Mechanisms of Oxidative Stress in Organisms Under Heat Stress Conditions (Postprint)

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Abstract

Heat stress refers to the collective non-specific physiological responses of an organism to heat exposure under high-temperature conditions. Studies have demonstrated that heat stress induces redox imbalance in the organism, resulting in oxidative stress that damages cells and tissues, thereby affecting growth, development, and health status. Heat stress has long been a focal point of research worldwide; with increasing global temperatures, issues related to heat stress will become more pronounced. This review summarizes the mechanisms underlying oxidative stress under heat stress conditions, aiming to provide a reference for future research.

Full Text

Mechanism of Oxidative Stress in the Body under Heat Stress

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Abstract

Heat stress is defined as the sum of nonspecific physiological responses of an organism to heat exposure at high ambient temperatures. Studies have shown that heat stress can disrupt redox homeostasis, induce oxidative stress, damage cells and tissues, and consequently affect growth, development, and health status. Research on heat stress has long been a focus worldwide, and with rising global temperatures, this issue will become increasingly prominent. This paper reviews the mechanisms of oxidative stress under heat stress conditions to provide a reference for future research.

Keywords: heat stress; oxidative stress; antioxidant system; reactive oxygen species; reactive nitrogen species

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Introduction

With rising global temperatures, heat stress has caused substantial economic losses to the livestock industry. Heat stress occurs when the heat released by the body to the environment is in negative balance with the heat produced by the body (production exceeds release). This imbalance is influenced by many factors, such as ambient temperature, humidity, lighting, and exercise [1]. In modern livestock production, the impact of heat stress on livestock is almost unavoidable. Heat stress reduces feed intake, increases nutritional metabolic consumption, and impairs immune function, leading to decreased growth and reproductive performance [2-3].

Although the decline in production and reproductive performance due to heat stress is generally considered to be directly related to reduced feed intake, an increasing number of studies have shown that heat stress first decreases feed intake and nutrient absorption, thereby affecting metabolic levels, particularly by generating excessive free radicals that disrupt antioxidant function [4-5]. Oxidative damage to cells and mitochondria ultimately leads to reduced production and reproductive performance in livestock. Under heat stress conditions, animal body temperature rises, which affects the activity of metabolic enzymes and increases metabolic rate. This elevated metabolic rate increases free radical production, which can react with many macromolecules such as lipids, proteins, and nucleic acids.

Many studies have shown that when animals suffer from heat stress, the activities of catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) are affected, influencing the antioxidant system and altering

free radical content, which demonstrates that heat stress can induce oxidative stress [5-7]. Oxidative stress refers to the imbalance between oxidation and antioxidant systems, resulting in excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) that damage tissues, cells, proteins, and nucleic acids [8]. All organisms, including simple life forms such as yeast and bacteria, possess complex antioxidant systems to balance continuously generated oxidants. Two antioxidant systems exist in the body: the enzymatic antioxidant system and the non-enzymatic antioxidant system. Many studies have found that oxidative stress can cause lipid oxidation and DNA damage, and the resulting excessive free radicals can induce intestinal diseases such as enteritis and increased intestinal mucosal permeability, thereby affecting growth and development [9]. Heat stress has long been a key factor limiting the development of animal husbandry. Investigating changes in oxidative stress under heat stress conditions can not only comprehensively reveal the mechanisms by which heat stress induces oxidative stress but also provide a theoretical basis for alleviating oxidative stress under heat stress. This paper reviews the mechanisms by which heat stress induces oxidative stress.

1. Heat Stress and Oxidant/Antioxidant Balance

When oxidant/antioxidant balance is maintained, the body remains in a normal physiological state. Once this balance is disrupted, oxidative stress responses are triggered. Heat stress breaks the body's oxidant/antioxidant balance [Figure 1: see original paper], inducing oxidative stress responses that cause harm to the body and substantial economic losses to the livestock industry.

2. Heat Stress and Free Radicals

Under heat stress, livestock exhibit decreased feed intake, which affects metabolic levels, particularly by generating excessive free radicals. Free radicals are defined as any atoms or atomic groups containing one or more unpaired electrons that can exist independently. Some ROS are produced during free radical reactions and, although not strictly free radicals themselves, can directly or indirectly trigger free radical reactions. Therefore, the main free radicals in the body are generally considered to be ROS and RNS, which participate in various metabolic reactions.

