Vol.66: e23210726, 2023
https://doi.org/10.1590/1678-4324-2023210726
ISSN 1678-4324 Online Edition

Article - Human and Animal Health

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

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Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Andressa Novatski

Received: 06-Nov-2021; Accepted: 26-Jul-2022.

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HIGHLIGHTS

- The cereals are a significant food source for both humans and animals.
- The balanced nutrition is critical to the development of the reproductive system.
- The serum lipid profile is critical to the development of multiple diseases.
- Dietary gluten has effects on the immunohistochemical structure of the ovarian tissue.

Abstract: This study was aimed at determining the effects of corn and wheat glutens on the histopathological and immunohistochemical structure of the ovarian tissue, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, and lipid profile in rats. Twenty-day-old 24 female Sprague-Dawley rats were assigned to three groups, and were raised until 185 days of age. Three study groups, named as Wheat, Corn and Soybean Groups, were established and fed on wheat gluten, corn gluten and soybean meal, respectively, as a protein source. At the end of the trial, ovarian tissue specimens and serum samples were taken from the animals, and analyzed. Compared to Soybean Group, in Wheat Group, of the ovarian histopathological parameters investigated, values pertaining to the primordial, primary, secondary, and Graafian follicles and corpora lutea (CL) were numerically smaller, and for the immunohistochemical parameters investigated, in the transglutaminase 2 (TGM2), gliadin, IgA, IgM, CD4 and CD8 were immunopositivity higher (P>0.05). It was determined that, in Wheat Group, ovarian tissue LH levels had significantly decreased, whilst serum

FSH levels had significantly increased (P<0.05). Wheat Group also displayed reduced ovarian tissue cholesterol levels and increased serum monoacylglycerol levels (P<0.05). In result, it was ascertained that wheat and corn glutens had limited effects on the histopathological and immunohistochemical structure of the ovarian tissue, but showed distinct effects on ovarian tissue LH and serum FSH levels.

Keywords: gluten; immunohistochemistry; LH; ovarian; transglutaminase.

INTRODUCTION

Wheat, barley, corn, oats and rice are some of the major cereals grown worldwide, and are a significant food source for both humans and animals [1]. The carbohydrates, proteins and lipids contained in cereals are important components of the diet and are considered critical to nutritional quality in both the food and feed sectors [2]. The storage proteins of wheat are classified as albumins, globulins, gliadins and glutenins, based on their solubility, and of these fractions, gliadins and glutenins constitute the gluten proteins and are stored in the endosperm of the seed together with starch [2,3]. While corn is highly demanded at global level, due to it lacking functional properties as a food ingredient, corn gluten is mostly used in animal nutrition [1,4]. Zein, a prolamin class, is the most abundant protein fraction of corn gluten, and has been determined within a range of 62-74%, depending on the isolation method and corn variety [4]. In some individuals, glutens cause disorders and allergies [5]. The most common disorders related to gluten ingestion are celiac disease, gluten intolerance, non-celiac gluten sensitivity (NCGS), wheat allergy, and dermatitis herpetiformis [2,6].

Dietary composition is an important lifestyle factor and has potential effects on the histopathological and immunohistochemical structure of the ovarian tissue [7]. It has also been indicated that gluten and certain substances in gluten-containing diets may increase reproductive activity in both humans and animals [8,9]. This study was aimed at investigating the effects of high levels of dietary wheat and corn glutens on the histopathological and immunohistochemical structure of the ovarian tissue, LH and FSH levels, and the lipid profile in rats.

MATERIAL AND METHODS

Animal Material, Experimental Groups, and Feed

This study was approved by the Local Ethics Board for Animal Experiments of Ataturk University, pursuant to Decision 6/96 dated 28.07.2017 and the study was conducted at the Medical Experimental Application and Research Center of Ataturk University.

In this study, 20-day-old 24 healthy female Sprague-Dawley rats were randomly divided into 3 groups, each of 8 rats. In this study, rats were raised until 185 days (the trial period lasted for 165 days) of age by being fed on experimental rations. The rations provided to the study groups were isonitrogenic and isocaloric (Table 1). Three study groups, referred to as Wheat Group, Corn Group and Soybean Group, were established and provided with dietary wheat gluten, corn gluten and soybean meal, respectively. In this study, the animals were fed for a period of 165 days, provided with feed and water ad libitum, and housed at a comfort temperature (22°C) throughout the study period. In breeding during rats (female: male=2:1) were housed in cages for 2 days. During this period, 2 female rats and 1 male rat were housed together for 2 days and, with 3 different male rats for a total of 6 days.

Table 1. Ingredients and nutrient composition of rat diet in the study.

