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Investigation of the Effect of Wheat and Corn Gluten on Inflammation, Transglutaminase, Gliadin and IgA Levels in Healthy Rat Intestines

Sağlıklı Rat Bağırsaklarında Buğday ve Mısır Gluteninin İnflamasyon, Transglutaminaz, Gliadin ve IgA Düzeyleri Üzerine Etkisinin Araştırılması

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ABSTRACT

The aim of this study was to evaluate the effects of wheat and corn gluten on some histopathologic parameters such as villus atrophy, crypt hyperplasia, lymphocyte plasma neutrophils and immunohistochemical parameters such as trans glutaminase, gliadin and IgA in the small intestine of healthy male rats without HLA-DQ2 and HLA-DQ8 genes. In the study, 21 healthy newborn male Sprague Dawley rats were fed wheat, corn and soy with the addition of 7 rats in each group from one-day age to 60 days of age. Histopathological (villous atrophy, lymphocyte plasma neutrophil, crypt hyperplasia) and immunohistochemical (transglutaminase, gliadin, IgA) parameter analyses were performed in small intestinal tissue samples. As a result of the study, it was found that the small intestinal villus lengths of the wheat gluten group were longer than the other groups ($P < .05$). Cryptic hyperplasia was detected most in the soybean group and the lowest in the wheat group ($P < .05$). Gliadin antibody levels were found to be in the soybean group with the highest and the lowest in the wheat group ($P < .05$). In healthy male rats lacking HLA-DQ2 and HLA-DQ8 genes, the effect of wheat gluten on crypt hyperplasia and gliadin levels in small intestinal tissue was significantly lower than in soy and corn gluten groups, while its effect on villous atrophy, lymphocyte plasma neutrophil and transglutaminase was limited. In addition, the intestinal villus lengths of the wheat gluten group were significantly higher than those of the corn and soybean groups.

Keywords: IgA, gliadin, gluten, tranglutaminase, villi.

ÖZ

Bu çalışmada HLA-DQ2 ve HLA-DQ8 genlerine sahip olmayan sağlıklı erkek ratlarda buğday ve mısır gluteninin ince bağırsaklarda villus atrofisi, kript hiperplazisi, lenfosit plazma nötrofil gibi bazı histopatolojik parametreler ile trans glutaminaz, gliadin, IgA gibi immünhistokimyasal parametrelere etkisinin değerlendirilmesi hedeflenmiştir. Çalışmada, 21 adet sağlıklı yeni doğmuş Sprague Dawley cinsi erkek rat bir günlük yaştan 60 günlük yaşa kadar her grupta 7 rat olmak üzere buğday, mısır ve soya eklenerek beslenmişlerdir. İnce bağırsak doku örneklerinde histopatolojik (villöz atrofi, lenfosit plazma nötrofil, kript hiperplazi) ve immunohistokimyasal (transglutaminaz, gliadin, IgA) parametre analizleri yapılmıştır. Çalışma sonucunda buğday gluteni grubunun ince bağırsak villus uzunluklarının diğer gruplardan daha uzun olduğu saptanmıştır ($P < ,05$). Kript hiperplazisi en fazla soya grubunda, en düşük buğday grubunda tespit edilmiştir ($P < ,05$). Gliadin antikor seviyesi en yüksek soya grubunda iken en düşük buğday grubunda olduğu tespit edilmiştir ($P < ,05$). HLA-DQ2 ve HLA-DQ8 genlerine sahip olmayan sağlıklı erkek ratlarda buğday gluteninin ince bağırsak dokusunda kript hiperplazisi ve gliadin değeri soya ve mısır gluteni verilen gruplardan önemli derecede düşük olduğu belirlenirken, villöz atrofisi, lenfosit plazma nötrofil ile transglutaminaz üzerine etkisi sınırlı düzeyde kalmıştır. Ayrıca buğday gluteni verilen grubun bağırsak villus uzunlukları mısır ve soya verilen gruplardan önemli oranda yüksek olduğu tespit edilmiştir.

Anahtar Kelimeler: IgA, gliadin, gluten, tranglutaminaz, villus.