2.1 Heat Stress and ROS

ROS include superoxide anion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$), and their levels are the most direct markers of oxidative stress. Superoxide anion production in the body has both enzymatic and non-enzymatic sources. The enzymatic source is nicotinamide adenine dinucleotide phosphate oxidase (NADPHox) located in macrophages and endothelial cells [10]. NADPHox is activated in many cellular systems, producing large amounts of $\cdot O_2^-$. Non-enzymatic sources refer to the direct single-electron transfer to oxygen (O_2)

when coenzymes or prosthetic groups are reduced or when some exogenous substances decrease enzyme activity, generating $\cdot O_2^-$. Superoxide anion is the precursor of most ROS, producing H_2O_2 through dismutation and partially reducing $\cdot OH$ [11]. Hydrogen peroxide has less impact than other ROS but plays an important role in carcinogenesis, as it can diffuse throughout mitochondria and cross cell membranes into other cells [12]. H_2O_2 is converted to water by GSH-Px, which simultaneously converts oxidized glutathione (GSH) to reduced glutathione (GSSG). Studies have found that GSH levels decrease under heat stress, leading to increased H_2O_2 [13]. However, H_2O_2 is a relatively stable molecule, and its toxicity is determined by the highly reactive $\cdot OH$ generated through the Fenton reaction [H_2O_2 + ferrous ion (Fe^{2+}) $\rightarrow$ $\cdot OH$ + hydroxide ion (OH^-)]. Hydroxyl radical production is related to Fe^{2+}, which is bound to proteins in mammals. Studies have found that heat stress induces carbonylation of serum transferrin, releasing Fe^{2+} and causing its accumulation [14]. This also provides the source of Fe^{2+} for the Fenton reaction in cells, ultimately leading to increased $\cdot OH$ [15].

ROS are normal cellular metabolites that can react with sugars and lipids, directly react with proteins, and cause mutations in some amino acid residues [16]. Studies have found that excessive ROS production under heat stress damages mitochondrial membranes, releasing cytochrome C (Cyto-C) into the cytoplasm. Once Cyto-C enters the cytoplasm, it rapidly completes assembly, activating a cascade of cysteine reactions, followed by increased expression of cysteine aspartate protease 3 (Caspases-3), leading to apoptosis [17]. Other studies have found that heat shock protein 70 (Hsp70) inhibits the expression of apoptotic protease-activating factor-1 (Apaf-1) [18] and Cyto-C release [19], decreasing Caspases-3 expression and reducing apoptosis. Research has also shown that Hsp70 can reduce ROS production by activating antioxidant enzymes such as SOD [20]. Many studies have indicated that Hsp70 expression increases under heat stress [21-22], while Reeg et al. [23] found that ROS causes Hsp70 degradation and inactivation, exacerbating heat stress and forming a vicious cycle that causes severe damage to the body. Although heat shock proteins can activate antioxidant enzymes, some are degraded, and the amount of functional heat shock proteins remains small. Additionally, studies have found that Nrf2 expression increases in dairy cows under summer heat stress [24]. Huang [25] also found that heat stress decreases Keap1 expression, allowing Nrf2 to be released into the nucleus, increasing its expression and producing SOD1 and heme oxygenase-1 (HO-1) to inhibit ROS production and alleviate oxidative stress. Sholomskas et al. [26] showed that heat stress in mice causes overexpression of redox factor-1 (Ref-1), activating activator protein-1 (AP-1), which both inhibits apoptosis and increases manganese superoxide dismutase (Mn-SOD) activity, thereby negatively regulating and inhibiting ROS production, alleviating oxidative stress, and reducing cellular and tissue damage. Uncoupling proteins (UCPs) are mitochondrial inner membrane proteins, with five types identified in mammals (UCP1, UCP2, UCP3, UCP4, and UCP5), among which UCP2 and UCP3 mainly regulate oxidative stress responses [27-28]. The primary function

of UCPs is to reduce ROS production by regulating proton leak. Affourtit et al. [29] reported that increased UCP2 expression in islet cells reduces mitochondrial ROS generation, suggesting that UCP2 negatively regulates insulin and modulates ROS production through energy metabolism. Studies have shown that UCPs expression is downregulated under heat stress, and this downregulation is associated with increased ROS. Heat stress causes excessive ROS production that cannot be timely inhibited by UCPs, leading to their downregulated expression [30].

