https://doi.org/10.52973/rcfcv-e34434 Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34434

# Effect of protein sources on the antioxidant metabolism of visceral organs of Morkaraman lambs

## Efecto de las fuentes de proteicas en el metabolismo antioxidante de los órganos viscerales de corderos Morkaraman

***Mazhar Burak Can*[1]*, *Aybuke İmik*[2]**

[1]*Bayburt Provincial Directorate of Agriculture and Forestry, Animal Health Breeding and Aquaculture Branch Directorate. Bayburt, Türkiye.*
[2]*Selcuk University, Faculty of Health Sciences, Department of Nutrition and Dietetics. Konya, Türkiye.*
**Corresponding author: drmazharburakcan@gmail.com*

### ABSTRACT

The selection of protein sources plays a significant role in meeting the dietary requirements of animals and addressing specific nutritional needs. This study was designed to determine the effects of different protein sources incorporated into lamb diets on the antioxidant metabolism of the lung, heart and kidney tissues by means of the measurement of GSH and LPO levels and SOD, CAT and GPx activities. For this purpose, 24 male Morkaraman lambs were randomly assigned to 3 groups, each of 8 animals. The dietary protein sources provided to the animals were soybean meal + safflower meal in the control group (SSG), wheat gluten in the wheat group (WG), and corn gluten in the corn group (CG). The diets fed to each group were formulated to be isonitrogenous (17% crude protein/CP) and isocaloric (2700 kcal·kg$^{-1}$ ME). In the lambs fed on the diet supplemented with wheat gluten, it was determined that SOD activity in the lung ($P$<0.05) and heart ($P$<0.01) tissues, CAT activity in the lung and heart tissues ($P$<0.01), and GPx activity in the kidney and heart tissues ($P$<0.01) had significantly increased. In the lambs fed on the diet supplemented with corn gluten, statistically significant increases were detected in the SOD activity of the lung ($P$<0.05) and heart ($P$<0.01) tissues, CAT activity of the lung, heart and kidney tissues ($P$<0.01, $P$<0.05), and GPx activity of the kidney and heart tissues ($P$<0.01, $P$<0.05). The lambs fed on the gluten-supplemented diets presented with statistically significant decreases in the LPO levels of the lung tissue ($P$<0.01, $P$<0.05), and the GSH levels of the lung, heart and kidney tissues ($P$<0.01). In result, it was ascertained that, when fed on diets supplemented with wheat gluten and corn gluten, the antioxidant metabolism of the lung, heart and kidney tissues were significantly affected in lambs.

**Key words:** Antioxidant; catalase; glutathione peroxidase; lipid peroxidation; superoxide dismutase

### RESUMEN

La selección de las fuentes de proteínas desempeña un papel importante a la hora de satisfacer los requisitos dietéticos de los animales y de atender necesidades nutricionales específicas. Este estudio se diseñó para determinar los efectos de diferentes fuentes proteicas incorporadas a las dietas de corderos sobre el metabolismo antioxidante de los tejidos pulmonar, cardíaco y renal mediante la medición de los niveles de GSH y LPO y las actividades de SOD, CAT y GPx. Para ello, se distribuyeron aleatoriamente 24 corderos machos Morkaraman en 3 grupos de 8 animales cada uno. Las fuentes de proteínas alimentarias suministradas a los animales fueron harina de soja + harina de cártamo en el grupo de control (SSG), gluten de trigo en el grupo de trigo (WG) y gluten de maíz en el grupo de maíz (CG). Las dietas suministradas a cada grupo se formularon para ser isonitrogenadas (17 % proteína bruta/CP) e isocalóricas (2700 kcal·kg$^{-1}$ EM). En los corderos alimentados con la dieta suplementada con gluten de trigo, se determinó un aumento significativo de la actividad SOD en los tejidos pulmonares ($P$<0,05) y cardíacos ($P$<0,01), de la actividad CAT en los tejidos pulmonares y cardíacos ($P$<0,01) y de la actividad GPx en los tejidos renales y cardíacos ($P$<0,01). En los corderos alimentados con la dieta suplementada con gluten de maíz, se detectaron aumentos estadísticamente significativos en la actividad SOD de los tejidos pulmonares ($P$<0,05) y cardíacos ($P$<0,01), la actividad CAT de los tejidos pulmonares, cardíacos y renales ($P$<0,01, $P$<0,05), y la actividad GPx de los tejidos renales y cardíacos ($P$<0,01, $P$<0,05). Los corderos alimentados con dietas suplementadas con gluten presentaron descensos estadísticamente significativos en los niveles de LPO del tejido pulmonar ($P$<0,01, $P$<0,05), y en los niveles de GSH de los tejidos pulmonar, cardíaco y renal ($P$<0,01). En consecuencia, se comprobó que, cuando los corderos se alimentaban con dietas suplementadas con gluten de trigo y gluten de maíz, el metabolismo antioxidante de los tejidos pulmonar, cardíaco y renal se veía significativamente afectado.