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INTRODUCTION

Cereals are important plant-based foods and wheat, rice, corn are the most consumed cereals worldwide. It also contains protein, starch, vitamins and minerals in their structure. In cereals, proteins are classified as gluten-forming and non-gluten-forming proteins, and the proteins that make up gluten are glutelin and prolamine proteins. Glutelines are called glutenin in wheat and hordenine in barley whereas prolamins are called gliadin in wheat, hordein in barley, avenin in oats, secalin in rye and zein in corn. Gluten is the main storage form of wheat proteins, which makes up 85-90% of wheat proteins, and is basically a structure consisting of gliadin and glutenin complex. It is stated that 5-20 g/day of gluten is taken with the Western diet.[1] The increase in the production and consumption of gluten-containing products has led to the awareness of gluten-related diseases. Celiac disease is seen in at least 1% of the general adult population.[2] Gluten-related diseases are classified as immune, allergic and autoimmune according to pathogenesis. While celiac disease is in the autoimmune class, gluten sensitivity is in the autoimmune and non-allergic class.[3] Gastrointestinal symptoms such as celiac disease, diarrhea, steatorrhea, abdominal distention, abdominal pain and gas, and non-gastrointestinal symptoms such as abnormal liver function tests, iron deficiency anemia, bone and skin diseases can be seen.[4] Celiac disease is usually detected by serological tests and celiac specific antibodies and diagnosed by duodenal mucosal biopsies.[5] The primary treatment for celiac disease is a gluten-free diet.[6,7] In individuals with HLA-DQ2 and HLA-DQ8 genes in celiac disease, gluten intake may cause inflammatory response and villi damage. This leads to a new anti-inflammatory response in the intestine with the release of anti-gliadin, anti-endomysial antibodies and tissue transglutaminase. Although the general population prevalence of non-celiac gluten sensitivity is not clearly known, celiac-like symptoms may occur after gluten intake and may occur in the absence of celiac specific antibodies, villi atrophy and human leucocyte antigen (HLA) change. Therefore, anti-tissue transglutaminase and endomysial antibodies are negative and there is no change in the intestinal mucosa. However, an increase in interferon (IFN)-γ and CD3+ T cells can be seen.[8,9] In a study with rats, wheat gluten was found to increase the level of CD3 and CD8 in the intestines, although not statistically significant.[10] In another study with rats, it was found that the immunohistochemical parameters CD4, CD8, IgA, gliadin and transglutaminase in ovarian tissues were lower in the wheat group than in the soy group.[11] As a result, gluten can affect histopathological and immunohistochemical structures in many tissues, especially in the intestine. In this study, it was aimed to evaluate the effect of wheat and corn gluten on some histopathological parameters such as villus atrophy, crypt hyperplasia, lymphocyte plasma neutrophil in the small intestine and immunohistochemical parameters such as transglutaminase, gliadin, IgA in healthy male rats without HLA-DQ2 and HLA-DQ8 genes.

MATERIALS AND METHODS

Animal Working Groups

The animal supply used in the study, the care and feeding of the animals during the trial period were carried out at Atatürk University Medical and Experimental Application and Research Center (ATADEM). This study was approved by the Eastern Mediterranean University Scientific Research and Publication Ethics Board Health Ethics Sub-Committee with the decision dated 18.11.2020 and numbered 2020/07.

In the study, a total of 21 healthy Sprague Dawley male rats were fed soy, corn or wheat feeds isonitratically and isocalorically according to the experimental group in which they took part for a total of 60 days, with their mothers from one day old to 30 days of age, and separately from their mothers from 30 days to 60 days of age (Table 1). At the end of sixty days, rats in the soybean group (7 pieces), corn group (7 pieces) and wheat group (7 pieces) were sacrified under general anesthesia and tissue samples were taken from their small intestines for histopathological and immunohistochemical examinations.

Table 1. Feed given to rats.

