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# The effect of adding wheat and corn gluten to the diet of rats on the autoimmune and histopathological parameters in the intestine and liver

## El efecto de la adición de gluten de trigo y maíz a la dieta de ratas sobre los parámetros autoinmunes e histopatológicos en el intestino y el hígado

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**ABSTRACT**

This study investigated the histopathological and immunohistochemical effect on the intestine and liver tissues with addition of the soybean meal (SBM), wheat Gluten meal (WGM) and Corn gluten meal (CGM) to rat diet. A total of 24 average twenty–day–old male rats (Wistar albino) were used in the study. The rats were randomly divided into 3 groups with 8 animals in each group (Control, Wheat and Corn groups). The diet provided to all three groups contained proteins, which were SBM, WGM and CGM in the Control, Wheat and Corn groups, respectively. In the study, the group fed with SBM was used as the Control group. Rats were fed a diet containing 22% crude protein and 2,598 kcal·kg$^{-1}$ metabolic energy throughout the experimental period. The feeding trial was continued for a period of 50 days. Degenerative changes of varying severity in intestinal epithelial cells and atrophy in villi were observed. Similarly, the degenerative changes, especially vacuolar or hydropic degeneration were determined in hepatocytes. It was determined that the CD4 level were statistically significantly increased in the Wheat and Corn groups compared to the Control group ($P$<0.01) on intestine tissue. Also, it was determined that the IgA level was statistically significantly increased of the Wheat and Corn groups in liver tissue. ($P$<0.05). As a result, it was observed that the histopathological and immunohistochemical parameters of the intestine and liver tissues of the rats fed with diets containing highly WGM and CGM were limitedly affected.

**Key words:** Autoimmune; gluten; histopathology; intestine; liver

**RESUMEN**

Este estudio investigó el efecto histopatológico e inmunohistoquímico en los tejidos del intestino y el hígado con la adición de harina de soja (SBM), harina de gluten de trigo (WGM) y harina de gluten de maíz (CGM) a la dieta de ratas. En el estudio se utilizaron un total de 24 ratas macho (Wistar albino) de veinte días de edad promedio. Las ratas se dividieron aleatoriamente en 3 grupos con 8 animales en cada grupo (grupos Control, Trigo y Maíz). La dieta proporcionada a los tres grupos contenía proteínas, que eran SBM, WGM y CGM en los grupos Control, Trigo y Maíz, respectivamente. En el estudio, el grupo alimentado con SBM se utilizó como grupo Control. Las ratas fueron alimentadas con una dieta que contenía 22% de proteína cruda y 2.598 kcal·kg$^{-1}$ de energía metabólica durante todo el período experimental. La prueba de alimentación continuó durante un período de 50 días. Se observaron cambios degenerativos de diversa gravedad en las células epiteliales intestinales y atrofia de las vellosidades. De manera similar, en los hepatocitos se determinaron los cambios degenerativos, especialmente la degeneración vacuolar o hidrópica. Se determinó que el nivel de CD4 aumentó de manera estadísticamente significativa en los grupos de trigo y maíz en comparación con el grupo de control ($P$<0,01) en el tejido intestinal. Además, se determinó que el nivel de IgA aumentó estadísticamente de manera significativa en el tejido hepático de los grupos Trigo y Maíz. ($P$<0,05). Como resultado, se observó que los parámetros histopatológico e inmunohistoquímico de los tejidos del intestino y del hígado de las ratas alimentadas con dietas que contenían un alto contenido de WGM y CGM se vieron afectados de forma limitada.

**Palabras clave:** Autoinmune; gluten; histopatología; intestino; hígado

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## INTRODUCTION

Cereals are one of the main foods for both humans and animals. Wheat *(Triticum aestivum L.)* and corn *(Zea mays L.)*, which are the main cereal products, are among the most important food products in the World that are grown and consumed Worldwide [1]. Protein in wheat consists of 10–15% of albumin/globulin and 85–90% of glüten [2]. Gluten is a complex mixture of hundreds of different proteins, primarily gliadin and glutenin. Gliadin and glutenin proteins are termed prolamins, which represent seed proteins that are insoluble in water but can be extracted in aqueous ethanol and are characterized by high levels of glutamine (38%) and proline residues (20%) [2, 3]. Corn is the most produced product among cereals after wheat, and corn gluten meal obtained from corn is accepted as a high quality protein source with desired functionality for food application [4]. Corn gluten contains approximately 62–74% zein as a protein fraction [5].