2.2 Heat Stress and RNS

RNS mainly include nitric oxide (NO) and peroxynitrite ($ONOO^-$). NO is produced in cells through the catalysis of nitric oxide synthase (NOS) using L-arginine as a substrate and can directly reflect the level of oxidative stress in the body (Figure 1). Studies have found that NOS expression is upregulated in mice under heat stress stimulation, catalyzing the production of large amounts of NO from L-arginine and causing damage to the body [31]. NO is a highly reactive free radical and also an important signaling molecule in cell signaling pathways, participating in various physiological responses. NO can react with oxyhemoglobin to effectively prevent the direct high-speed reaction of NO with oxygen to form nitrogen dioxide (NO_2), and can also react with ROS to produce highly oxidizing $ONOO^-$, inactivating Mn-SOD and iron superoxide dismutase (Fe-SOD) [32]. Moreover, NO can attack polyunsaturated fatty acids, and the unstable intermediate lipid peroxides (LOO^- and LOOH) produced in this process can cause lipid peroxidation and damage to proteins and DNA [33]. Meanwhile, under heat stress conditions, increased ROS production reacts with NO, increasing peroxynitrite and potentially causing poisoning in the body [31]. $ONOO^-$ can modify proteins by nitrating tyrosine residues and oxidizing tryptophan and cysteine, causing them to lose activity and resulting in tissue damage [34]. Additionally, studies have found that damage to mitochondrial structural integrity and increased mitochondrial membrane permeability cause increased mitochondrial calcium ion (Ca^{2+}) influx. Ca^{2+} can bind to calmodulin to enhance NOS activity and increase NO production [35]. RNS play an important role in oxidative stress induced by free radicals. In human studies, RNS have been found to cause many diseases, such as thrombosis and cancer. However, research on RNS under heat stress conditions in animals is scarce and requires further investigation.

Figure 1. Mechanism of oxidative stress under heat stress. HS: heat stress; complex I: complex I; complex II: complex II; complex III: complex III; complex IV: complex IV; monoamine oxidase: monoamine oxidase; mitochondrion: mitochondrion; oxidation: oxidation; induction: induction; binding: binding; degradation: degradation; apoptosis: apoptosis; lysine: lysine; ARE: antioxidant response element; $\Delta\Psi$: membrane permeability; citrulline: citrulline; nucleus: nucleus.

3. Effects of Heat Stress on the Antioxidant Enzyme System

The antioxidant enzyme system includes SOD, CAT, and GSH-Px. The body contains copper-zinc-containing CuZn-SOD and manganese-containing Mn-SOD. CuZn-SOD is mainly distributed in the cytoplasm, while Mn-SOD is primarily located in the mitochondrial matrix. SOD can combine $\cdot O_2$ with hydrogen ions to generate H_2O_2 and O_2, and can also catalyze the dismutation of $\cdot O_2^-$ to produce O_2 and water. CAT is an iron-containing enzyme that decomposes H_2O_2 into water and O_2. The active center of GSH-Px is selenocysteine, and this enzyme can catalyze the conversion of GSH to GSSG, reducing toxic H_2O_2 to non-toxic hydroxyl compounds.

Heat stress can be divided into acute and chronic heat stress, with different effects on antioxidant enzyme activities under different conditions (Table 1). Under acute heat stress, the body is suddenly stimulated, causing a rapid increase in free radicals, and the antioxidant enzyme system responds with significantly elevated activities of CAT, SOD, and GSH-Px to scavenge excess free radicals. Chronic heat stress, however, causes prolonged exposure to heat stress, leading to decreased activities of CAT, SOD, and GSH-Px, destruction of the antioxidant enzyme system, and inability to timely scavenge large amounts of free radicals, breaking the body' s oxidative balance and causing oxidative stress. Different types of heat stress result in different antioxidant enzyme activities, and animals with different heat tolerance capacities also show differences in antioxidant enzyme activities.