Feed Additives %	Soy	Corn	Wheat
Wheat Bran	6	5	2
Oat, 11% Crude Protein	6	5	3
Sunflower Meal, 28% Crude Protein	6	4	1
Corn Gluten, 62% Crude Protein	5	3	1
Wheat Gluten, 75% Crude Protein	6	4	2
Soy Meal, 51% Crude Protein	5	4	1
Animal Fat	6	4	2
Vitamin-Mineral Combination			
Feed Nutritional Values			
Crude Protein,%	22.0	22.0	22.0
Metabolic Energy, ccal/kg	2598.0	2657.0	2599.0
Calcium%	0.14	0.11	0.15
Methionine + Cysteine,%	0.68	0.83	0.66
Lysine,%	1.15	0.63	1.17

Histopathological Examination

The eight animals from each group were sacrificed under anesthesia end of the sixty days. The tissue samples were fixed in 10% buffered formalin and routinely processed for histological examination by embedding in paraffin wax. Tissue sections were cut (thickness 4 µm), stained (Haematoxylin-Eosin) and observed under a light microscope.[12,13]

Villi Lengths

After the small intestine tissues of rats were stained with the above-mentioned method for histopathological analysis, the villi lengths were measured with the image analysis system (Leica Q Win Standard) from the tip of the villi to the villi crypt junction.[14]

Immunohistochemical Examinations

Tissue sections (thickness 4 µm) from all of the tissue samples were processed by a standard avidin-biotin-peroxidase method which is described by producer. Rabit policlonal antibodies that react with rat transglutaminase 2 (TG2) antibody (Catalog No:NB600-547), gliadin antibody (Catalog No: BS-13374-R), IgA antibody (Catalog No: BS-0648-R10491-R) were used for 60 minutes. A secondary antibody was used in compliance with protocol of the manufacturer (expose mouse and rabbit-specific HRP/DAB detection IHC Kit, Abcam Cat. No. ab80436). Then tissue sections was washed three times with 0.1% Tween 20 in PBS and were incubated with 3,3-diaminobenzidine (Dako Cytomation) and counterstained with Mayer's hematoxylin (Dako Cytomation).[12,15]

Image Analysis

High-power light microscopic examination (Olympus Bx51 with a DP72 camera system) was used for tissue section evaluation. Each specimens were examined in 10 randomly selected areas with an X40 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).[16]

Statistical Analysis

SPSS 10.01 program was used in the statistical evaluation of the obtained findings.[17] Histopathological parameters and immunohistochemical parameters were calculated in the statistical evaluation and median values and standard error (SE) values were calculated in the soy, corn and wheat groups. One Way ANOVA was used for small intestinal villus lengths and Duncan test was used for the difference between groups. Kruskal Wallis analysis was used for changes in histopathological and immunohistochemical parameters and Duncan test was used for the difference between the groups. Statistical significance was accepted as $P < .05$.

RESULTS

Histopathological Findings

At the end of sixty days, the mean villus length of wheat group was higher than soybean and maize groups ($P < .05$) (Figure 1).

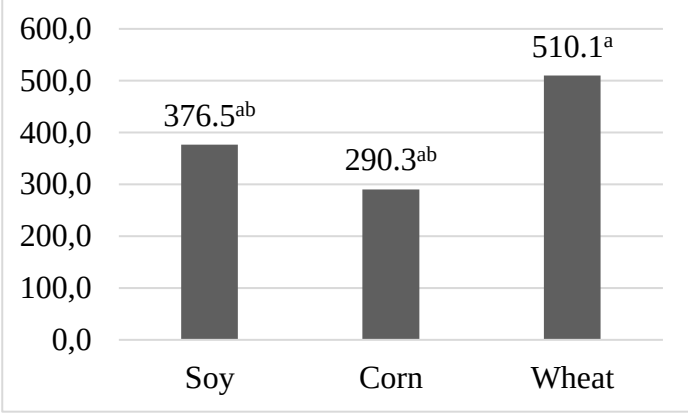

Figure1. Small intestine villus lengths (µm) [a,b] P < 0.05

Histopathological values are shown in Table 2 and the statistical results of these values are shown in Table 3. Villus atrophy was highest in the corn gluten group and lowest in the soybean group, and lymphocyte plasma neutrophil values were highest in the soybean group and lowest in the corn group ($P > .05$). Cryptic hyperplasia was highest in the soybean group and lowest in the wheat group ($P < .05$) (Table 3, Figure 2).

Immunohistochemical Findings

The immunohistochemical values of the groups are shown in Table 2 and the statistical results of these values are shown in Table 3. While there was no statistically significant difference between the transglutaminase and IgA values of the groups, the soy group with the highest transglutaminase value was determined in the wheat group with the lowest and the corn group with the highest IgA value was determined in the lowest soybean group. The highest gliadin level was found in the soybean group and the lowest in the wheat group ($P < .05$) (Table 3, Figure 3).

