increased total antioxidant capacity and SOD and GSH-Px activities and decreased levels of MDA in serum and liver of birds [95]. Notably, birds fed diets containing 50 mg/kg of LA and 50 mg/kg of ALC had higher serum and liver SOD activities than those fed diets containing 100 mg/kg of LA or ALC alone. In laying hens reared in a hot and humid climate L-threonine supplementation at 0.2% maximised the SOD activity in both serum and liver [96]. Serum SOD increased linearly and quadratically in laying hens receiving excess dietary tryptophan (0·4 g/kg) [97]. Broilers given a diet containing 5.9 g/kg methionine had enhanced serum SOD activity and decreased hepatic MDA content at day 7 [98].

Selenium

Low-Se diet caused a significant decrease in the activities of SOD and GSH-Px, and an increased MDA content in thymus, spleen, Bursa of Fabricius and serum [99]. Interestingly, not only Se deficiency (0.03 mg Se per kg of diet) but also Se excess (3 mg/kg) in chickens significantly lowered SOD and CAT activities in the liver and serum [100]. It seems likely that SOD in adult birds is also affected by Se status. For example, laying hens fed the Se-supplemented diet showed higher SOD and GSH-Px activity and lower MDA content in plasma compared with those fed the control (non-supplemented) diet [101]. Positive effects of dietary Se on SOD activities in avian species depend not only on Se concentration, but also on the form of Se used, with organic Se being more effective than sodium selenite. In fact, the activities of serum GSH-Px, SOD and total antioxidant capacity were significantly higher in selenium yeast than sodium selenite-fed chickens [102]. Similarly, dietary Se-Met significantly elevated T-AOC, GPX, T-SOD, CAT activities, contents of GSH and reduced carbonyl protein content in chicken breast muscle [103]. It was shown that dietary organic Se significantly increased the Se content and the activities of CAT and SOD, but decreased the MDA content in chicken breast muscle at 42 days of age [104].

Phytochemicals

Polyphenolic compounds and various plant extracts have received substantial attention as an important means of decreasing oxidative stress in vitro and in vivo. For example, in cultured muscle cells of embryonic broilers, pretreatment with low-dosage phytoestrogen equol (1μM) restored altered (decreased) by H_2O_2 intracellular SOD activity. However, pretreatment with high-dosage equol (10 and 100 μM) showed a synergistic effect with H_2O_2 in inducing cell damage, but had no effect on MDA content, SOD or GSH-Px activity [105]. Similarly, in chicken HD11 macrophages challenged with LPS activity of SOD increased in cells treated with the higher concentration of equol (80 μmol/L or 160 μmol/L, but not in 10, 20 or 40 μmol/L groups; [106]). In a chicken erythrocyte model both curcumin and cyanidin-3-rutinoside were shown to significantly attenuate apoptosis and hemolysis, decreasing MDA content, and increasing SOD activity in a time- and dose-dependent manner [107]. Similarly, feeding diets with added flavonoids (hesperetin and naringenin) to laying hens increased the blood serum SOD activity [108]. There was a significant increase in the activities of SOD chicken blood due to Brahma Rasayana supplementation [109]. Dietary xanthophyll (lutein+zeaxanthin) supplementation (20 or 40 mg/kg) for 3 or 4 weeks was shown to increase serum SOD activity in chickens [110]. However, the SOD activity was not affected in the chicken liver or jejunal mucosa. Inclusion into the chicken diet of polysavone (1·5 g/kg), a natural extract from alfalfa, for 6 weeks increased serum and liver SOD activity, while breast muscle SOD activity at 6 weeks of age were significantly higher and MDA content was significantly lower in 1·0 and 1·5 g/kg polysavone groups than in the control group [111]. Notably, effects of plant extracts added to chicken diets on the SOD activity would depend on many factors including polyphenol composition, concentration and bioavailability. In fact, low availability of polyphenolic compounds for growing chickens, breeders and layers [112] is an important limiting factor of their biological efficacy and nutritive value. For example, there was no effect of dietary turmeric rhizome powder (0.25- 0.75%) on the activities of GSH-Px and SOD in thigh muscle [113] or serum [114]. Feeding to broiler chicks diets enriched with selected herbal supplements failed to affect the growth performance of chickens at 42 days of age. In addition, this supplementation had no influence on the activities of SOD and GSH-Px, concentration of vitamin A and selected lipid metabolism indices [115].

