

# Biosynthesis and Roles of Glutathione in heat Stressed Animals

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## ABSTRACT

Oxidative stress commonly occurs following heat stress in tropical regions and affects negatively on animals performance. The adverse effect of the heat stress had a negative impact on enzymatic activity. Glutathione (GSH) is an antioxidant in animals protects cells from oxidative damages and is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals. GSH has been mediating the initial response for acquiring tolerance to heat stress. GSH serves vital functions in animals, antioxidant defense, scavenging free radicals and other reactive species, removing hydrogen and lipid peroxides, preventing oxidation of bio-molecules, signal transduction and gene expression and DNA and protein synthesis, and proteolysis. Different kinds of stress result in reduction in the concentration of reduced GSH in animal organs. GSH synthesis is impaired under stress conditions, leading to GSH deficiency. A decrease in GSH and an increase in GSSG were found in the blood of heat-stressed cattle.

**Keywords :** Glutathione, Antioxidant, Oxidative Stress, Heat Stress, Animals.

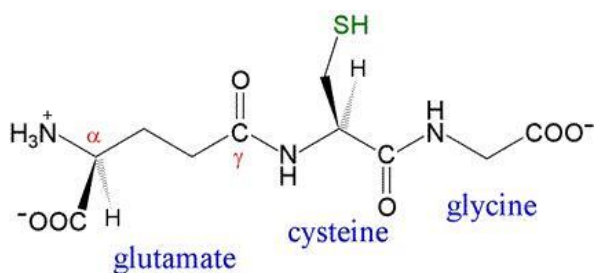
## I. INTRODUCTION

High ambient temperature and humidity are the major constraint on animal productivity in tropical and subtropical areas [1] [2] [3]. Oxidative stress commonly occurs following heat stress in tropical regions and affects animals and Glutathione protects cells from oxidative damages. The oxidative balance is affected during heat stress periods. Fast production of free radicals and reactive oxygen species and/or a decrease in antioxidant defense mechanisms result in oxidative stress [4]. The effect of heat stress is known to induce oxidative stress which induces production of Reactive Oxygen Species (ROS). The high production of ROS and a decrease in antioxidant defense, leads to cause of many diseases and leading to the onset of health disorders in cattle [5]. Roles for glutathione (GSH) in signal transduction, gene expression, apoptosis, protein glutathione-ylation and nitric oxide metabolism are discovered at the past two

decades [6]. Specifically, several studies have led to the free radical theory of human diseases and to the advancement of nutritional therapies to improve GSH status under various pathological conditions [7]. Most recently, studies of GSH turnover were initiated to provide much needed information about quantitative aspects of GSH synthesis and catabolism in the animal body and specific cell types. Adequate protein nutrition is crucial for the maintenance of GSH homeostasis in animal enteral or parenteral cysteine, methionine, N-acetyl-cysteine, and 1-2-oxothiazolidine-4-carboxylate are effective precursors of cysteine for tissue GSH synthesis [8].

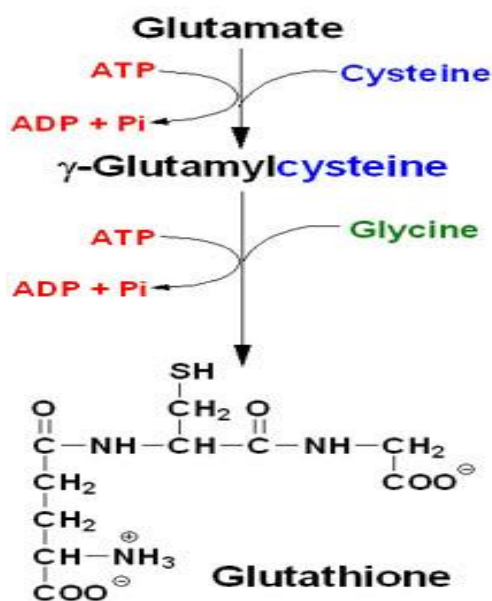
### 1-Glutathione biosynthesis

Glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine; GSH) is the most abundant low-molecular-weight thiol and GSH / glutathione disulfide is the major redox couple in animal cells (Figure 1).



**Figure 1.** Chemical structure of Glutathione  
Journal of Nutrition [8].

GSH is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side chain and the amine group of cysteine, and the carboxyl group of cysteine is attached by normal peptide linkage to a glycine. The synthesis of GSH from glutamate, cysteine, and glycine is catalyzed sequentially by two cytosolic enzymes,  $\gamma$ -glutamyl cysteine synthetase and GSH synthetase. Compelling evidence shows that GSH synthesis is regulated primarily by  $\gamma$ -glutamyl cysteine synthetase activity, cysteine availability, and GSH feedback inhibition [8]. The synthesis of GSH from glutamate, cysteine and glycine is catalyzed sequentially by two cytosolic enzymes,  $\gamma$ -glutamylcysteine synthetase (GCS) and GSH synthetase [8].