Table 2. Histopathological and immunochemical results of intestinal tissue.

	Histopathological			Immunochemical		
	Villous Atrophy	Lymphocyte Plasma Neutrophil	Crypt Hyperplasia	Transglutaminase	Gliadin	IgA
Soy						
1	++	+++	++	-	++	++
2	++	+++	++	+	++	-
3	+	++	++	-	++	++
4	+	+++	+	+	++	+
5	+	++	+	+	+	-
6	++	++	++	-	+++	+
7	+	+++	++	+	+	-
Corn						
1	+	++	+	-	++	++
2	++	+++	-	-	++	++
3	+	+	-	-	+	+
4	+++	+	+	-	+	+
5	+++	+	++	+	+	+
6	+++	++	-	+	+	++
7	++	+	+	+	++	++
Wheat						
1	-	+++	+	-	+	+
2	++	+	-	-	-	+++
3	+++	+++	+	-	+	+
4	+	+	-	-	+	+
5	-	+++	-	-	-	+
6	+	+	-	+	++	++
7	++	+	+	-	-	+

Table 3. Histopathological and immunohistochemical values of small intestine tissue

Parameter		Soy	Corn	Wheat	
Histopathological		$\bar{x}$±SH (median)	$\bar{x}$±SH (median)	$\bar{x}$±SH (median)	*P*
Villous Atrophy		1.42±0.202 (1.00)	2.14±0.340 (2.00)	1.50±0.428 (1.00)	.205
Lymphocyte Neutrophil	Plasma	2.57±0.202 (3.00)	1.57±0.297 (1.00)	1.66±0.421 (1.00)	.098
Crypt Hyperplasia		1.71±0.184^{a} (2.00)	0.71±0.286^{a}(1.00)	0.33 ± 0.202^{a} (0.00)	.008*
Immunohistochemical					
Transglutaminase		0.57±0.202 (1.00)	0.42±0.202 (0.00)	0.16±0.167 (0.00)	.345
Gliadin		1.85±0.261^{b} (2.00)	1.42±0.202^{bc}(1.00)	0.83±0.307^{c} (1.00)	.031*
IgA		0.85±0.340 (1.00)	1.57±0.202 (2.00)	1.50±0.341 (1.00)	.226

*,a,b,c, $P < .05$

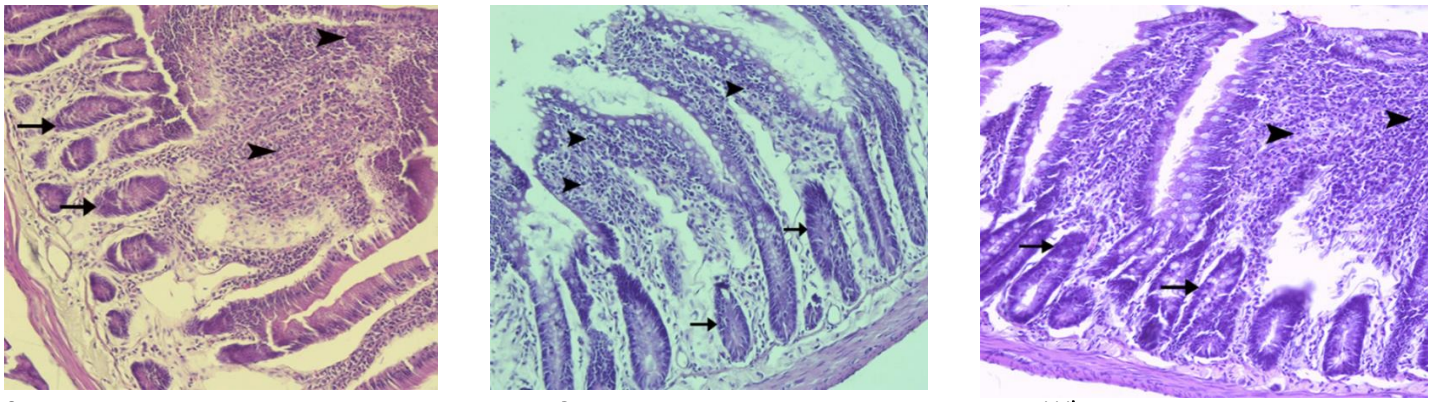

Figure 2. Hyperplasia of crypts in small intestine tissue (→)lymphocyte plasma neutrophil infiltration (➡)atrophy of villi (H&E, Bar: 100 µm)