**Palabras clave:** Antioxidante; catalasa; glutatión peroxidasa; peroxidación lipídica; superóxido dismutasa

Received: 03/04/2024 Accepted: 07/05/2024 Published: 21/07/2024

## INTRODUCTION

Viability depends on the continuous proceeding of metabolic activities in the body tissues. On the other hand, health maintenance depends on the balance of these metabolic activities. Elimination of free radicals in the body by antioxidants ensures metabolic balance. Multiple factors, including among others, management conditions such as heat stress, as well as climatic factors, diseases and nutrition, are known to affect the generation of free radicals in the body [1].

In the body, there is a balance between free radicals and the antioxidant system. The disturbance of this balance in favor of free radicals results in the development of oxidative stress [2]. Cellular damage resulting from the unavoided increase of free radicals in cells disrupts the intracellular signaling pathways [3]. The free radicals generated in cells, referred to as reactive oxygen species (ROS), are either eliminated or prevented from being generated by antioxidants, such that cell protection is ensured.

Antioxidants synthesized by tissue cells may fall short or fail in eliminating free radicals generated in the body. Such cases require antioxidant supplementation. Antioxidant supplementation can be provided by incorporating certain feed additives or feedstuffs into the feed ration. Soybean meal not only contains a high level of crude protein (40–49% CP) and is rich in certain elements, but also contains several antioxidant compounds, including phenols, saponins and flavonoids [4, 5, 6]. Corn gluten is the residue of proteinaceous compounds after the removal of starch and other compounds from corn grains. Corn gluten, which is composed of nearly 60% of crude protein, is also a feedstuff rich in hydrophobic amino acids such as leucine, alanine and phenylalanine. Corn gluten is composed 60–75% protein, 15–20% residual starch, 1% crude fibre and 2% minerals [7]. Previous studies have revealed that protein peptides and hydrolysates derived from corn protein exhibit potent antioxidant activity in the elimination of free radicals [8, 9, 10, 11].

Wheat not only feeds most of the human population, but is also commonly used as a feedstuff in animal nutrition. A wheat kernel (*Triticum*) contains nearly 5.4% of gluten. Wheat gluten is composed of 70–85% of crude protein, 5–15% of carbohydrates, 3–10% of lipids and 1–2% of crude ash [12]. Recent research has shown that, given its low production cost, wheat gluten is increasingly used as an alternative to milk and soybean protein (*Glycine max*) [8, 13]. The second most common use of wheat gluten is for animal nutrition, yet there are no examples of its use for the feeding of ruminants [14]. Literature reports indicate that both wheat gluten and corn gluten have limited effect on the immunohistochemical structure of the liver and intestinal tissues [15, 16]. Nonetheless, to the authors' knowledge, there is no previous study presenting a comprehensive investigation of the effects of the use of wheat gluten and corn gluten as alternative feedstuffs on the antioxidant metabolism of tissues.

Many literature reports have been published on the incorporation of antioxidant substances into animal feed. The aim of this study was to determine the effects of different protein sources included in lamb (*Ovis aries*) diets on antioxidant metabolism of various internal organs.

## MATERIALS AND METHODS

### Animal material, experimental groups and nutrition

Twenty-four 9-month-old male Morkaraman lambs, which had similar body condition scores and live weights, were used in this study. The lambs were fed on isocaloric (ME: 2700 kcal·kg$^{-1}$) and isonitrogenous (CP 17%) diets, which were incorporated with 15.93% of soybean meal and 22% of safflower meal in Group SSG, 10.3% of wheat gluten in Group WG, and 14.78% of corn gluten in Group CG as different protein sources. After their arrival to the farm, the study animals were vaccinated against enterotoxaemia, treated for internal and external parasites, acclimatized for 21 days, and fattened for a period of 56 days. The composition of the diets provided to the lambs throughout the study period is presented in TABLE I.

***TABLE I***
**Composition of concentrate feeds containing different protein sources in lambs (%)**

| Ingredients, % | Groups | | |
|---|---|---|---|
| | SSG | WG | CG |
| Wheat gluten (75% CP) | – | 10.30 | – |
| Corn gluten (61% CP) | – | – | 14.78 |
| Soybean meal (45% CP) | 15.93 | – | – |
| Safflower meal (22% CP) | 7.47 | – | – |
| Rice bran | 10.00 | – | – |
| Barley | 60.00 | 52.50 | 60.00 |
| Wheat | – | 30.00 | – |
| Corn | – | – | 18.22 |
| Molasses | 3.00 | 3.00 | 3.00 |
| Marble dust | 2.40 | 1.65 | 2.35 |
| Dicalcium phosphate | – | 1.51 | 0.96 |
| Soy oil | 0.60 | 0.33 | – |
| Salt | 0.30 | 0.31 | 0.30 |
| Ammonium chloride | 0.20 | 0.30 | 0.28 |
| Vitamin–Mineral premix | 0.10 | 0.10 | 0.10 |
| Total | 100 | 100 | 100 |
| Crude protein, % | 17 | 17 | 17 |
| Metabolisable energy, (kcal·kg$^{-1}$) | 2700 | 2700 | 2700 |

SSG: Soybean meal–Safflower meal group. WG: Wheat gluten group. CG: Corn gluten group