**Figure 2.** Glutathione biosynthesis  
Journal of Nutrition [8].

This pathway occurs in virtually all cell types, with the liver being the major producer and exporter of GSH. In the GCS reaction, the  $\gamma$ -carboxyl group of glutamate reacts with the amino group of cysteine to form a peptidic- $\gamma$ -linkage, which protects GSH from hydrolysis by intracellular peptidases [8]. Although  $\gamma$ -glutamyl- cysteine can be a substrate for  $\gamma$ -glutamylcyclotransferase, GSH synthesis is favored in animal cells because of the much higher affinity and activity of GSH synthetase and changes in plasma GSH levels may not necessarily reflect changes in GSH synthesis in specific cell types [9].

## 2-Regulation of Glutathione synthesis

### 2-1-Regulation of Glutathione synthesis by $\gamma$ -glutamylcysteine synthetase (GCS):

Oxidant stress, nitrosative stress, inflammatory cytokines, cancer, cancer chemotherapy, ionizing radiation, heat shock, inhibition of GCS activity, GSH depletion, GSH conjugation, prosta- glandin, heavy metals, antioxidants and insulin increase GCS transcription or activity in a variety of cells [9]. In contrast, dietary protein deficiency, dexamethasone, erythropoietin, tumor growth factor  $\beta$ , hyperglycemia and GCS phosphorylation decrease GCS transcription or activity [7]. In addition, S-nitrosation of GCS protein by NO donors reduces enzyme activity suggesting a link between NO and GSH metabolism [9]. Glucosamine, taurine, phyto-estrogens, polyphenols, carotenoids and zinc, which inhibit the expression of inducible NO synthase and NO production may prevent or attenuate GSH depletion in cells. However, conversely, high-fat diet, saturated long-chain fatty acids, low-density lipoproteins, linoleic acid and iron, which enhance the expression of inducible NO synthase and NO production may exacerbate the loss of GSH from cells [10].

## **2-2-Regulation of Glutathione synthesis by amino acids:**

Cysteine is generally the limiting amino acid for GSH synthesis in humans, animals and chickens [11]. Insulin and growth factors that stimulate cysteine (cystine) uptake by cells generally increase intracellular GSH concentrations [9]. In addition, increasing the supply of cysteine or its precursors (cystine, N-acetyl-cysteine, and L-2-oxothiazolidine-4-carboxylate) via oral or intravenous administration enhances GSH synthesis and prevents GSH deficiency in humans and animals under various nutritional and pathological conditions [7]. Glutamate can be used for GSH synthesis because dietary glutamate is almost completely utilized by the small intestine and plasma glutamate is derived primarily from synthesis and protein degradation [12]. Glutamine is an effective precursor of the glutamate for GSH synthesis in many cell types, including enterocytes, neural cells, liver cells, and lymphocytes. Thus, glutamine supplementation to total parenteral nutrition maintains tissue GSH levels [13]. Glutamate plays a regulatory role in GSH synthesis through two mechanisms: 1) the uptake of cystine, and 2) the prevention of GSH inhibition of GCS [8]. Glutamate and cystine share the system of amino acid transporter, therefore, when extracellular glutamate concentration is high, cystine uptake is competitively inhibited by glutamate, resulting in reduced GSH synthesis [14]. GSH synthesis is enhanced and its concentration is particularly high when intracellular glutamate concentration is high [15]. When hepatic glycine oxidation is enhanced in response to high levels of glucagon, glycine may become a limiting factor for GSH synthesis. Dietary glycine supplementation enhances the hepatic GSH concentration in protein deficient rats [16]. The evidence indicates that the dietary amino acid balance has an important effect on protein nutrition and therefore on GSH homeostasis [9]. In particular, the adequate provision of sulfur-containing amino acids as

well as glutamate (glutamine) and glycine (or serine) is critical for the maximization of GSH synthesis [8].