Wheat gluten is the main factor that causes some diseases and allergies in living beings with carrying the HLA-DQ2/8 genes [6]. Due to the harmful immune response to the gluten proteins found in wheat, cases of gluten intolerance have been reported which people cannot tolerate wheat consumption [7]. The most common disorders associated with gluten intake are celiac disease, gluten intolerance, non-celiac gluten sensitivity (NCGS), wheat allergy, and dermatitis herpetiformis [3]. It has been reported that gluten causes intestine and liver damage with an increase in the transglutaminase enzyme and gliadin level due to liver dysfunctions plays a role in the pathogenesis in celiac patients with an autoimmune disease [8, 9].

This study was aimed to determine the effects of glutens (WGM and CGM), incorporated as protein sources into rats *(Wistar albino)* diet, on histopathological and immunohistochemical effects on intestine and liver tissues.

## MATERIALS AND METHODS

### Animals, experimental design and diets

The experimental protocol of the study was approved by the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee's decision dated 2021 and numbered 462.

A total of 24 average twenty-day-old male rat (Wistar albino) were used in the experiment. The rats were randomly divided into 3 groups with 8 animals in each group. The feed diets provided to all three groups contained of proteins, which were SBM (24.85%), WGM (24.85%) and CGM (16.80%) in the Control, Wheat and Corn groups, respectively (TABLE I). The feeding trial was continued for a period of 50 days. Feed and water were provided *ad libitum*. The animals were housed at the comfort temperature (22°C) and were fed on a diet containing 22% of crude protein and 2,598 kcal·kg$^{-1}$ of metabolic energy throughout the experiment period.

### Histopathological examination

At the end of the study eight animals from each group were sacrificed under anesthesia. The tissue samples were fixed in 10% buffered formalin and routinely processed for histological examination by embedding in paraffin wax. Tissue sections were cut 4 µm in thickness (Leica RM2125 RTS microtome) and stained by the Haematoxylin-Eosin for observation under a light microscope (Olympus Bx51 with a DP72, Tokyo, Japan) [10].

***TABLE I***
**Contents and nutrient composition of diets used in the study (%)**

| Composition (%) | Groups | | |
|---|---|---|---|
| | Control | Wheat | Corn |
| Wheat bran | 3.24 | 1.8 | 4.55 |
| Oat, 11% CP[1] | 62.11 | 68 | 64.00 |
| Sunflower meal, 28% CP[1] | 6 | 13 | 13 |
| Corn gluten meal, 62% CP[1] | – | – | 16.80 |
| Wheat gluten meal, 75% CP[1] | – | 24.85 | – |
| Soybean meal, 51% CP[1] | 24.85 | – | – |
| Animal fat | 2.8 | 2.2 | 0.65 |
| Vitamin-mineral premix* | 1 | 1 | 1 |
| *Nutrient composition (calculated)* | | | |
| Metabolisable energy, (kcal·kg$^{-1}$) | 2598 | 2598 | 2598 |
| Crude protein (%) | 22 | 22 | 22 |

*The vitamin-mineral premix provides the following (per kg): vitamin A: 6,000,000 IU, vitamin D3: 800,000 IU, vitamin E: 8,000 mg, vitamin K3: 2,000 mg, vitamin B1: 1,200 mg, vitamin B2: 3,000 mg, vitamin B6: 2,000 mg, vitamin B12: 8 mg, niacin: 10,000 mg, folic acid: 400 mg,; d-biotin: 20 mg, choline chloride: 160,000 mg, manganese: 32,000 mg, iron: 16,000 mg, zinc: 24,000 mg, copper: 2,000 mg, iodine: 800 mg, cobalt 200 mg, selenium: 60 mg, Cal-D-Pan: 4,000 mg, antioxidant: 4,000 mg. [1]CP: Crude protein.

### Image analysis

Tissue sections were evaluated by high-power light microscopic examination using an Olympus Bx51 with a DP72 (Tokyo, Japan) camera system. Each specimens were examined in 10 randomly selected areas with an 40× objective. The scores were derived semi-quantitatively using light microscopy on the preparations from each rat and were reported as follows: Grade 0 = - (negative); Grade 1 = +1 (mild); Grade 2 = +2 (moderate); Grade 3 = +3 (severe); Grade 4 = +4 (most severe) [11].

### Immunohistochemical examinations

Four µm sections from all of the tissue samples were cut and processed for immunohistochemical examination by a standard avidin-biotin-peroxidase method that the producer described. Rabit policlonal antibodies that react with rat transglutaminase 2 (TG2) (Catalog No:NB600-547), gliadin (Catalog No: BS-13374-R), IgA (Catalog No: BS-0648-R10491-R), IgG (Catalog No: BS- 0392-R), CD4 (Catalog No: BS-0647R, ThermoFisher), and CD8 antibodies (Catalog No: BS-0648-R) were used for 60 min. A secondary antibody was used according to the manufacturer's protocol (expose mouse and rabbit-specific HRP/DAB detection IHC Kit, Abcam Cat. No. ab80436, Cambridge, UK). After three washes with 0.1% Tween 20 in PBS, the sections were incubated with 3,3-diaminobenzidine (Dako Cytomation, Glostrup, Denmark) and counterstained with Mayer's hematoxylin (Dako Cytomation) [12, 13].