### Oxidative stress and lipid peroxidation indicators in the lung, kidney and heart tissues

To obtain tissue homogenates, the lung, kidney and heart tissues were ground using liquid nitrogen. Tissues were pulverized using a homogenizer (Tissue Lyser II, Qiagen, Netherlands) with liquid nitrogen. The powdered tissues were used in all analyses. After this process, the tissues were homogenized with 1.15 % potassium chloride diluted 1:10 (w/v). The tissues were then centrifuged at +4°C and 3500 rpm for 15 min. The supernatant was analyzed by the method based on the measurement of the absorbance at 532 nm of the color formed by the reaction of malondialdehyde (MDA) with thiobarbituric acid [17]. To determine glutathione peroxidase (GPx) activity and glutathione (GSH) level in tissues, homogenates were centrifuged (NUVE NF 800R, Turkey) at 10310 G for 20 min. In the supernatants obtained, GPx activity and GSH levels in tissues were analyzed by the method of Lawrence and Burk [18] and Sedlak and Lindsay [19],

respectively. For superoxide dismutase (SOD) and catalase (CAT) activity, homogenates were centrifuged (NUVE NF 800R, Turkey) at 4392 G for 15 min at +4 °C and the supernatants were used for analysis. The method of Sun *et al.* [20] and Aebi [21] was used for the determination of SOD and CAT activities, respectively.

### Statistical Analyses

Statistical analysis of the data obtained in the study was performed using SPSS 20.0 package programme [22]. One-way analysis of variance (ANOVA) was used for statistical calculations and significance control of the difference between the mean values of the groups, and Duncan multiple comparison test was used for pairwise comparisons between groups. The results were given as mean ± standard error of the mean. In TABLE II, feed raw materials were considered as the independent variable, and tissues as the dependent variable. In Table III, feed raw materials and tissues were both treated as independent variables, while antioxidant parameters were regarded as dependent variables. General Linear Model (GLM) method was used for statistical analysis of the interaction between diet, organ and diet*organ.

The model used was: $Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + E_{ijk}$

$Y_{ijk}$ = Response variable,

$\mu$ = Population mean,

$a_i$ = Diet (soybean meal, wheat gluten and corn gluten),

$b_j$ = Organ (kidney, lung and heart),

$ab_{ij}$ = a × b interaction,

$E_{ijk}$ = Experimental error.

## RESULTS AND DISCUSSION

### Findings of antioxidant parameters in tissues

At the end of the study period, the ameliorative effects of wheat gluten and corn gluten on oxidative stress-induced damage in the lung, kidney and heart tissues were assessed by means of the measurement of the activity of the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), as well as the levels of glutathione (GSH) and malondialdehyde (MDA), all of which are part of the antioxidant defense system of the body (TABLE II and III).

When compared to Group SSG, it was found that in Groups WG and CG, SOD activity in the lung and heart tissues, CAT activity in the lung and heart tissues, and GPx activity in the kidney and heart tissues had significantly increased ($P<0.01$, $P<0.05$). In comparison to the other groups, CAT activity in kidney tissue was significantly increased in Group CG ($P<0.05$). Compared to Group SSG, Groups WG and CG displayed significantly decreased LPO levels in the lung tissue ($P<0.01$). On the other hand, heart tissue LPO levels were determined to have significantly decreased in Group CG, when compared to Groups SSG and WG ($P<0.05$). Furthermore, Groups WG and CG displayed a significant increase in GSH levels in all tissues compared to Group SSG ($P<0.01$).

Upon the analysis of Table III, it was observed that SOD and GPx activities, as well as GSH levels, decreased significantly in the SSG group compared to the WG and CG groups ($P<0.001$). Conversely, the LPO level exhibited a significant increase in the SSG group compared to the WG and CG groups ($P<0.001$). Meanwhile, CAT enzyme activity significantly decreased in the SSG group and increased significantly in the CG group ($P<0.001$). As for the organs, LPO and GSH levels, along with GPx and SOD activities, showed a significant increase in the SSG group but a significant decrease in the CG group ($P<0.001$). CAT enzyme activity significantly increased in the SSG group and significantly decreased in the WG group ($P<0.001$). The diet*organ interaction did not show any statistically significant differences between the groups ($P>0.05$).

Although the impact of diet and organs on tissue antioxidant levels was significant, the interaction between diet and organs was found to be insignificant. These findings indicate that the antioxidant effect of diet on tissues remains consistent across all organs examined (TABLE III).

Proteins are known to play a major role in the healthy development and growth of living beings. The metabolism of proteins in tissue cells varies with several factors. The primary factors affecting protein metabolism include species, management conditions, environmental conditions, feed amino acid profile, feed protein content and tissue metabolic activity. Liver tissue cells are a primary site for metabolic activity in the body. The liver tissue plays a critical role in the metabolism of dietary nutrients. Therefore, the healthy functioning of the liver tissue is vital for living beings. Although at a