## **3- Glutathione in cells and plasma**

Glutathione is the predominant low molecular weight thiol (0.5-10 mmol/L) in animal cells. Most of the cellular GSH (85-90%) is present in the cytosol with the remainder in many organ cells (including the mitochondria, nuclear matrix and peroxisomes) [9]. With the exception of bile acid, which may contain up to 10 mmol/L GSH, extracellular concentrations of GSH are relatively low (2-20  $\mu$ mol/L in plasma) [6]. GSH is readily oxidized nonenzymatically to glutathione disulfide (GSSG) by electrophilic substances (free radicals and reactive oxygen/nitrogen species). The GSSG efflux from cells contributes to a net loss of intracellular GSH. Cellular GSH concentrations are reduced markedly in response to protein malnutrition, oxidative stress, and many pathological conditions [15]. The GSH + 2GSSG concentration is usually denoted as total glutathione in cells and a significant amount of which (up to 15%) may be bound to protein [17]. The GSH: GSSG ratio, which is often used as an indicator of the cellular redox state, is >10 under normal physiological conditions [15]. GSH/GSSG is the major redox couple that determines the antioxidative capacity of cells, but its value can be affected by other redox couples, including NADPH/NADP and thioredoxin/thioredoxinox. GSH/GSSG plays crucial roles in antioxidant defense, nutrient metabolism and the regulation of pathways essential for whole body homeostasis and glutathione deficiency contributes to oxidative stress [6]. Plasma GSH originates primarily from the liver, but some of the dietary and intestinally derived GSH can enter the portal venous plasma [9]. Glutathione molecules leave the liver either intact or as  $\gamma$ -Glu-(Cys)<sub>2</sub> owing to  $\gamma$ -glutamyltranspeptidase activity on the outer plasma membrane. The extreme concentration gradient across the plasma membrane makes the transport of extracellular GSH or GSSG into

cells thermodynamically unfavorable. However,  $\gamma$ -Glu-(Cys)<sub>2</sub> is readily taken up by extrahepatic cells for GSH synthesis. The kidney, lung, and intestine are major consumers of the liver derived GSH [9].

#### **4-Role of Glutathione**

Glutathione deficiency contributes to oxidative stress. Oxidative stress (a deleterious imbalance between the production and removal of reactive oxygen/nitrogen species) plays a key role in the pathogenesis of many diseases [8]. Roles of glutathione in animals are antioxidant defense, scavenging free radicals and other reactive species, removing hydrogen and lipid peroxides, preventing oxidation of biomolecules, metabolism, synthesis of leukotrienes and prostaglandins, conversion of formaldehyde to formate, production of D-lactate from methylglyoxal, formation of mercapturates from electrophiles, formation of glutathione-NO adduct, storage and transport of cysteine, regulation intracellular redox status, signal transduction and gene expression, DNA and protein synthesis, and proteolysis, cell proliferation and apoptosis, cytokine production and immune response, protein glutathionylation and mitochondrial function and integrity [8]. Glutathione as an antioxidant in animals is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals [18]. Glutathione as antioxidant either in reduced (GSH) or oxidized (GS-SG) form is one of the key constituent in the antioxidative system with a significant function in reactive oxygen species scavenging and act as redox buffer to keep the cellular redox state in balance [8]. GSH is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. GSH may also function in maintaining the integrity of the red cell by reducing sulphhydryl groups of hemoglobin, membrane proteins and enzymes that may have become oxidized [19]. An important function of Glutathione in the red

cell is the detoxification of low levels of hydrogen peroxide which may form spontaneously or as a result of drug administration [20]. GSH participates in leukotriene synthesis and is a cofactor for the enzyme glutathione peroxidase [21]. GSH effectively scavenges free radicals and other reactive oxygen species (hydroxyl radical, lipid peroxy radical, peroxynitrite and H<sub>2</sub>O<sub>2</sub>) directly and indirectly through enzymatic reactions and in such reactions. GSH is oxidized to form GSSG, which is then reduced to GSH by the NADPH-dependent glutathione reductase [21]. In addition, GSH plays a role in diverse biological processes as protein synthesis, enzyme catalysis, trans-membrane transport, receptor action, intermediary metabolism and cell maturations [22]. Glutathione is also needed for the detoxification of methylglyoxal, a toxin produced as a by-product of metabolism. GSH is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms [23]. GSH has a vital function in regulation of the nitric oxide cycle, which is critical for life but can be problematic if unregulated [24]. GSH is used in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation [25]. Glutathione is also important as a hydrophilic molecule that is added to lipophilic toxins and waste in the liver during biotransformation before they can become part of the bile. GSH also conjugates with NO to form S-nitroso-glutathione which is cleared by the thioredoxin system to release GSH and NO and the targeting of endogenous NO is mediated by intracellular GSH. Both NO and GSH are necessary for the hepatic action of insulin-sensitizing agents, indicating their critical role in regulating lipid, glucose and amino acid utilization [8]. In addition, GSH plays a role in diverse biological processes as protein synthesis, enzyme catalysis, trans-membrane transport, receptor action, intermediary metabolism

