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CHANGES IN ANTIOXIDANT ENZYME ACTIVITY DURING FEEDING WITH WHEY POWDER AND CHITOSAN

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Abstract. *This scientific article provides a systematic literature-based analysis of changes occurring in the enzymatic components of the antioxidant defense system under feeding conditions involving whey powder (whey protein) and chitosan. The discussion focuses on the role of key antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—in neutralizing reactive oxygen species (ROS), their modulation through glutathione metabolism supported by whey proteins, and the influence of chitosan on intestinal barrier function and antioxidant response pathways. The cysteine-rich composition of whey proteins is considered as a factor strengthening the substrate basis for glutathione synthesis, thereby promoting optimal GPx function and potentially reducing lipid peroxidation. Evidence is also presented that chitosan, particularly chitosan oligosaccharides, may increase SOD, CAT, and GPx activity in intestinal tissues and activate Nrf2-dependent antioxidant gene expression. Overall, the reviewed findings indicate that combined supplementation with whey powder and chitosan represents a promising approach for lowering oxidative stress, supporting mitochondrial stability, and improving immunometabolic adaptation.*

Keywords: *whey powder, chitosan, antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase, glutathione, oxidative stress, mitochondria, ROS.*

INTRODUCTION

Oxidative stress is biochemically interpreted as an imbalance between the production of reactive oxygen species and the capacity of antioxidant systems that neutralize them. When oxidative stress intensifies, lipid peroxidation increases, protein oxidation and nucleic acid damage become more pronounced, and direct impairment of cell membranes and mitochondrial function may occur. The enzymatic antioxidant defense of the organism is largely ensured by coordinated activity of SOD, CAT, and GPx; these enzymes interrupt ROS-driven chain reactions at early stages and limit downstream oxidative damage [3; 4]. Whey powder is widely used in applied nutrition and as a bioactive supplement, and its fractions—β-lactoglobulin, α-lactalbumin, and

others—are described as supporting antioxidant potential in addition to providing high biological protein value [11; 12]. Chitosan, as a natural biopolymer, has attracted scientific attention due to its ability to protect the intestinal mucosa, modulate inflammatory responses, and strengthen antioxidant defenses [15; 16]. Therefore, combined feeding with whey powder and chitosan constitutes a relevant theoretical and practical model for evaluating antioxidant enzyme activity and mitochondrial redox balance.

Main part. The biological significance of antioxidant enzymes is closely linked to controlling ROS formation at different stages. SOD converts the superoxide anion into hydrogen peroxide, thereby “attenuating” the earliest step of oxidative processes; catalase decomposes hydrogen peroxide into water and oxygen, preventing excessive accumulation of peroxides. GPx, in turn, detoxifies hydrogen peroxide and lipid hydroperoxides at the expense of glutathione; thus, GPx effectiveness directly depends on glutathione reserves and the stability of glutathione cycling [4; 5]. Reviews authored by researchers from the CIS also emphasize that this triad functions as a basic “enzymatic framework” maintaining cellular stability [3].

The impact of whey powder on antioxidant enzyme activity is largely explained by its amino acid profile, particularly cysteine. Cysteine is one of the rate-limiting substrates in glutathione synthesis, and an increase in glutathione expands the capacity of GPx to neutralize peroxides. Reviews dedicated to the antioxidant action of whey proteins systematically describe mechanisms involving the glutathione system (GSH/GSSG), as well as changes in SOD and catalase activity [11]. Experimental data at cellular and tissue levels suggest that whey protein administration may increase glutathione reserves and elevate SOD and catalase activity, which aligns with stronger protection under peroxide-induced oxidative injury [12]. In animal models exposed to physical load, whey protein has been interpreted as reducing oxidative effects while supporting muscle mass, with these outcomes explained through antioxidant system markers [13]. In addition, certain clinical and experimental studies report that whey protein intake may lower MDA (malondialdehyde) and increase “core antioxidant enzyme activity,” consistent with reduced oxidative stress markers [14]. From this perspective, whey powder may influence antioxidant enzyme systems through two complementary routes—substrate support for glutathione synthesis and signaling modulation of redox responses—thereby enhancing the overall antioxidant capacity [11; 12].