Sod up- and down-regulation in stress conditions

Heat stress

High environmental temperature is one of the most important stressors causing economic losses to the poultry industry, including poor growth performance, immunosuppression, high mortality, decreased reproductive performance and deterioration of meat quality [116]. Since SOD is an inducible enzyme, depending on conditions, stresses can tissue-specifically increase or decrease SOD activity in various avian species. For example, acute heat stress (34°C) in chickens was shown to induce a significant production of ROS, and antioxidant enzymes, including SOD, CAT and GSH-Px [117]. On exposure to chronic heat stress, GSH-Px activity remained relatively constant, though a temperature-dependent elevation in Cu,Zn-SOD activity was observed in skeletal muscle of broiler chickens [118]. Chicken exposure to heat stress increased SOD activity and MDA levels in skeletal muscle and vitamin E or vitamin E+Se dietary supplementation further enhanced SOD activity in muscles in heat-stressed birds [119]. In broiler chickens, plasma activity of SOD was increased, whereas GSH-Px was suppressed by heat stress (32 ± 1°C). Furthermore, heat exposure increased SOD and catalase activities in breast muscle but the reverse was true in thigh muscle. On the other hand, heat stress increased SOD and decreased GSH-Px activities of mitochondria regardless of muscle types [120]. Interestingly, in restrictedly fed broiler breeder's plasma MDA, protein carbonyl content, activity of SOD and corticosterone content were not altered after acute (33°C) and prolonged heat challenges [121]. Probably the stress intensity was not high enough to upregulate SOD. On the other hand, if stress is too high adaptive functions of SOD can be overwhelmed with the following SOD decrease. For example, heat stress in black-boned chickens reduced daily feed intake and BW gain; decreased serum GSH and inhibited GSH-Px, SOD and CAT activities compared with birds subjected to thermo-neutral circumstances [122]. Similarly, in chickens heat stress induced higher levels of TNF-α, IL-4, HSP27, HSP70, and MDA levels but lower level of IFN-γ, IL-2, GSH-Px, and SOD in spleen [123-124]. These responses were ameliorated by the treatment of Se, polysaccharide of Atractylodes macrocephala Koidz alone or in combination [124].

Cold stress

Environmental temperature either below or above the comfort zone causes discomfort in avian species. In fact, the increase in metabolic rate at temperatures below the comfort zone (cold stress) is a significant cause of increased mortality from the pulmonary hypertension syndrome (ascites) in broilers [125]. Initially, it was shown that when broilers were exposed to a cool environment for 3 weeks, plasma SOD activity was decreased [126]. Similarly, cold exposure reduced chicken plasma SOD and supplemental L-carnitine (100 mg/kg) was shown to restore the SOD activity in cold-stressed birds [127]. Broilers with cold-induced ascites were characterised by a significantly decreased SOD activity in the liver [128]. Opposite results were also reported. In fact, during acute cold stress, the SOD activity of the lung increased compared with their control group at each stress time point [129]. Similarly, there was a significant decrease in CAT and SOD in blood, but increased SOD activity was evident in the liver [130]. A complexity of the SOD response to various stresses is also illustrated in the next two papers. In chick duodenum, under acute cold stress MDA level increased and the activity of SOD and iNOS first increased and then decreased. In contrast, under chronic cold stress the activity of SOD, NO, and NOS in duodenum first decreased and then increased, whereas the MDA level increased [131]. In immune organs, the activities of SOD and GSH-Px were first increased then decreased, and activity of total antioxidant capacity was significantly decreased at the acute cold stress in chicks [132].

Other environmental stresses

Effects of environmental stresses on SOD activity is, probably, tissue-specific and depend on many factors, including strength and duration of the stress. For example, in broilers corticosterone administration caused decreases in serum SOD activity as well as in the apparent digestibility of energy, relative weight of bursa and thymus, total antioxidant capacity, and antibody titers to Newcastle disease virus [133]. In contrast, there was an increase in SOD activity in the chicken heart during short-term corticosterone administration [134]. In growing chickens exposed to high ammonia and low humidity blood antioxidative capacities and pectoral muscle SOD and GSH-Px activities were significantly reduced [135]. Hepatic mitochondrial SOD activity decreased at 14 d in feed-restricted broiler chicks [136]. However, the plasma SOD activity of feed-restricted birds was markedly higher than those fed ad libitum on d 35 and d 42 [126].