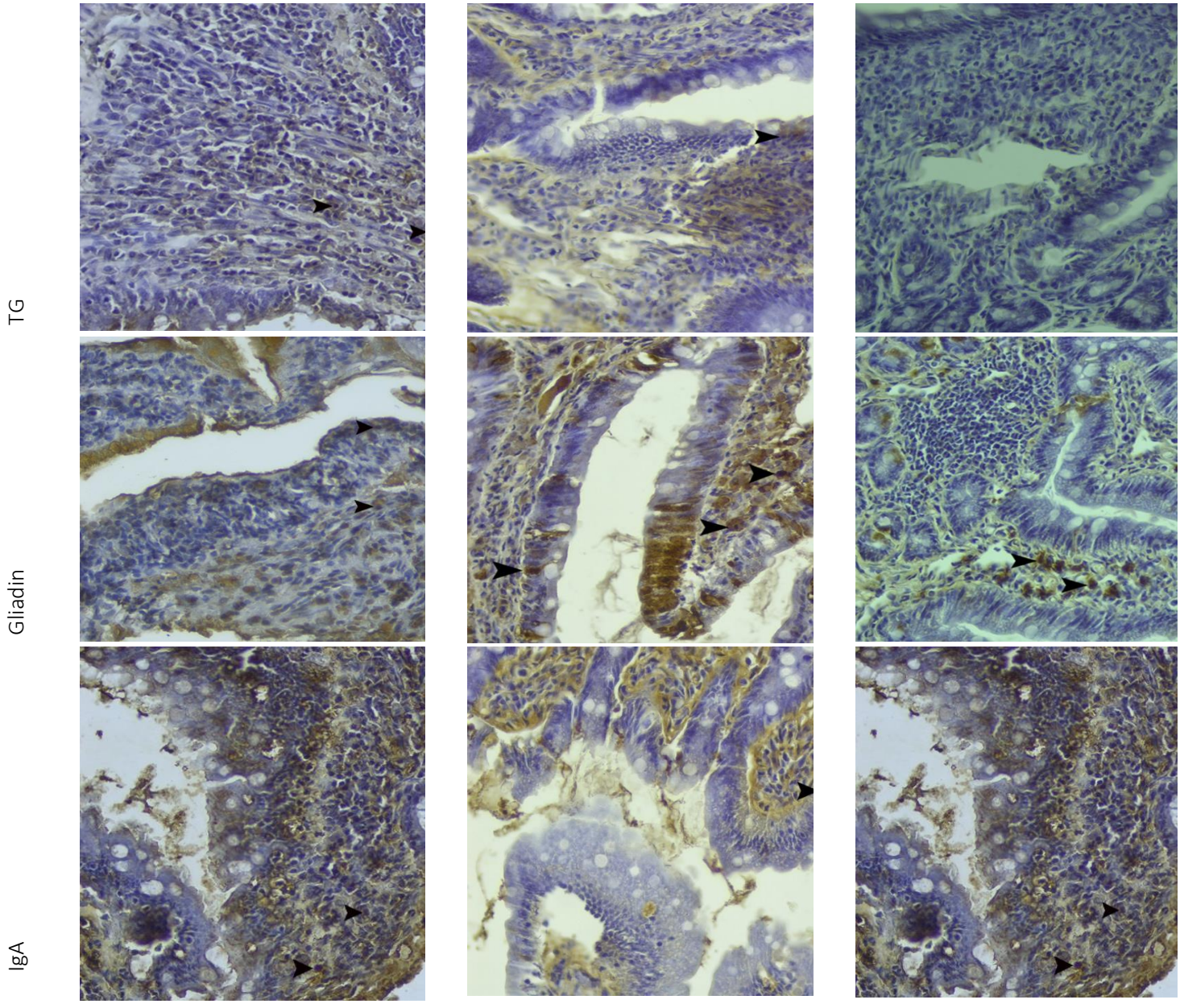

Figure 3. Transglutaminase formed in the small intestines (TG), gliadin and IgA immunohistochemistry (➡) (IHC, Bar: 50µm)

DISCUSSION

Gliadin peptides, which are formed by the breakdown of gluten, combine with HLA molecules to cause the initiation of clinical manifestations and a chain of immunological events. It is known that the peptides that make up the structure of gliadin initiate the cellular, humoral and inflammatory response in tissues.[18,19] The peptides found in the structure of gliadinin cannot be fully digested because they are resistant to proteases and proteolysis found in the gastrointestinal tract of celiac patients.[20] In this study, healthy male rats without HLA-DQ2 and HLA-DQ8 genes were given wheat and corn gluten and their effect on histopathological and immunohistochemical parameters in small intestine tissue was examined.