### Statistical analysis

For all analyses, SPSS® 22.0 (IBM, New York, ASA) for Windows was used and $P<0.05$ was considered significant [14]. The nonparametric Kruskal-Wallis test was used to detect the differences for histopathological and immunohistochemical parameters. The results obtained in this study are expressed as mean ± standard error of the mean (SEM).

## RESULTS AND DISCUSSION

### Histopathological findings in intestine tissue

Degenerative changes of varying severity in intestinal epithelial cells and atrophy in villi were observed. Inflammatory cell infiltration, mostly accompanied by lymphocytes, plasma and eosinophils and a small number of neutrophil leukocytes, was observed in the lamina propria. In addition, hyperplasia in the crypts was among the observed findings (FIG. 1).

The villous atrophy, inflammation and crypt hyperplasia parameters results are presented in FIG. 2 and TABLE II. The study results showed that villous atrophy, inflammation and crypt hyperplasia parameters ($P$>0.05) did not statistically affect with WGM and CGM supplementation (TABLE II).

### Histopathological findings in liver tissue

While degenerative changes, especially vacuolar or hydropic degeneration were observed in hepatocytes, necrosis in some

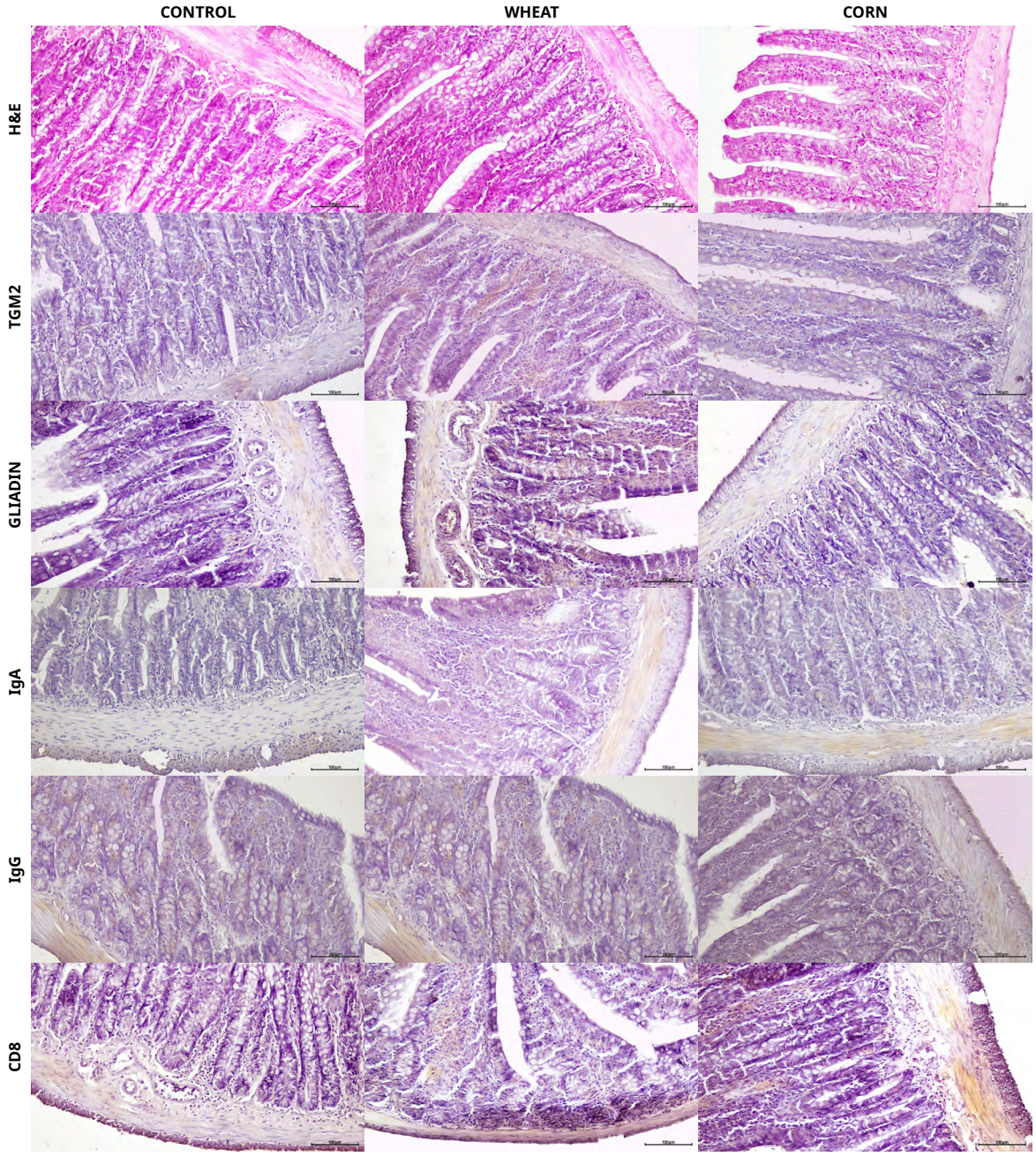


**FIGURE 1. H&E: Hematoxylin–eosin staining of the intestine tissue of experimental groups. Apperance of degenerative changes and necrosis in intestine. Bar: 20 µm. Immunohistochemistry of Transglutaminase 2/TG2, IgG, IgA and CD4 expressions in the intestine tissue of experimental groups, Bar: 20 µm**

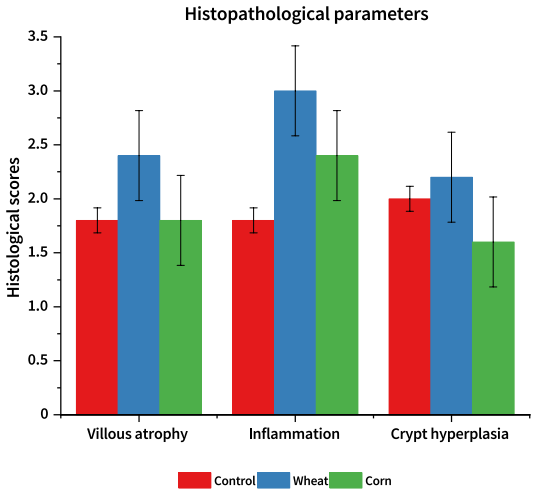