and cell maturations [8]. Moreover, GSH is necessary for the hepatic action of insulin-sensitizing agents indicating their critical role in regulating lipid, glucose and amino acid utilization [8]. Free glutathione is mainly present in its reduced form (GSH), which can be converted to the oxidized form (GSSG) during oxidative stress. Glutathione reductase can convert GSSG to GSH. Reduction and conjugation reactions are the most important functions of GSH [26]. The GSH protects cell membranes from oxidative damage by removing of produced reactive species [27]. Therefore, adequate GSH concentrations are necessary for the proliferation of cells, including lymphocytes and intestinal epithelial cells [28]. Glutathione also plays an important role in spermatogenesis and sperm maturation [29]. GSH serves as a substrate for formaldehyde dehydrogenase, which converts formaldehyde and GSH to S-formyl-glutathione. The removal of formaldehyde (a carcinogen) is of physiological importance, because it is produced from the metabolism of methionine, choline, methanol, sarcosine and xenobiotics [7]. GSH is essential for the activation of T-lymphocytes and polymorphonuclear leukocytes as well as for cytokine production, and therefore for mounting successful immune responses when the host is immunologically challenged [7]. GSH is required for the conversion of prostaglandin H<sub>2</sub> into prostaglandins D<sub>2</sub> and E<sub>2</sub> by endoperoxidase [9]. GSH is involved in the glyoxalase system, which converts methylglyoxal to d-lactate, a pathway active in microorganisms [8]. Glutathionylation of proteins plays an important role in cell physiology and shifting the GSH/GSSG redox toward the oxidizing state activates several signaling pathways thereby reducing cell proliferation and increasing apoptosis [7]. Moreover, GSH is necessary for the hepatic action of insulin-sensitizing agents indicating their critical role in regulating lipid, glucose and amino acid utilization. Both NO and GSH are necessary for the hepatic action of insulin-sensitizing agents, indicating their critical role in

regulating lipid, glucose and amino acid utilization [30].

### **5-Heat stress and glutathione level**

The adverse effect of the heat stress had a negative impact on enzymatic activity. Different kinds of stress result in reduction in the concentration of reduced glutathione (GSH) in animal organs [31]. GSH synthesis is impaired under stress conditions, leading to GSH deficiency. A decrease in GSH and an increase in GSSG were found in the blood of heat-stressed cattle [32]. The authors found that mean GSH concentrations for thermo-neutral and heat stress were  $3.2 \pm 0.65$  and  $2.7 \pm 0.62$  mmol/L of RBC, respectively and reduced GSH concentrations were associated with reduced feed intake during heat stress period. The same authors concluded that heat stress reductions in feed intake and thermoregulatory effects may induce oxidative stress in cattle. The concentrations GSH in the liver and kidney of three genetic groups of rabbits decreased insignificantly due to stress of the influence of displacement of animals from cage to cage daily for 30 days [33]. Same authors concluded that this decrease in the concentrations of GSH in the liver and kidney of rabbits is due to adaptive processes in biochemical reaction of stress and/or by long time of experiments. Short-term heat stress was found to decrease GSH levels and increase lipid peroxidation in killifish and when increased acclimation temperature increased total GSH levels and increased glutathione peroxidase and GSH reductase activities [34]. Plasma total antioxidant activity decreases when cows are placed in environmentally controlled chambers and exposed to 29.5 °C temperatures for a period of 7 days [35]. The same authors reported that as the temperature humidity index (THI) approaches levels dangerous to livestock, total antioxidant activity declines. In addition same authors reported that hyperthermia results in a transient decrease followed by an increase in GSH levels in the blood, an increase in excretion of

hepatic GSH, and an increase in lipid peroxidation [36]. Liver GSH in male rats was significantly decreased after heat stress in both age groups treated with N-acetyl cysteine [37]. Glutathione has been mediating the initial response for acquiring tolerance to heat stress [38]. Heat exposure results in decreases in GSH levels and consequently increase in free radicals leading to increase lipid peroxidation (oxidative stress) in rats [39]. Serum glutathione level in the ram and its changes during normal and heat stress conditions were determined and reported that glutathione levels change during different environmental conditions [40]. The decreased values of GSH on long exposures to temperature stress indicate utilization of this antioxidant, either to scavenge oxiradicals or act in combination with other enzymes, was more than its production capacity under heat stress [41]. Liver supplies most of the plasma glutathione and it is removed from plasma by transpeptidase action which is mostly located in the kidney therefore, glutathione levels may be influenced by different physiological conditions [42].

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