Toxicological stresses

Administration of cadmium to chickens decreased SOD activities in various tissues, including liver [137-138], kidney [139], blood [140], ovary [141], testes [142] and splenic lymphocytes in vitro [143]. Usually, decreased SOD activity was accompanied by decreased GSH-Px activity and increased lipid peroxidation in the same tissues. In contrast to the aforementioned results, Cd oral administration produced peroxidative damage in chickens, as indicated by increase in TBARS, reduction in GSH concentration in liver and kidney, but increased CAT and SOD activities were observed in erythrocytes [144]. Dietary nickel chloride is also shown to have a negative effect on SOD and other antioxidant enzymes (GSH-Px and CAT) in the intestine [145], cecal tonsil [146] or splenocytes [147]. Similarly, vanadium inhibited SOD activity in chicken liver and kidney [148]. The list of chicken SOD inhibitors includes aluminium [149-150], fluorine [151], polychlorinated biphenyls [152-153], 4-nitrophenol [154], dioxin [155], organophosphate [156], thiram [157], furazolidone [158], valproic acid [159], oxidised oil [160]. It seems likely that mycotoxins can also decrease SOD activity in various chicken tissues. In particular, DON decreased SOD activity in embryo fibroblast DF-1 cells [161] and AFB1 feed contamination was associated with decreased SOD in the chicken liver [162-163] and erythrocytes [164]. However, the activities of SOD, GST and non-protein thiol levels in the chicken liver were not altered by the FB1-containing (100 mg/kg) diet fed for 21 days [165].

Diseases and gut health

Various avian diseases also negatively affect antioxidant defenses including decrease SOD activity in jejunal and ileal parts of the gut challenged with Salmonella pullorum [166], brain and liver of Newcastle disease virus-infected chickens [167], erythrocytes of the Eimeria acervulina infected birds [168] and plasma of E. tenella challenged birds [169]. Since antioxidant-pro-oxidant balance in the gut plays an important role in chicken health and immunity [5], special emphasis should be given to this area of research. For example, in vitamin-D-replete chicks, Cu,Zn-SOD was shown to be associated with the apical border (microvilli) of the duodenal absorptive cells [170]. Furthermore, inclusion of γ-aminobutyric acid (GABA) in laying hen diet was associated with significant increasing the activity of SOD and GSH-Px and decreasing MDA levels in serum [171]. Similarly, serum SOD and catalase activities were significantly increased, and MDA was decreased by dietary sodium butyrate at 0.5 or 1.0 g/kg feeding to chickens from hatch for 21 days [172]. Broilers fed a diet supplemented with 1×10^9 cfu Clostridium butyricum/kg diet had greater SOD activity in the ileal mucosa on d21 and in jejunal mucosa on d42 than those in the other groups fed antibiotic aureomycin or lower doses of the probiotic [173].

Clinical significance of SOD activity in different tissues

When studying SOD, results interpretation could be a challenging task. First of all, plasma is easily obtained material; however, the meaning of increased or decreased total SOD in plasma sometimes could be misleading. Indeed, in normal human plasma three forms of SOD are found with the lowest amount of SOD1 (5.6-35.5 ng/ml), somehow higher amount of SOD2 (47-150 ng/ml) and even more SOD3 (79-230 ng/ml; [174]). Therefore, ideally individual SODs should be determined in plasma to have maximum information to analyse. However, practically in all studies related to SOD in avian plasma only total SOD was determined. Secondly, in

tissues Mn-SOD and Cu,Zn-SOD should be distinguished. However, similar to plasma SOD, in most of poultry-related studies only total SOD was analysed. Thirdly, since Mn-SOD is an inducible enzyme, an increased SOD activity in tissues could mean an adaptive response to stress situation or could indicate a potential of the antioxidant defense in the stress conditions. Indeed, when natural antioxidants are supplemented with diets there could be upregulation of SOD indicating an increase in antioxidant defenses or downregulation of SOD reflecting a decreased need for SOD because of other antioxidant mechanisms are increased. However, as mentioned above SOD is the main enzyme dealing with superoxide production in mitochondria, a primary site of ROS formation, and most likely it cannot be replaced by other antioxidants. Furthermore, when stress is too strong there is a decrease in SOD activity indicating that the antioxidant defense network was overwhelmed by increased production of free radicals and the body is not able to adequately adapt to the situation. Clearly, there is a need for additional research on individual forms of SOD in avian species with specific emphasis to various transcription factors, including NF-κB and Nrf2, responsible for or involved in SOD activation in stress conditions.

In general, the free radical-initiated oxidative damage of lipids, proteins, and DNA as part of the unspecific immune response caused by some viral (Marek's disease, Newcastle diseases, or infectious bursal disease), bacterial diseases (Salmonella, Staphylococcus, Clostridium, or E. coli), or parasitic infections (coccidiosis) has been recently reviewed [175]. Indeed, roles of superoxide production and SOD activity in many of those diseases in poultry await investigations. In fact, it has been suggested that oxidative damage may regulate the occurrence and development of avian infectious bronchitis and SOD activity in the serum of chickens inoculated with infectious bronchitis virus significantly decreased [176]. Similarly, blood SOD was shown to be significantly decreased in broiler birds infected with Eimeria tenella [177].