**FIGURE 2. Histological score of villous atrophy, inflammation and crypt hyperplasia parameters in intestine tissue of experimental groups. The values are given as mean ± SEM. (*P*>0.05) n=5**

***TABLE II***
**Values obtained with the hematoxylin–eosin and immunohistochemical staining of the rats intestine tissue samples**

| | Histopathological Parameters | | | Immunohistochemical Parameters | | | | |
|---|---|---|---|---|---|---|---|---|
| n | Villous atrophy | Inflammation | Crypt hyperplasia | TG2 | Gliadin | IgA | IgG | CD4 |
| | | | Control Group | | | | | |
| 1 | +2 | +2 | +2 | +1 | +1 | +1 | +1 | +1 |
| 2 | +2 | +2 | +2 | +1 | +1 | +2 | +1 | +1 |
| 3 | +2 | +2 | +2 | 0 | +1 | +1 | +1 | +1 |
| 4 | +2 | +2 | +2 | 0 | 0 | 0 | 0 | 0 |
| 5 | +1 | +1 | +2 | 0 | 0 | 0 | 0 | 0 |
| | | | Wheat Group | | | | | |
| 1 | +3 | +4 | +3 | +2 | +1 | +2 | +2 | +3 |
| 2 | +3 | +4 | +2 | +1 | +1 | +2 | +2 | +3 |
| 3 | +2 | +2 | +2 | +1 | +2 | +3 | +3 | +2 |
| 4 | +2 | +3 | +2 | +1 | +1 | +1 | +1 | +2 |
| 5 | +2 | +2 | +2 | +1 | 0 | +2 | +1 | +2 |
| | | | Corn Group | | | | | |
| 1 | +2 | +3 | +2 | +1 | +1 | +2 | +2 | +2 |
| 2 | +2 | +3 | +2 | +1 | +1 | +2 | +2 | +3 |
| 3 | +2 | +2 | +2 | +1 | +2 | +1 | +1 | +2 |
| 4 | +2 | +2 | +1 | 0 | +1 | +1 | +1 | +2 |
| 5 | +1 | +2 | +1 | 0 | 0 | 0 | 0 | +1 |

TG2: Transglutaminase 2, IgA: Immunoglobulin A, IgG: Immunoglobulin G, n=5

hepatocytes were remarkable findings. Also, lymphocytes and macrophage had infiltrated in intralobuler and portal areas and hyperplasia of bile ducts (FIG. 3).

The degeneration, inflammation and biliary hyperplasia parameters results are presented in FIG. 4 and TABLE III. The study results showed that WGM and CGM supplementation did not statistically affect degeneration, inflammation and biliary hyperplasia ($P > 0.05$)(TABLE III). However, it was determined that these parameters increased numerically in Wheat and Corn groups (FIG. 4).