***TABLE II***
**CAT, SOD and GPx activities and GSH and LPO levels in various tissues in the study groups**

| Parameters | Groups | | | *P*–value |
|---|---|---|---|---|
| | SGG Mean±SEM | WG Mean±SEM | CG Mean±SEM | |
| SOD, mmol·min$^{-1}$·mg tissue$^{-1}$ | | | | |
| Kidney | 35.97±0.60 | 38.13±0.63 | 37.84±1.01 | NS |
| Lung | 31.00±0.44$^{b}$ | 33.22±0.70$^{a}$ | 34.09±0.93$^{a}$ | <0.05* |
| Heart | 27.25±0.58$^{b}$ | 30.22±0.65$^{a}$ | 30.47±0.64$^{a}$ | <0.01** |
| CAT, mmol·min$^{-1}$·mg tissue$^{-1}$ | | | | |
| Kidney | 52.94±1.03$^{b}$ | 54.92±0.93$^{ab}$ | 56.34±1.23$^{a}$ | <0.05* |
| Lung | 36.26±0.48$^{b}$ | 38.71±1.15$^{a}$ | 40.73±0.51$^{a}$ | <0.01** |
| Heart | 41.61±1.02$^{b}$ | 44.52±0.97$^{a}$ | 47.12±0.71$^{a}$ | <0.01** |
| GPx, IU·g prot$^{-1}$ | | | | |
| Kidney | 33.31±0.65$^{b}$ | 36.32±0.77$^{a}$ | 36.29±0.75$^{a}$ | <0.05* |
| Lung | 27.09±0.84 | 29.17±0.87 | 29.67±0.93 | NS |
| Heart | 22.47±0.63$^{b}$ | 24.78±0.49$^{a}$ | 24.49±0.55$^{a}$ | <0.05* |
| LPO, nmol MDA·g tissue$^{-1}$ | | | | |
| Kidney | 21.06±0.48 | 19.70±0.36 | 19.66±0.65 | NS |
| Lung | 18.64±0.31$^{a}$ | 16.75±0.65$^{b}$ | 15.84±0.63$^{b}$ | <0.01** |
| Heart | 15.02±0.28$^{a}$ | 13.92±0.42$^{ab}$ | 13.67±0.43$^{b}$ | <0.05* |
| GSH, nmol·g tissue$^{-1}$ | | | | |
| Kidney | 4.28±0.060$^{b}$ | 4.68±0.053$^{a}$ | 4.63±0.057$^{a}$ | <0.01** |
| Lung | 4.07±0.055$^{b}$ | 4.48±0.041$^{a}$ | 4.40±0.081$^{a}$ | <0.01** |
| Heart | 3.40±0.060$^{b}$ | 3.75±0.043$^{a}$ | 3.80±0.044$^{a}$ | <0.01** |

All values are given as mean ± standard error (n=6) (SEM). $^{a,b}$: The difference between means indicated with different letters in the same row is significant (*: $P<0.05$; **: $P<0.01$). NS: Not significant, *: Significant differences at $P<0.05$ level, **: Significant differences at $P<0.01$ level. CAT: Catalase, SOD: Superoxide dismutase, GSH: Glutathione, GPx: Glutathione peroxidase, LPO: Lipid peroxidation

*TABLE III*
**Effects of Diet, Organ and Diet×Organ interaction on antioxidant enzyme activity and ratio**

| Parameters | Diet Groups | | | Organ Groups | | | Diet *P*–value | Organ *P*–value | Diet×Organ *P*–value |
|---|---|---|---|---|---|---|---|---|---|
| | SGG Mean±SEM | WG Mean±SEM | CG Mean±SEM | SGG Mean±SEM | WG Mean±SEM | CG Mean±SEM | | | |
| SOD, mmol·min$^{-1}$·mg tissue$^{-1}$ | 31.41±3.93$^{b}$ | 33.87±3.78$^{a}$ | 34.14±3.88$^{a}$ | 37.31±2.30$^{a}$ | 32.77±2.35$^{b}$ | 29.31±2.25$^{c}$ | 0.001 | 0.001 | 0.580 |
| CAT, mmol·min$^{-1}$·mg tissue$^{-1}$ | 43.61±7.50$^{c}$ | 46.05±7.39$^{b}$ | 48.07±6.96$^{a}$ | 54.73±3.23$^{a}$ | 38.56±2.81$^{b}$ | 44.41±3.37$^{c}$ | 0.001 | 0.001 | 0.801 |
| GPx, IU·g prot$^{-1}$ | 27.63±4.94$^{b}$ | 30.09±5.25$^{a}$ | 30.16±5.35$^{a}$ | 35.30±2.44$^{a}$ | 28.65±2.65$^{b}$ | 23.91±1.84$^{c}$ | 0.001 | 0.001 | 0.942 |
| LPO, nmol MDA·g tissue$^{-1}$ | 18.24±2.73$^{a}$ | 16.79±2.76$^{b}$ | 16.39±2.98$^{b}$ | 20.14±1.54$^{a}$ | 17.07±1.92$^{b}$ | 14.20±1.20$^{c}$ | 0.001 | 0.001 | 0.837 |
| GSH, nmol·g tissue$^{-1}$ | 3.92±0.41$^{b}$ | 4.31±0.43$^{a}$ | 4.28±0.39$^{a}$ | 4.53±0.23$^{a}$ | 4.32±0.24$^{b}$ | 3.65±0.22$^{c}$ | 0.001 | 0.001 | 0.862 |

All values are given as mean ± standard error (n=3) (SEM). $^{a,b}$: The difference between means indicated with different letters in the same row is significant. CAT; Catalase, SOD; Superoxide dismutase, GSH; Glutathione, GPx: Glutathione peroxidase, LPO: Lipid peroxidation

level secondary to that in the liver, metabolic activities also take place in other visceral organs. The study data provides input on the impact of nutritional strategy on antioxidant metabolism and should be taken into consideration in the development of animal nutrition strategies.