Nutritional modulation of vitagenes

The aforementioned data clearly indicate that vitagenes can be modulated by nutritional means. Indeed, vitamins E, A, carnitine, selenium and some phytochemicals can affect SOD expression and concentration in various stress conditions. It is interesting that the same compounds can affect other vitagenes, namely thioredoxins, sirtuins and heat shock proteins [2,178]. Therefore, it would be of considerable interest to develop an antioxidant-based composition/ supplement for decreasing negative consequences of various stresses in poultry and pig production. Such a composition should meet at least five important requirements [1-2]:

Vitagene activation and redox-signaling (carnitine, betaine, vitamins A, E, D, C, Se, Zn, Mn, silymarin and possibly other phytochemicals);

Maintenance of the vitamin E recycling system (vitamin C, Se, Vitamin B1 and B2);

Provision of nutrients required for carnitine synthesis (lysine and methionine, ascorbic acid, vitamin B6 and niacin);

Supporting the liver, a main site of T-2 toxin, ochratoxins, fumonisins and aflatoxins detoxification and gut, responsible to DON detoxification (vitamins E and C, selenium, carnitine, betaine, organic acids, methionine and lysine);

Possessing immunomodulating properties (vitamins A, E, D, C, carnitine, Se, Zn and Mn).

Inclusion of various protective compounds into the diet of farm animals and poultry to decrease negative consequences of stress conditions is quite complicated, firstly, by a decreased feed consumption at time of stress. Secondly, such an approach has a low flexibility, since existing feeding systems do not allow to include anything into the feed loaded into the feed storage bins located near the poultry/pig house (usually several tons of feed for several days feeding). Therefore, before the previous feed is consumed, nothing can be added to the feed. However, sometimes it is necessary to supplement animals/poultry with specific additives very quickly to deal with consequences of unexpected stresses (e.g. mycotoxins in the feed, immunosuppression, high temperature, etc.). In such a case, additive supplementation via drinking system is a valuable option [178]. In fact, modern commercial poultry and pig houses have water medication equipment installed, which can be perfectly used for the aforementioned supplementations. For example, an attempt to address the aforementioned option was implemented in a commercial product PerforMax, containing a vitagene-regulating mixture of 28 compounds, including antioxidants (vitamins E and C), carnitine, betaine, minerals (Zn and Mn) and essential amino acids, and supplied via drinking water. Its efficacy in fighting stresses in commercial poultry production has been recently reviewed [4] and prospects of its use to maintain gut health in weaned piglets and newly hatched chicks was considered [5]. Indeed, it is well known that commercial animal/poultry production is associated with a range of stress conditions including environmental (high temperature), nutritional (mycotoxins and oxidized fat) or internal (vaccinations, disease challenges, etc.) stresses [4, 19-20]. In such conditions, supplying the PerforMax with drinking water was shown to have protective effects in growing birds [179-180] as well as in adult birds [4] helping maintain their health, productive and reproductive performance. Therefore, the aforementioned results are the first step to go from the development of the vitagene concept to designing a commercial product and testing it in the commercial conditions of poultry and pig production. We can suggest that this idea could be realized in human nutrition as well. Clearly more research is needed to understand a fundamental role of vitagenes in adaptation to various stresses.

Conclusions and future directions

From the aforementioned analysis of the data related to SOD in poultry physiology and adaptation to stresses it is possible to conclude:

SOD as important vitagene is the main driving force in cell/body adaptation to various stress conditions. Indeed, in stress conditions additional synthesis of SOD is an adaptive mechanism to decrease ROS formation;

If the stress is too high SOD activity is decreased and apoptosis is activated;

There are tissue-specific differences in SOD expression which also depends on the strength of such stress-factors as heat, heavy metals, mycotoxins and other toxicants;

SOD is shown to provide an effective protection against lipid peroxidation in chicken embryonic tissues and in semen;

SOD is proven to be protective in heat and cold stress, toxicity stress as well as in other oxidative stress- related conditions in poultry production;

There are complex interactions inside the antioxidant network of the cell/body to ensure an effective maintenance of homeostasis in stress conditions. Indeed, in many cases nutritional antioxidants (vitamin E, selenium, phytochemicals, etc.) in the feed can increase SOD expression;

Regulating effects of various phytochemicals on HSPs need further investigation;

Nutritional means of additional SOD upregulation in stress conditions of poultry production and physiological and commercial consequences await investigation. Indeed, in medical sciences manipulation of SOD expression and usage of SOD mimics are considered as an important approach in disease prevention and treatment;

Vitagene upregulation in stress conditions is emerging as an effective means for stress management.

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