***TABLE III***
**Values obtained with the hematoxylin–eosin and immunohistochemical staining of the rats liver tissue samples**

| | Histopathological Parameters | | | Immunohistochemical Parameters | | | | |
|---|---|---|---|---|---|---|---|---|
| n | Degeneration | Inflammation | Biliary hyperplasia | TG2 | IgA | IgG | CD4 | CD8 |
| | | | Control Group | | | | | |
| 1 | +2 | +1 | +2 | +1 | +1 | +1 | +1 | +1 |
| 2 | +2 | +1 | +2 | +2 | +1 | +1 | +1 | +1 |
| 3 | +2 | +2 | +2 | +1 | +1 | +1 | +1 | +1 |
| 4 | +2 | +1 | +1 | +1 | +1 | +1 | +1 | +1 |
| 5 | 0 | 0 | 0 | +1 | 0 | 0 | +1 | +1 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | +1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | Wheat Group | | | | | |
| 1 | +2 | +2 | +2 | +3 | +2 | +2 | +2 | +2 |
| 2 | +3 | +1 | +2 | +3 | +2 | +2 | +2 | +3 |
| 3 | +2 | +1 | +2 | +2 | +2 | +2 | +2 | +2 |
| 4 | +2 | +1 | +1 | +2 | +3 | +1 | +2 | +2 |
| 5 | +2 | +2 | 0 | +2 | +3 | +1 | +1 | +1 |
| 6 | +2 | 0 | +1 | +1 | +2 | +1 | +1 | +1 |
| 7 | +1 | +1 | 0 | +1 | +2 | +1 | +1 | +1 |
| 8 | 0 | 0 | +1 | 0 | 0 | 0 | 0 | 0 |
| | | | Corn Group | | | | | |
| 1 | +2 | +2 | +2 | +2 | +2 | +1 | +2 | +2 |
| 2 | +2 | +1 | +2 | +2 | +2 | +1 | +2 | +2 |
| 3 | +2 | +2 | +2 | +1 | +2 | +1 | +1 | +2 |
| 4 | +1 | +1 | +1 | +1 | +2 | +1 | +1 | +1 |
| 5 | +1 | +1 | +1 | +1 | +1 | +1 | +1 | +1 |
| 6 | +1 | +1 | +1 | +1 | +1 | +1 | +1 | +1 |
| 7 | +1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | +1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TG2: Transglutaminase 2, IgA: Immunoglobulin A, IgG: Immunoglobulin G, n=8

## Immunohistochemical findings in intestine tissue

Immunopositivity was observed in intestinal villi and crypt epithelial cells and inflammatory cells (TG2, gliadin, IgG, IgA and CD4) in all experimental groups (FIG. 1).

The TG2, gliadin, IgG, IgA and CD4 parameters results are presented in FIG. 5 and TABLE II. In the study, it was determined that CD4 parameter