Villus atrophy, crypt hyperplasia and lymphocyte plasma neutrophil parameters, which are indicators of histopathological structure, were examined. Villuses are structures that protrude towards the lumen in the small intestine tissue and increase the absorption area of the small intestine. The average small intestine length of rats is 110 cm.[21] In this study, the longest villi length was observed in the wheat gluten group ($P < .05$). Accordingly, it was observed that wheat gluten had no negative effect on the small intestine villi lengths of healthy rats. Villus atrophy, although not statistically significant, was observed to be the highest in the group given corn gluten. Lymphocyte plasma neutrophils distributed in the mucosal structure of performed as immunohistochemical parameters to determine the sensitivity of healthy male rats to gluten. Gliadins in the structure of grain proteins are perceived as antigens by the tissue and stimulate the development of T-cells to ensure the production of antibodies against it.[26-28] This leads to an increase in the transglutaminase antibody. The enzyme transglutaminase is intracellular in nature and stimulates the secretion of fibroblasts (from inflammatory and endothelial cells) after mechanical irritation or inflammation response. Gliadin is a protein that is resistant to proteolic enzymes that is deamidated by the enzyme transglutaminase in the digestive tract.[29] It is stated that Gliadin antibody is formed in 90% of untreated celiac patients. The presentation of gliadin protein by HLA-DQ2 and HLA-DQ8 to reactive CD4+T cells increases the level of pro-inflammatory cytokines that cause tissue damage, leading to the release of B lymphocytes and the formation of plasma cells. Plasma cells cause the release of gliadin and transglutaminase antibodies.[30,31]

In this study, transglutaminase ($P > .05$) and gliadin levels the entire intestinal tissue are known as defense barriers against viruses and bacteria.[22] The increase in antigenic agents in the digestive system leads to an increase in the number of lymphocyte plasma neutrophils.[23]

In this study, although the low number of lymphocyte plasma neutrophils in the corn and wheat group compared to the soy group was not statistically significant, it shows that the sensitivity of the small intestine tissue of healthy rats to wheat and corn gluten was less compared to the soy group. The most important task of the cells that settle in the crypts in the intestinal tissue is to help the intestinal tissue to perform its function more functionally by making secretions. Cryptic hyperplasias adversely affect the localization and secretion of these cells.[23,24] In this study, crypt hyperplasia was mostly observed in the soy group ($P <.05$). Accordingly, low crypt hyperplasia of wheat and corn gluten in healthy rats does not adversely affect the histopathological structure of intestinal tissue.

It is also very important to examine immunohistochemical parameters to support histopathological findings due to gluten sensitivity in tissues.[25] Therefore, antibodies are particularly used as immunohistochemical parameters for the detection of gluten sensitivity. In this study, transglutaminase, gliadin and IgA antibody analyzes were ($P < .05$) were observed in the wheat group with the lowest levels. This suggests that healthy rats lacking the HLA-DQ2 and HLA-DQ8 genes are not sensitive to wheat gluten. IgA is mainly present in many external secretions. Secretory IgA molecules are particularly effective against microbial agents on mucosal surfaces, preventing and neutralizing bacterial pathogens or their toxins on the mucosal surface by preventing them from adhering to epithelial cells.[32,33] In the study, IgA antibody parameters were found to be similar between the groups. However, it can be stated that the literature is not in agreement with the findings.[10,11,34] The most obvious reason for these differences can be attributed to the fact that the immune system metabolism has not been fully elucidated. It has done an extensive review of animal studies on gluten sensitivity and how to read their results, where he has provided invaluable information.[35]

As a conclusion in this study, the longest villus length, least crypt hyperplasia and lowest gliadin level were in the wheat gluten group and it was observed that wheat gluten had no negative effect on small bowel villus lengths. The fact that transglutaminase and gliadin levels, which are among the immunohistochemical parameters, were in the lowest

wheat group indicates that healthy male rats were not sensitive to wheat gluten. In addition, lymphocyte plasma neutrophil count and crypt hyperplasia are highest in the soy group, which means that wheat and corn gluten do not adversely affect the histopathological structure of small intestinal tissue. According to the findings obtained in this study, it was determined that wheat gluten added to the diet of healthy rats without HLA-DQ2 and HLA-DQ8 genes did not have a negative effect on the histopathological and immunohistochemical parameters of small intestinal tissue.