Owing to their structural and functional roles, proteins are nutrients of critical importance for animal nutrition. In particular, amino acids being the main constituents of the majority of body structures, including among others the visceral organs, muscles and hormones, makes the dietary intake of high–quality proteins a basic requirement for the sustainability of metabolic functions [23].

Free radicals generated in the body may be scavenged either by means of antioxidant substances ingested in feed, such as vitamins C and E, or by means of the defense systems of the body tissues. The enzymes SOD, GPx and CAT, and the antioxidant GSH, are the main parameters effective in preventing the accumulation of free radicals and the induction of lipid peroxidation [24, 25]. The measurement of the level of MDA, the end–product of lipid peroxidation, as well as the activities of antioxidant enzymes in the blood and tissues enables the determination of the extent and severity of oxidative damage [26]. In this respect, the present study was designed to measure SOD, CAT and GPx activities in various visceral organs (lungs, kidneys and heart), as well as GSH and MDA levels, the last having been selected for the detection of the severity of lipid peroxidation.

The enzyme SOD is the first line of defense against reactive oxygen species in the body [27, 28]. Described as being essential to the organism, SOD is the enzyme that is first activated within the antioxidant system. Acting as a catalyzer, it facilitates the dismutation of the toxic superoxide anion into hydrogen peroxide ($H_2O_2$) and molecular oxygen ($O_2$). Jiang *et al.* [29] reported that the incorporation of 5% of fermented corn gluten into the feed of weaned Holstein calves increased SOD activity. Fang *et al.* [30] determined that, the replacement of soybean meal by 2% of wheat gluten in the diet of broiler chickens did not cause any difference on day 21 due to the glutamic acid content of the feed, but reduced oxidative stress by day 42, although insignificantly.

On the contrary to most studies, in research conducted by Han *et al.* [31] on weaned piglets, no change having been determined to occur in SOD activity with the incorporation of 2% of wheat gluten into the soybean meal–containing basal diet was attributed to the oxidative stress factors not having been induced. On the other hand, the group fed on a diet incorporated with enzymatically hydrolyzed gluten displayed significantly increased SOD activity on the 28$^{th}$ day of the trial. In the present study, the replacement of the soybean meal–containing basal diet by diets supplemented with wheat gluten and corn gluten did not result in any effect on the kidney tissue.

However, dietary supplementation with gluten was associated with increased SOD activity in the heart and lung tissues, resulting from cellular damage. Gao *et al.* [32] reported that extracellular SOD, synthesized and secreted by fibroblasts, glial cells and endothelial cells, occurred at high levels in the pulmonary epithelial cells, as well as in the smooth muscle cells of the respiratory ducts and blood vessels. These researchers also described SOD as the only antioxidant capable of inactivating oxygen at the extracellular level, and highlighted its important role in protection from several pulmonary disorders, including among others oxidative damage, inflammation and fibrosis. In this respect, the present study having demonstrated lung tissue SOD activity to be significantly affected by dietary supplementation with wheat gluten and corn gluten is considered to be an interesting finding.

Catalase is found in various cell organelles, primarily the mitochondria and endoplasmic reticulum (ER). Despite being found at low levels in the brain, skeletal muscle and heart, the enzyme catalase is found at high levels in the bone marrow, blood, kidneys and liver [33]. CAT is a highly active enzyme, which acts as a catalyzer for the dismutation of hydrogen peroxide into water ($H_2O$) and oxygen ($O_2$).

The generation of hydrogen peroxide, in response to the effect of microbial or pathogenic stimulants, causes either oxidative damage or oxidative injury [34]. The maintenance of hydrogen peroxide at low levels by means of catalase activity contributes to the regulation of various physiological processes such as mitochondrial function, signal transfer and carbohydrate metabolism [35]. Jiang *et al.* [29] reported that the replacement of soybean meal by corn gluten in the basal diet of Holstein calves increased CAT activity. The present study showed that while both wheat gluten and corn gluten increased CAT activity in the lung and heart tissues, corn gluten was more effective than wheat gluten in inducing the antioxidant defense system in the kidney tissue.

The enzyme GPx, found in the cytoplasm, enables the reduction of intracellular hydrogen peroxide by means of the glutathione reaction, prevents hydrogen peroxide–induced oxidative damage, and thereby, avoids the generation of the hydroxyl radical (OH) [36]. Glutathione peroxidase is also responsible for the elimination of several peroxides, including hydrogen peroxide. In their study on weaned piglets, Han *et al.* [31] determined that while the incorporation of 2% of wheat gluten into a basal diet containing soybean meal did not alter serum GPx activity, dietary supplementation with enzymatically hydrolyzed wheat gluten (HWG) significantly increased enzyme activity.