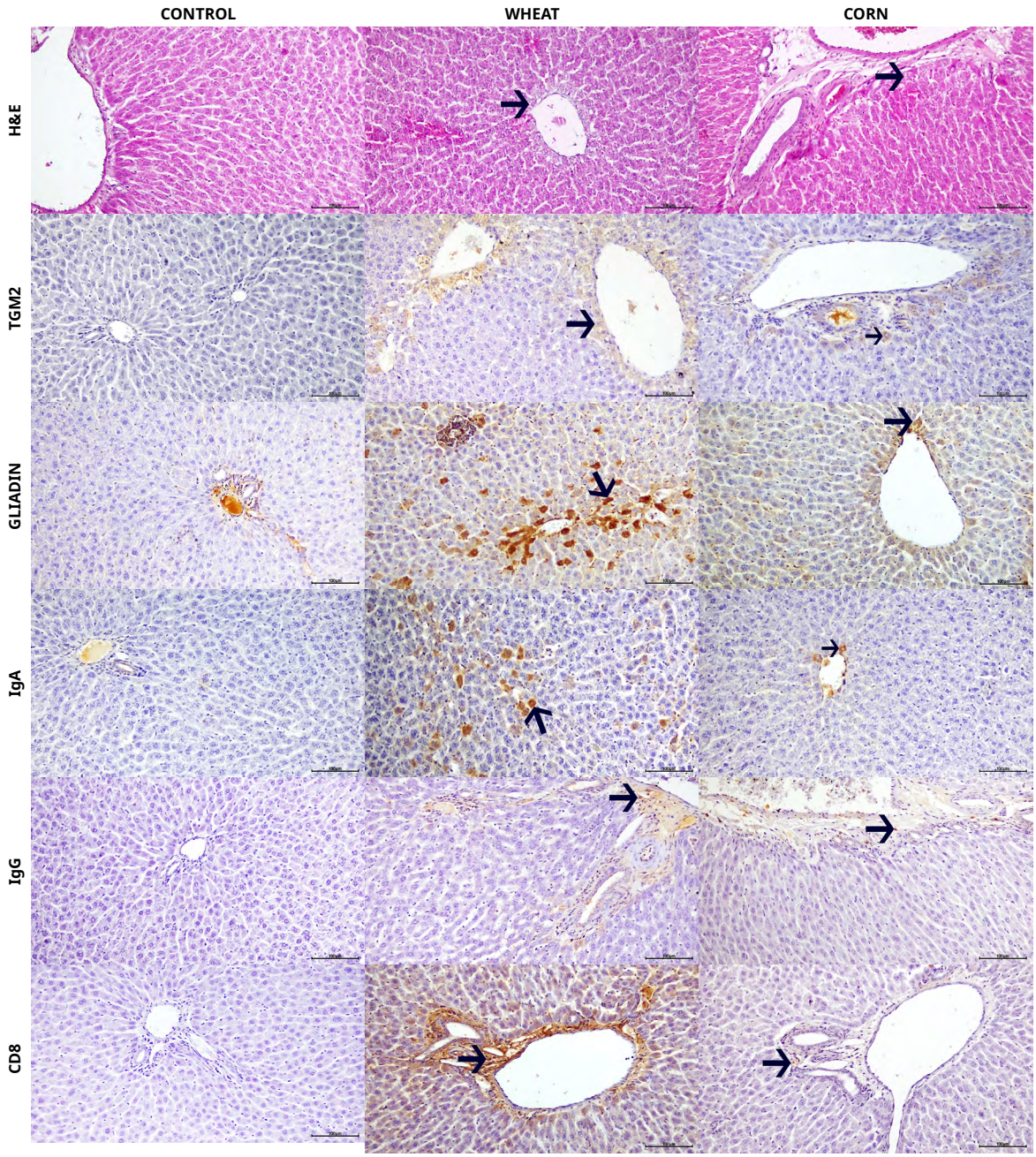


**FIGURE 3. Hematoxylin–eosin (H&E) staining of the liver tissue of experimental groups. Apperance of degenerative changes and necrosis in hepatocytes. Bar: 20 µm. Immunohistochemistry of Transglutaminase 2/TG2, IgG, IgA, CD4, and, CD8 expressions in the liver tissue of experimental groups, Bar: 20 µm**

were statistically increased in Wheat and Corn groups compared to the Control group ($P<0.01$) (TABLE II). The TG2, gliadin, IgA and IgG parameters were statistically similar to all groups ($P>0.05$) (TABLE II).

### Immunohistochemical findings in liver tissue

The TG2, IgG, IgA, CD4 and CD8 parameters results are presented in FIG. 6 and TABLE III. In the study, it was determined that IgA parameter was statistically increased ($P<0.05$), TG2, IgG, CD4 and CD8 parameters were numerically increased in Wheat and Corn groups compared to the Control group ($P>0.05$) (TABLE III).

All groups showed immunopositivity for CD4 antibody, CD8 antibody, IgA antibody, IgG antibody, and, TG2 antibody in the hepatocytes, inflammation cells, and epithelial cells of the glands (FIG. 3).

Gluten and other protein fractions, which make up the protein part of wheat, rye and barley, have antigenic properties that can trigger adverse reactions in genetically susceptible individuals [3]. Celiac

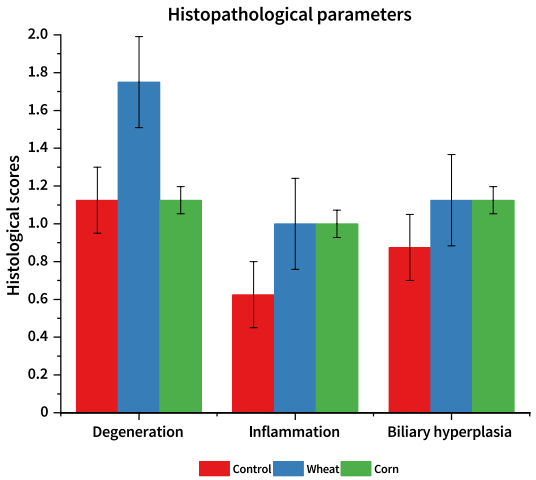


**FIGURE 4. Histological score of degeneration, inflammation and biliary hyperplasia parameters in liver tissue of experimental groups. The values are given as mean ± SEM. (*P*>0.05) n=8**