As a resort in the studies to be carried out in this regard, in rats with and without HLA-DQ2 and HLA-DQ8 genes, immunohistochemical parameters IgA, gliadin, tansglutaminase in addition to IgG, CD3, CD8 levels and gluten effect and inflammatory cytokines and serological parameters can be examined. Apart from intestinal cells, it may be useful to examine the histopathological and immunohistochemical effects of gluten on bone, skin, nerve cells. It can also be stated that it will shed light on new studies on the use of gluten as a protein source.

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REFERENCES

1. Biesiekierski JR. What is gluten?. *J Gastroenterol Hepatol*. 2017;31(Suppl 1):78-81.
2. Aziz I, Branchi F, Sanders DS. The rise and fall of gluten!. *Proc Nutr Soc*. 2015;74(3):221-226.
3.Sapone A, Bai JC, Ciacci C, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med.* 2012;10(1):1-12.
4.Machado MV. New developments in celiac disease treatment. *Int J Mol Sci*. 2023;24(2):945.
5. Catassi GN, Pulvirenti A, Monachesi C, Catassi C, Lionetti E. Diagnostic accuracy of IgA anti-transglutaminase and IgG anti-deamidated gliadin for diagnosis of celiac disease in children under two years of age: a systematic review and meta-analysis. *Nutrients.* 2021;14(1):7.
6. Rubio-Tapia A, Hill ID, Semrad C, Kelly CP, Lebwohl B. American college of gastroenterology guidelines update: Diagnosis and management of celiac disease. *Am J Gastroenterol*. 2023;118(1):59-76.
7. Abdi F, Zuberi S, Blom JJ, Armstrong D, Pinto-Sanchez MI. Nutritional considerations in celiac disease and non-celiac gluten/wheat sensitivity. *Nutrients.* 2023;*15*(6):1475.
8. Sümer SAG, Harmandar FA, Uyar S, Çekin AH. Non-Çölyak gluten duyarlılığı. *Güncel Gastroenteroloji*. 2015;19(2); 91-97.
9. Sergi C, Vincenzo V, Antonio C. Non-celiac wheat sensitivity: rationality and irrationality of a gluten-free diet in individuals affected with non-celiac disease: a review. *BMC Gastroenterol.* 2021; 21(1):5.
10. 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. *Brazilian Archives Biol Technol*. 2021;64: e21210256.
11. İmik H, Kapakin KAT, Karabulutlu Ö, Gümüş R, Çomaklı S, Özkaraca M. The effects of dietary wheat and corn glutens on the histopathological and immunohistochemical structure of the ovarian tissue and serum and ovarian tissue LH and FSH levels and lipid profiles in rats. *Brazilian Archives Biol Technol.* 2023;66:e23210726.
12. Kapakin KAT, Kapakin S, Imik H, Gumus R, Eser G. The investigation of the relationship between HSP-27 release