Furthermore, in a study by Fang *et al.* [30] on broiler chickens, the replacement of soybean meal by 2% of wheat gluten was observed not to cause any change in serum GPx activity on days 21 and 42. Likewise, in agreement with the previous studies referred to above, the present study showed that dietary supplementation with wheat gluten and corn gluten did not affect GPx activity in the lung tissue [30, 31]. However, GPx activity was increased in kidney and heart tissues by dietary supplementation of both corn gluten and wheat gluten. Thus, it was ascertained that the different protein sources incorporated into the diets of the lambs affected the GPx activity of the various tissues at different levels.

Lipid peroxidation is defined as the breakdown of free radicals into by-products by means of their entering into reaction with unsaturated fatty acids [37]. In such cases, free radicals cause damage to the proteins found in the structure of the cell membrane, which in return, leads to decrease in membrane permeability, as well as enzyme and cell permeability [38]. Malondialdehyde (MDA), which is generated as a result of the peroxidation of fatty acids and is of toxic nature, is considered one of the main products of lipid peroxidation (LPO).

The increase of the level of free radicals to a point, which cannot be compensated by cells, indicates the failure of the antioxidant defense system in preventing oxidative stress [24]. Differently, Fang *et al.* [30] reported that the replacement of soybean meal by 2% of wheat gluten in broiler chicken feed did not cause any alteration in serum MDA concentrations on days 21 and 42. Liao *et al.* [39] reported that oxidized wheat gluten added to the diet triggers oxidative stress, which led to an increase in MDA levels in the crop. In our study, MDA concentrations in the lung and heart tissues having increased in the lambs fed on the control basal ration containing soybean meal showed that these animals had developed oxidative stress.

While glutathione (GSH) is mostly found in the cytoplasm, after being synthesized, part of it may be found in the mitochondria, nucleus, peroxisomes and ER [40]. Known to be synthesized in many eukaryotic cells, GSH occurs at abundantly high levels. This antioxidant detoxifies lipid peroxides and hydrogen peroxide, and by means of its catalytic effect, eliminates singlet oxygen and the hydroxyl anion. GSH is mainly synthesized in the liver and 40% of it is eliminated from the body in bile [36]. The non-enzymatic antioxidant GSH is involved in several cellular and metabolic functions in the body. Although primarily acting as an antioxidant, it also undertakes different tasks in detoxification, oxidation-reduction (redox) reactions, the signaling mechanism, apoptosis and gene expression [41]. GSH also serves in amino acid transport and the regulation of vitamins E and C as part of cellular metabolic processes [42]. More than 99% of GSH, which is described as a major antioxidant, is found in reduced form within cells [43]. Showing a reductant function, GSH is also involved in the protection of intracellular molecules, including proteins, cysteine and coenzyme A, as well as antioxidants such as ascorbate and a-tocopherol. Research has shown that when ingested, glutathione and its precursors prevent the development of several pathophysiological disorders or enable the regression of these disorders to a former stage [44]. Ölmez *et al.* [45] reported that when Tuj (Tushin) lambs were given a SOD-rich dietary antioxidant supplement (30 g/t feed), their GSH levels increased. In the present study, when compared to the control group, the groups fed on diets supplemented with wheat gluten and corn gluten (Groups WG and CG) displaying significantly increased GSH levels in the lung, kidney, and heart tissues showed that these supplements were effective in preventing free radical-induced oxidative damage.

## CONCLUSIONS

Knowledge on the metabolism of feedstuffs used for animal nutrition is critical to the development of effective nutrition strategies. This study demonstrated the effects of soybean meal, wheat gluten and corn gluten, used as different dietary protein sources, on the antioxidant metabolism of the heart, lung and kidney tissues. A notable finding was the decrease in MDA levels with wheat gluten and corn gluten supplementation. Wheat gluten and corn gluten having been determined to show varying effects on the antioxidant metabolism of various tissues, and other findings of this study are expected to provide a foundation for future research in this area.

### Ethical Approval

The study was approved by Ataturk University, Faculty of Veterinary Medicine Ethics Committee (Decision number: 2024/08).

### Conflict of Interest

The authors declare there is no conflict of interest.

## BIBLIOGRAPHIC REFERENCES


[1] Puppel K, Kapusta A, Kuczyńska B. The etiology of oxidative stress in the various species of animals, a review. J. Sci. Food. Agric. [Internet]. 2015; 95(11):2179-2184. doi: https://doi.org/f7jdnq

[2] Halliwell B. Antioxidant defence mechanism: from the beginning to the end (of the beginning). Free Radic. Res. [Internet]. 1999; 31(4):261-272. doi: https://doi.org/dhq3mn

[3] López-Alarcón C, Denicola A. Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. Anal. Chim. Acta. [Internet]. 2013; 763:1-10. doi: https://doi.org/f4njsc

[4] Banaszkiewicz T. Nutritional Value of Soybean Meal. In: El-Shemy H, editor. Soybean and Nutrition [Internet]. London: IntechOpen Limited; 2011. p. 1-20. doi: https://doi.org/m8q4

[5] Silva-de Oliviera F, Perrone D. Characterization and stability of bioactive compounds from soybean meal. LWT Food Sci. Technol. [Internet]. 2015; 63(2):992-1000. doi: https://doi.org/mkgh