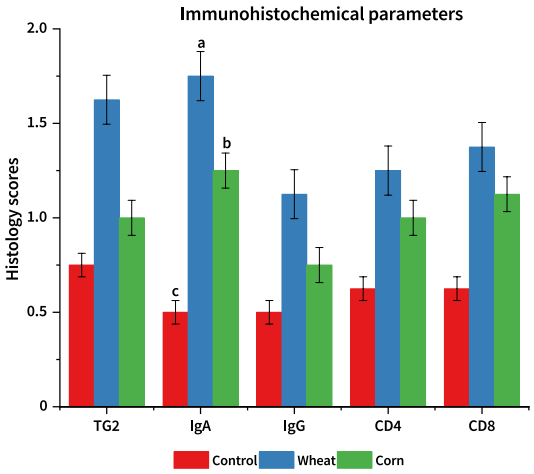


**FIGURE 6. Histological score of TG2, IgA, IgG, CD4 and CD8 parameters in liver tissue of experimental groups. TG2: transglutaminase 2. The values are given as mean ± SEM. a, b, c: Means in the column with different superscripts differ significantly, (*P*<0.05). n=8**

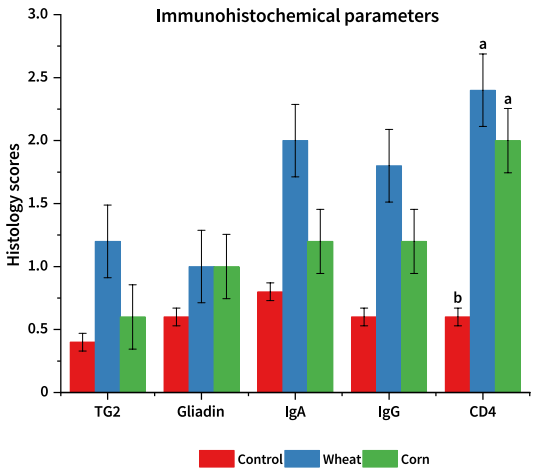


**FIGURE 5. Histological score of TG2, gliadin, IgA, IgG and CD4 parameters in intestine tissue of experimental groups. TG2: transglutaminase 2. The values are given as mean ± SEM. a, b: Means in the column with different superscripts differ significantly, (*P*<0.01). n=5**

disease was thought to only affect the intestines in previous studies. However, in recent studies, it has been reported that the disease also affects organs such as the liver, skin, brain and ovarian [13, 15]. For this reason, it is observed that celiac disease causes multiple organ disorders [16]. The use of rats that do not carry the HLA-DQ2/8 genes, which cause gluten sensitivity, and the determination of the non-celiac effects of high gluten consumption makes this study different from other studies in the literature.

The digestive system is one of the organs most affected by gluten-containing foods [17]. It is known that these health problems begin with the digestion and metabolism of gliadin and gluten in the gastrointestinal tract [18]. Dietary gluten has been reported to cause gradual villous atrophy and crypt hyperplasia of the small intestinal mucosa in celiac disease [19, 20]. It has been reported that this damage changes depending on the dose and duration of gluten ingested and causes an increase in exposure time and dose increase [21]. In a study, it was reported that intragastric gliadin administration to rats from birth to 63 days of age caused various morphological changes resembling human celiac disease, such as shortening of jejunal villi, crypt hyperplasia, and increased amount of mitosis in the crypt epithelium [22]. In another study, it was reported that in intestinal sections from duodenum and jejunum of rats were observed infiltration of immune cells in the lamina propria, edema in the villus tips, and mild inflammation characterized by macrophages and neutrophils in the intestinal lumen [23]. In this study, although there was no statistical difference between the values of villus atrophy, inflammation, and crypt hyperplasia in intestinal tissue, it can be said that these values were numerically higher in the wheat group and this effect would increase with the prolongation of gluten exposure.

Although liver damage occurs in many celiac patients, these damages are usually moderate and nonspecific signs. Nonspecific findings such as inflammatory reaction in the periportal areas, obstruction in the bile ducts, increase in the number of Kupffer cells, mononuclear cell infiltrations in the parenchyma, moderate fibrosis and fatty can be observed [24]. Severe fibrosis and cirrhosis have been reported in some cases [25]. It has been reported that liver histology typically shows a preserved architecture with mild mononuclear infiltrate and hyperplasia of Kupffer cells in the portal and lobular tract, and intraepithelial lymphocytes are also seen in the interlobular bile ducts as well as the small intestine in some studies [9]. Hyperplasia of Kupffer cells also known as celiac hepatitis is typical of nonspecific reactive hepatitis [26]. The term celiac hepatitis specifically refers to liver damage in patients with confirmed celiac disease that resolves after a gluten-free diet [27]. Degeneration, inflammation and biliary hyperplasia were observed in this study in liver tissue similar to the above findings; it was determined that the degeneration parameter increased only in the Wheat group while

the inflammation and biliary hyperplasia parameters increased numerically in both the Wheat and Corn groups compared to the Control group when these histopathological findings were examined.