and oxidative DNA damage in broiler chickens with tibial dyschondroplasia by using histopathological and immunohistochemical methods. *Braz J Poultry Sci*. 2019;eRBCA-2019-1091.
13. Iskender H, Dokumacioglu E, Terim Kapakin KA, et al. Effects of oleanolic acid on inflammation and metabolism in diabetic rats. *Biotech Histochem.* 2022;97(4):269-276.
14. Uni Z, Gal-Garber O, Geyra A, Sklan D, Yahav S. Changes in growth and function of chick small intestine epithelium due to early thermal conditioning. *Poultry Sci*. 2001;80(4):438-445.
15. Kapakin KAT, Sahin M, Buyuk F, Kapakin S, Gursan N, Saglam YS. Respiratory tract infection induced experimentally by Ornithobacterium rhinotracheale in quails: effects on heat shock proteins and apoptosis. *Revue de Med Vet*. 2013;164(3):132-140.
16. Kapakin KAT, Gümüş R, İmik H, Kapakin S, Sağlam YS. Effects of ascorbic and α-lipoic acid on secretion of HSP-70 and apoptosis in liver and kidneys of broilers exposed to heat stress. *Ankara Univ Vet Fak Derg.* 2012;59(4):279-287.
17. SPSS: Statistical Packages for the Social Sciences for Windows release 10.01. *SPSS Inc*., Chicago.1996.
18.Marsh MN. Gluten, major histocompatibility complex, and the small intestine: a molecular and immunobiologic approach to the spectrum of gluten sensitivity (‘celiac sprue’). *Gastroenterol.* 1992;102(1):330-354.
19. Sapone A, Lammers KM, Mazzarella G, et al. Differential mucosal IL-17 expression in two gliadin-induced disorders: gluten sensitivity and the autoimmune enteropathy celiac disease. *Int Arch Allergy Immunol*. 2010;152(1):75-80.
20. Molberg O, Mcadam SN, Körner R, et al. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat Med*. 1998;4(6):713-717.
21. Yakan B, Fötüs BG. Yeni doğmuş ve erişkin farede ince bağırsakların histolojik ve histokimyasal kıyaslı yapısı. *Erciyes Tıp Derg*. 2000;22(1):7-15.
22. Engel E, Guth PH, Nishizaki Y, Kaunitz JD. Barrier function of the gastric mucus gel. *Am J Physiol-Gastrointest Liver Physiol*. 1995;269(6):G994-G999.
23. Solakoğlu S, Aytekin Y. Temel Histoloji, Junqueira LC, Carneiro J*: Basic Histology*, text and atlas, eleventh edition. *Nobel Tip Kitabevleri*, ISBN: 978-975-420-699-9. 2009; 281-317.
24. Kagnoff, MF. "Immunology and inflammation of the gastrointestinal tract." *Gastrointestinal and liver disease. Sixth ed. Philadelphia: WB Saunders Company* (1998):19-48.
25. Greco N, Pisano A, Mezzatesta L, et al. New Insights and Evidence on “Food Intolerances”: Non-celiac gluten sensitivity and nickel allergic contact mucositis. *Nutr.* 2023;15(10):2353.
26. Korponay–Szabo IR, Sulkanen S, Halttunen T, et al. Tissue transglutaminase is the target in both rodent and primate tissues for celiac disease-specific autoantibodies. *J Pediatric Gastroenterol Nutr*. 2000;31(5): 520-527.
27. Kalliokoski S, Piqueras VO, Frias R, et al. Sulic AM, Maatta JA, Kahkönen N, Lindfors K. Transglutaminase 2-specific coeliac disease autoantibodies induce morphological changes and signs of inflammation in the small-bowel mucosa of mice. *Amino Acids*. 2017;49(3):529-540.
28. Iversen Rasmus, Ludvig MS. The immunobiology and pathogenesis of celiac disease. *Ann Rev Pathol.* 2023;24(18):47-70.
29. Arentz-Hansen H, Körner R, Molberg O, et al. The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J Exp Med.* 2000;191(4):603-612.
30. Bürgin-Wolff A, Gaze H, Hadziselimovic F, et al. Antigliadin and antiendomysium antibody determination for coeliac disease. *Arch Dis Childhood.* 1991;66(8):941-947.
31. Maiuri L, Ciacci C, Ricciardelli I, et al. Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. *The Lancet.* 2003;362 (9377):30-37.
32. Jaskowski TD, Schroder C, Martins TB, Litwin CM, Hill HR. IgA antibodies against endomysium and transglutaminase: a comparison of methods. *J Clin Lab Analysis*. 2001;15(3):108-111.
33. Matsumoto I, Uchida K, Nakashima K, et al. IgA antibodies against gliadin and tissue transglutaminase in dogs with chronic enteritis and intestinal T-cell lymphoma. *Vet Pathol.* 2018;55(1):98-107.
34. Smeekens JM, Kulis MD. Mouse models of food allergy in the pursuit of novel treatment modalities. *Front Allergy*. 2021;15(2):810067.
35.Marietta EV, Murray JA. Animal models to study gluten sensitivity. *Semin Immunopathol.* 2012;34(4):497-511.