[6] Singh P, Krishnaswamy K. Sustainable zero-waste processing system for soybeans and soy by-product valorization. Trends Food Sci. Technol. [Internet]. 2022; 128(10):331-344. doi: https://doi.org/grrg44

[7] Zhang R, Ma S, Li L, Zhang M, Tian S, Wang D, Liu K, Liu H, Zhu W, Wang X. Comprehensive utilization of corn starch processing by-products: A review. Grain Oil Sci. Technol. [Internet]. 2021; 4(3):89-107. doi: https://doi.org/gn6n7q

[8] Li G, Liu W, Wang Y, Jia F, Wang Y, Ma Y, Gu R, Lu J. Functions and applications of bioactive peptides from corn gluten meal. Adv. Food Nutr. Res. [Internet]. 2019; 87:1-41. doi: https://doi.org/mkgf

[9] Li XX, Han LJ, Chen, LJ. In vitro antioxidant activity of protein hydrolysates prepared from corn gluten meal. J. Sci. Food Agric. [Internet]. 2008; 88(9):1660-1666. doi: https://doi.org/fstq9s

[10] Wang Y, Chen H, Wang X, Li S, Chen Z, Wang J, Liu, W. Isolation and identification of a novel peptide from zein with antioxidant and antihypertensive activities. Food Func. [Internet]. 2015; 6(12):3799–3806. doi: https://doi.org/gjvsf4

[11] Yang Y, Tao G, Liu P, Liu JIA. Peptide with angiotensin I–converting enzyme inhibitory activity from hydrolyzed corn gluten meal. J. Agric. Food Chem. [Internet]. 2007; 55(19):7891–7895. doi: https://doi.org/fmzwxt

[12] Apper–Bossard E, Feneuil A, Wagner A, Respondek F. Use of vital wheat gluten in aquaculture feeds. Aquatic Biosyst. [Internet]. 2013; 9(1):21. doi: https://doi.org/gcbmcp

[13] Ooms N, Delcour JA. How to impact gluten protein network formation during wheat flour dough making. Curr. Opin. Food Sci. [Internet]. 2019; 25:88–97. doi: https://doi.org/gsbdg8

[14] Day L, Augustin MA, Batey IL, Wrigley CW. Wheat–gluten uses and industry needs. Trends Food Sci. Technol. [Internet]. 2006; 17(2):82–90. doi: https://doi.org/ckctn2

[15] Gümüş R, Terim Kapakin KA, Manavoğlu Kirman E, Bolat İ, İmik A, Ercan N. The effect of adding wheat and corn gluten to the diet of rats on the autoimmune and histopathological parameters in the intestine and liver. Rev. Cient. FCV–LUZ. [Internet]. 2024; 34(1):1–9. doi: https://doi.org/mpwq

[16] Gümüş R, Uslu S, Aydoğdu U, İmik A, Ekici M. Investigation of the effects of glutens on serum interleukin–1 beta and tumor necrosis factor–alpha levels and the immunohistochemical distribution of CD3 and CD8 receptors in the small intestine in male rats. Braz. Arch. Biol. Technol. [Internet]. 2021; 64(25):e21210256. doi: https://doi.org/mjtc

[17] Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem. [Internet]. 1966; 16(2):359–364. doi: https://doi.org/b96rpj

[18] Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium–deficient rat liver. Biochem. Biophys. Res. Commun. [Internet]. 1976; 71(4):952–958. doi: https://doi.org/d3vv59

[19] Sedlak J, Lindsay RH. Estimation of total, protein–bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 1968; 25(1):192–205. doi: https://doi.org/csbsfm

[20] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin. Chem. [Internet]. 1988; 34(3):497–500. doi: https://doi.org/gj74fn

[21] Aebi H. Catalase *in vitro*. Methods Enzymol. [Internet]. 1984; 105:121–126. doi: https://doi.org/dnf7v9

[22] IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk (NY, USA): IBM Corp. 2011

[23] Boye J, Wijesinha–Bettoni R, Burlingame B. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. Br. J. Nutr. [Internet]. 2012; 108(S2):S183–S211. doi: https://doi.org/gk6jhk

[24] Bratovcic A. Antioxidant enzymes and their role in preventing cell damage. Acta Sci. Nutr. Health. [Internet]. 2020; 4(3):01–07. doi: https://doi.org/mpwc

[25] Gümüş R, Erol HS, Imik H, Halici M. The effects of the supplementation of lamb rations with oregano essential oil on the performance, some blood parameters and antioxidant metabolism in meat and liver tissues. Kafkas Univ. Vet. Fak. Derg. [Internet]. 2017; 23(3):395–401. doi: https://doi.org/mp4r

[26] Marrocco I, Altieri F, Peluso I. Measurement and clinical significance of biomarkers of oxidative stress in humans. Oxid. Med. Cell. Longev. [Internet]. 2017; 6501046. doi: https://doi.org/gmpjns

[27] Sen S, Chakraborty R. The role of antioxidants in human health. In: Andreescu S, Hepel M, editors. Oxidative Stress: Diagnostics, Prevention, and Therapy [Internet]. Washington (DC, USA): American Chemical Society; 2011. p. 1–37. (ACS Symposium Series). doi: https://doi.org/c988zc