A series of studies have shown that heat stress alters antioxidant enzyme activities, but these studies only describe the changes in enzyme activities at the phenotypic level and cannot clearly explain the mechanisms affecting enzyme activity (Table 1). Many factors affect enzyme activity, such as acetylation, methylation, and the content of trace elements at the enzyme active center. Studies have found that acetylation of certain amino acids changes the expression of some proteins, affecting enzyme activity [36]. However, no studies have reported on the effects of methylation or phosphorylation of certain amino acids on enzyme activity under heat stress. Therefore, the authors hypothesize that heat stress may alter enzyme activity through protein methylation or amino acid phosphorylation, which requires further investigation.

4. Effects of Heat Stress on the Non-Enzymatic Antioxidant System

In addition to the antioxidant enzyme system, a non-enzymatic antioxidant system exists when oxidative stress occurs in the body. The non-enzymatic antioxidant system includes vitamin C, vitamin E, GSH, carotenoids, and trace elements such as copper, zinc, selenium, and manganese, which participate in biotransformation in the body.

Most non-enzymatic substances are obtained through feed intake. Under heat stress conditions, reduced feed intake decreases most non-enzymatic substances, weakening the antioxidant capacity of the non-enzymatic antioxidant system and causing oxidative stress. Different non-enzymatic substances have different mechanisms of action (Table 2). Vitamin E is an important antioxidant in biological systems that can insert into lipid bilayers and combine with vitamin C and other antioxidant systems to terminate lipid oxidation [33]. Glucocorticoids can promote protein decomposition and enhance the body' s adaptability to adverse external environments. Excessive glucocorticoid secretion under heat stress conditions causes a certain degree of cell damage, while vitamin C can regulate glucocorticoids in the body, thereby alleviating cell damage under heat stress. Zinc plays an important role in the antioxidant defense system in vivo, participating in the composition of CuZn-SOD to alleviate oxidative stress [44] and regulating metallothionein expression to improve antioxidant capacity [45]. Studies have found that chromium can promote RNA synthesis by accumulating in the nucleus to regulate nuclear synthesis, and it also has enzymatic functions, catalyzing DNA polymerization and preventing DNA damage [46]. Heat stress increases chromium excretion in urine, causing substantial chromium loss that fails to meet the body' s needs and results in DNA damage. Additionally, selenium is an important element in the body, and GSH-Px is the major selenium-dependent enzyme. When heat stress occurs, the body' s selenium requirements cannot be met, leading to decreased GSH-Px activity. SOD is another selenium-dependent antioxidant enzyme that works with GSH-Px to convert large amounts of free radicals into non-toxic hydroxyl compounds and catalyze the decomposition of H_2O_2 into water [47].

5. Antioxidants for Alleviating Oxidative Stress

Under heat stress conditions, excessive free radical production in the body causes oxidative stress in cells and tissues, harming the body. To alleviate oxidative stress induced by heat stress and reduce economic losses, numerous studies on antioxidant supplementation have been conducted both domestically and internationally (Table 3). Oxidative stress induced by heat stress mainly results from increased free radicals that exceed the body' s scavenging capacity. Antioxidant supplementation enhances the body' s ability to scavenge free radicals, thereby alleviating oxidative stress. However, research has found that more safe and effective antioxidants are needed in livestock production, which requires further research and development.

6. Summary and Outlook

Excessive free radical production under heat stress conditions can easily cause oxidative stress and harm animal health. This paper elaborates in detail on the mechanisms by which heat stress induces oxidative stress, which can serve as part of the reference basis for preventing heat stress. Some free radicals can act as signaling molecules to provide feedback inhibition of free radical production

in cell pathways. If the expression of genes in cell pathways under the action of free radicals during heat stress is clearly understood, the mechanisms can be better improved and oxidative stress induced by heat stress can be more effectively alleviated. Currently, reports on the actual effects of additives for alleviating oxidative stress vary, and there is no unified standard for dosage. Whether mixing multiple additives or developing more Chinese herbal medicine additives can better alleviate oxidative stress remains to be studied, as few research reports exist on this topic.

Note: Figure translations are in progress. See original paper for figures.