The antioxidant-related effects of chitosan, particularly chitosan oligosaccharides, can be explained through several mechanisms. First, chitosan may support intestinal barrier function and create conditions for reduced inflammation; decreased inflammatory mediator production can also reduce the intensity of ROS generation. Second, studies report that chitosan oligosaccharides can increase SOD, CAT, and GSH-Px activity in intestinal tissues and activate Nrf2-dependent antioxidant genes

[15]. Third, in various oxidative stress models, chitosan has been described as positively affecting parameters of the glutathione-dependent antioxidant system, suggesting strengthening of the “glutathione arm” of antioxidant responses [16]. Consequently, when whey protein and chitosan are used together, it is logically plausible that a synergistic effect emerges: on one side, the substrate basis for glutathione synthesis increases; on the other, the intestinal microbiota–barrier axis may limit sources of inflammation and oxidative stress.

At the mitochondrial level, regulation of oxidative stress is particularly important because the electron transport chain is one of the primary sources of ROS generation. The activity of SOD2 (mitochondrial isoform) and GPx-mediated detoxification protect mitochondria from peroxide accumulation, limit oxidation of membrane lipids, and help maintain the efficiency of ATP synthesis. Systematic analyses of oxidative stress markers in the context of protein supplementation and amino acid support indicate that under exercise and metabolic load, protein supplementation may normalize certain biomarkers, although the magnitude of effect depends on context and conditions [17]. Thus, the synergistic model of whey protein plus chitosan may also support mitochondrial stability by combining enhancement of the glutathione system with reduction of pro-oxidant burden originating from intestinal sources [11; 15].

Methodology. This article is based on a literature review. Experimental, clinical, and review-type data from national, CIS, and international sources were generalized using a comparative biochemistry approach. The analysis employed a classical biochemical model of antioxidant enzyme systems, identified limiting steps of glutathione metabolism, and considered the conceptual role of the intestinal microbiota–barrier axis in shaping oxidative stress. Theoretical and experimental approaches developed within CIS scientific schools regarding the first stage of glutathione synthesis and its enzymatic control were also taken into account [6]. In addition, commonly used methodological approaches for assessing antioxidant enzymes and interpreting their changes were considered as a general framework [12].

Analysis. Based on the aggregated literature, a coherent chain of reasoning can be outlined: the cysteine-rich nature of whey powder supports glutathione synthesis; increased glutathione reserves improve the efficiency of GPx in detoxifying peroxides; adaptive increases in SOD and catalase activity limit accumulation of superoxide and hydrogen peroxide. Evidence obtained at different levels (cell culture, poultry, liver, or muscle tissue) indicates that whey protein may increase GSH, SOD, and CAT activity and is associated with a potential reduction in oxidative injury markers [12; 13; 14]. This mechanism becomes especially relevant under sports loads, endocrine imbalance, or other oxidative stress states, since mitochondrial ROS production increases under such conditions [17].

Regarding chitosan, increased antioxidant enzyme activity in intestinal tissues and results linked to the Nrf2 pathway and expression of genes such as GPX1 have

been reported [15]. This suggests that chitosan may support antioxidant responses at the level of signal transduction as well. Reports that chitosan improves indicators of the glutathione-dependent system in various oxidative stress models complement the “substrate” mechanism associated with whey protein by adding a stabilizing, regulatory dimension [16]. Therefore, when whey protein and chitosan are combined, changes in antioxidant protection are likely to manifest not as isolated increases in a single enzyme but as a coordinated, system-level adaptation of the SOD–CAT–GPx cascade.

Results. The literature analysis indicates that feeding with whey powder and chitosan can be associated with consistent positive trends in antioxidant enzyme activity. Whey powder may enhance the amino acid substrate base supporting glutathione synthesis—especially via cysteine availability—thereby improving the functional efficiency of GPx. Chitosan, in turn, is characterized by increasing antioxidant enzyme activity in intestinal tissue and activating antioxidant gene responses (Nrf2 pathway). As a consequence of the combined approach, the likelihood of reduced oxidative stress markers, strengthened resistance to membrane lipid oxidation, and improved mitochondrial stability increases.

Conclusion. Based on available scientific sources, adaptive activation of enzymatic components of the antioxidant defense system (SOD, catalase, and GPx) may occur under feeding conditions involving whey powder and chitosan. Whey powder plays a key role by supporting glutathione synthesis and GPx efficiency due to its cysteine-rich composition, whereas chitosan contributes to strengthening antioxidant potential through intestinal barrier support and enhancement of antioxidant gene responses. This synergistic approach may serve as a scientific basis for preventive and rehabilitative nutrition concepts in conditions associated with oxidative stress (physical load, metabolic disorders, inflammatory states).

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