[28] Gümüş R, Kara A, Özkanlar S, İmik H, Celep NA. Effects of dietary thyme and rosemary essential oils on biochemical parameters, anti–oxidant metabolism, small intestinal morphology and myofiber structure of superficial pectoral and biceps femoris muscles in broilers. Vet. Res. Forum. [Internet]. 2023; 14(5):249–257. doi: https://doi.org/mp4q

[29] Jiang X, Liu X, Liu S, Li Y, Zhao HB, Zhang YG. Growth, rumen fermentation and plasma metabolites of Holstein male calves fed fermented corn gluten meal during the postweaning stage. Anim. Feed Sci. Technol. [Internet]. 2019; 249:1–9. doi: https://doi.org/mkgc

[30] Fang J, Martínez Y, Deng C, Zhu D, Peng H, Jiang H, Li A. Effects of dietary enzymolysis products of wheat gluten on the growth performance, serum biochemical, immune, and antioxidant status of broilers. Food Agric. Immunol. [Internet]. 2017; 28(6):1155–1167. doi: https://doi.org/mkf9

[31] Han F, Wang Y, Wang W, Cheng F, Lu Z, Li A, Xue X, Zeng Q, Wang J. Effects of enzymatically hydrolyzed wheat gluten on growth performance, antioxidant status, and immune function in weaned pigs. Can. J. Anim. Sci. [Internet]. 2017; 97(4):574–580. doi: https://doi.org/mkgb

[32] Gao F, Kinnula VL, Myllärniemi M, Oury TD. Extracellular superoxide dismutase in pulmonary fibrosis. Antioxid. Redox Signal. [Internet]. 2008; 10(2):343–354. doi: https://doi.org/b6tn3x

[33] Ścibior D, Czechot H. [Catalase: Structure, Properties, Functions]. Postepy Hig. Med. Dosw. [Internet]. 2006 [cited 20 Feb 2024]; 60:170–180. Polish. Available in: https://goo.su/ERAiXA PubMed PMID: 16618987.

[34] Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, Dhama K. Oxidative stress, prooxidants, and antioxidants: the interplay. BioMed Res. Int. [Internet]. 2014; 761264. doi: https://doi.org/gbfz3r

[35] Sies, H. Role of metabolic $H_2O_2$ generation: redox signaling and oxidative stress. J. Biol. Chem. [Internet]. 2014; 289(13):8735–8741. doi: https://doi.org/f53mvd

[36] Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D, Sharifi–Rad J. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Front. Chem. [Internet]. 2023; 11:1158198. doi: https://doi.org/mpwj

[37] Welker T, Congleton J. Effect of dietary α-tocopherol + ascorbic acid, selenium, and iron on oxidative stress in sub-yearling Chinook salmon (*Oncorhynchus tshawytscha* Walbaum). J. Anim. Physiol. Anim. Nutr. [Internet]. 2009; 93(1):15–25. doi: https://doi.org/bbd5sg

[38] Maritim AC, Sanders RA, Watkins III JB. Diabetes, oxidative stress, and antioxidants: a review. J. Biochem. Mol. Toxicol. [Internet]. 2003; 17(1):24–38. doi: https://doi.org/bw9t4d

[39] Liao R, Hua Y, Liu G, Zhang S, Chang W, Liu W, Lin CH, Huang XY, Cai H. Effect of gut stress induced by oxidized wheat gluten on the growth performance, gut morphology and oxidative states of broilers. J. Anim. Physiol. Anim. Nutr. [Internet]. 2018; 102(2):e849–e855. doi: https://doi.org/mp4p

[40] Kalinina E, Chernov N, Novichkova M. Role of glutathione, glutathione transferase, and glutaredoxin in regulation of redox-dependent processes. Biochemistry. [Internet]. 2014; 79(13):1562–1583. doi: https://doi.org/f69ztq

[41] Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed. Pharmacother. [Internet]. 2003; 57(3–4):145–155. doi: https://doi.org/fwwd74

[42] Aoyama, K. Glutathione in the Brain. Int. J. Mol. Sci. [Internet]. 2021; 22(9):5010. doi: https://doi.org/gn2mhk

[43] Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. Mitochondrial glutathione, a key survival antioxidant. Antioxid. Redox Signal. [Internet]. 2009; 11(11):2685–2700. doi: https://doi.org/cqfv8t

[44] Aksoy Y. [The Role of Glutathione in Antioxidant Mechanism] [Internet]. Türkiye Klinikleri J. Med. Sci. 2002 [cited 25 Feb 2024]; 22(4):442–448. Turkish. Available in: https://goo.su/PT9Jued

[45] Ölmez M, Riaz R, Karadağoğlu Ö, Şahin T, Şerbetçi İ, Yılmaz B, Uysal S, Yörük MA. Effect of SOD-Rich Melon Supplement on Performance, Serum Biochemical, Antioxidant and Meat Quality Characteristics of Tuj Lambs. Agriculture. [Internet]. 2023; 13(3):625. doi: https://doi.org/mkgg