Gluten-induced celiac disease may present with nonspecific hepatitis symptoms such as liver dysfunction, weakness and fatigue. However, patients are usually asymptomatic and may not have signs or symptoms of celiac disease [26]. Tissue transglutaminase is an enzyme that deamidates gliadin peptides and increases their immunogenicity [28]. Studies have shown that hypertransaminases are formed in most of the people with celiac disease [9, 29, 30] and their transglutaminase level decreases when a gluten-free diet is applied to them [9]. It has also been reported that intestinal permeability increases in patients with high transglutaminase levels in celiac patients although the liver tests are normal, but the gluten-free diet applied to the patients normalizes both intestinal permeability and transglutaminase levels [9, 31, 32]. Transaminase antibody secretion was increased, especially in endothelial cells and periportal hepatocytes, in immunohistochemical staining of liver tissues in celiac patients was reported in a study [33]. It was observed that liver tissue TG2 levels increased in the groups given wheat and corn gluten, and immunopositivity for transglutaminase antibody occurred in hepatocytes, inflammation cells and epithelial cells of glands similarly to this study.

Gliadin is one of the main proteins in gluten. The gliadin antibody test is used to determine the presence of celiac disease which an autoimmune disease, and have been reported in higher-than-normal levels of this antibody over 90% of untreated patients [8, 34]. Gliadin is presented to gliadin-reactive CD4+ T cells via a T cell receptor which resulting in the production of cytokines that cause tissue damage [35]. CD4+ T cells increase the level of T Helper 1 and T Helper 2 as a proinflammatory cytokines which causing the release of B lymphocytes and the formation of plasma cells. On the other hand plasma cells enable the release of gliadin and transglutaminase antibodies [36]. It has been stated that those with high antigliadin IgG levels represent a subgroup that may have gluten sensitivity [37]. In a study was reported that those who consume cereal-containing processed foods have higher IgG, IgA and IgM antibody responses [38]. TG2 is expressed in many organs, including the small intestine. Celiac patients on a gluten-containing diet produce TG2-specific IgA and IgG autoantibodies [20]. In this study, it was determined that TG2, IgA and IgG expressions were high in intestinal tissue. Also, in this study was observed that IgA and IgG levels increased numerically in liver tissue of Wheat and Corn groups, and immunopositivity for these antibodies occurred in hepatocytes, inflammation cells and epithelial cells of glands. These results are similar to other studies in the literature [20, 38].

Cytokines are a group of molecules that includes chemokines, interferons, interleukins, lymphokines and tumor necrosis factors have an important role in cell signaling [39]. Cytokines are produced by immune cells such as macrophages, B lymphocytes, T lymphocytes and mast cells as well as parenchymal cells [40]. Gluten-specific mucosal CD4+ T cells using the alpha/beta T-cell receptor appear to have a central role in the immunopathology of celiac disease [41]. The intestinal mucosa normally contains a large population of T lymphocytes that CD4+ T cells predominate in the lamina propria while CD8+ T cells are preferentially located in the villous epithelium (80-90%) [42]. It has been suggested that the mucosal changes found in untreated or treated patients after gluten loading are due to increased local production of certain cytokines [43]. It has been reported to induce the activation and simultaneous presence in blood of activated intestine-driven CD4+ and CD8+ T cells in treated celiac patients with loading of oral gluten [44]. In another study, it was reported that increased the number of CD8+ T and CD4+ T cells in the intestine with loading of gluten [45]. Also, it was stated that wheat gluten increased CD3 and CD8 values in the duodenal tissue of rats [46]. Similar to this study, it was observed that CD4 and CD8 levels in the liver tissue and CD4 levels in the intestines of the groups given wheat and corn gluten were increased, and immunopositivity against CD4 and CD8 antibodies was observed in hepatocytes, inflammation cells and epithelial cells of the glands. Also, similar to this study, it was reported that intragastric gliadin administration to rats from birth to 63 days of age caused an increase in the number of CD8 in the intestine and this increase may be the result of antigenic stimulation of lymphocytes in the intraepithelial compartment by gliadin [22].

## CONCLUSIONS

In conclusion, it was observed that dietary supplementation containing wheat Gluten meal and Corn gluten meal increased the CD4 parameter in the intestine tissue and the IgA parameters in the liver tissue of male rats. It was determined that it had no significant effect on other parameters. However, it was concluded that these results may be related to feeding time and may show parallelism with Gluten exposure time.

### Conflicts of interest

The authors have no declaration of competing interests.

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