Ingredients, %	Groups		
	Wheat	**Corn**	**Soybean**
Wheat bran	1.8	3.5	3.24
Oat, %11 CP	68	64	62.11
Sunflower meal, % 28 CP	13	13	6
Corn gluten meal, % 62 CP	-	17	-
Wheat gluten meal, % 75 CP	24.85	-	-
Soybean meal, % 51 CP	-	-	24.85
Animal fat	2.2	1.5	2.8
Vitamin-mineral premix*	1	1	1
Nutrient composition			
Crude protein, %	22	22	22
Metabolisable energy, (kcal/kg)	2599	2657	2598
Ca, %	0.15	0.11	0.14
Methionine + cysteine, %	0.66	0.83	0.68
Lysine, %	1.17	0.63	1.15

*The vitamin-mineral premix provides the following (per kg): vitamin A 6.000.000 IU; vitamin D3 800.000 IU; vitamin E 8000 mg; vitamin K3 2000 mg; vitamin B1 1200 mg; vitamin B2 3000 mg; vitamin B6 2000 mg; vitamin B12 8 mg; niacin 10000 mg; folic acid 400 mg; d-biotin 20 mg; choline chloride 160.000 mg; manganese 32000 mg; iron 16000 mg; zinc 24.000 mg; copper 2000 mg; iodine 800 mg; cobalt 200 mg; selenium 60 mg; Cal-D-Pan. 4000 mg; antioxidant 4000 mg. CP: Crude protein.

Pathological Analyses

At the end of the study period, the rats were sacrificed under anesthesia for the collection of tissue specimens. Ovarian tissue samples were taken for histopathological and immunohistochemical examination and lesions were scored semi-quantitatively, based on the microscopic examination of 10 different areas at 40X magnification. The scores were as follows: 0 (negative), +1 (slight), +2 (moderate), +3 (severe) and +4 (very severe) [10].

Histopathological Analyses

For histopathological examination, the tissue specimens were first fixed in 10% buffered formalin for 48-72 h, and then washed under running water for 6-8 h. Next, the specimens underwent routine tissue processing by being passed through graded alcohol (70°, 80°, 90°, 96° and 100°) and xylol series. After being embedded in paraffin, 4-mm-thick sections were cut from the paraffin blocks and mounted on glass slides. The histopathological sections were stained with hematoxylin-eosin (HE) [11], and were examined and imaged using an Olympus BX52 light microscope equipped with a DP72 camera system, at the laboratory of the Pathology Department of Atatürk University, Faculty of Veterinary Medicine.

Immunohistochemical Analyses

After being fixed in 10% buffered formalin, the tissue specimens were embedded in paraffin. Four-µm-thick sections were cut from the paraffin blocks and stained with the avidin-biotin-peroxidase complex (ABC) technique for immunohistochemical examination under a light microscope. All sections mounted on poly-L-lysine-coated adhesive glass slides for immunoperoxidase examination were passed through graded xylol and alcohol series for deparaffinization and dehydration, and were then washed in distilled water for 5 min. After being washed in phosphate buffer solution (PBS, pH 7.2) for 5 min, the slides were immersed in 3% H_2O_2 for 10 min to block endogenous peroxidase activity. Next, the slides were washed in PBS for 5-10 min, and incubated with a protein-blocking solution, which was compatible with all primary and secondary antibodies, for 5 min, with an aim to avoid non-specific background staining. At the end of the incubation period, the excessive blocking solution on the slides was removed, and without being washed, the slides were treated with primary antibodies, CD4 (Catalog No: BS-0647R, ThermoFisher), CD8 (Catalog No:BS-0648-R), IgA (Catalog No: BS-0648-R10491-R), IgG (Catalog No: BS- 0392-R), gliadin (Catalog No: NB600-54713374-R), transglutaminase 2/TGM2 (Catalog No: NB600-547), and in the control group with PBS.

Depending on the primary antibody used, incubation took place either for 1 h at room temperature or at +4 °C overnight. Subsequently, the slides were washed with PBS twice, each time for 5 min, and were then incubated with biotinylated secondary antibody at room temperature for 10-30 min. After being washed with PBS once more, the slides were treated with streptavidin-peroxidase for 10-30 min, and were again washed with PBS. Next, the sections were treated with DAB (3, 3-diaminobenzidine) as a chromogen for 5-10 min. Background staining was performed with Mayer's hematoxylin for 1-2 min, followed by washing with tap water and mounting with a water-based adhesive [12].

Collection of Serum Samples

At the end of the trial, venous blood samples were collected from eight animals per study group into 10 ml-glass tubes containing a coagulation accelerator. At the laboratory of the Biochemistry Department of Atatürk University, Faculty of Veterinary Medicine, the blood samples were centrifuged at 3000 x G and +4 °C for 5 min. Extracted sera were stored at -82 °C until being analyzed.

FSH and LH Analyses in the Serum and Ovarian Tissue

At the end of the trial, blood samples were collected by cardiac puncture from anesthetized animals into serum tubes, and were centrifuged at 3000 rpm for 10 min at +4 °C. The sera were pooled in Eppendorf tubes and stored at 20 ˚C. Blood serum samples were analyzed for FSH and LH levels.

FSH and LH analyses were performed using an ELISA kit specific to rats, in accordance with the manufacturer's instructions. Measurements for each parameter were made with a kit coated with an antibody specific to the animal species of concern.

Analysis of Serum and Ovarian Tissue Lipid Profiles (Thin-layer Chromatography)

Thin-layer chromatography, using a 20x10 cm Silica Gel 60 F254 HPTLC (High-performance thin-layer chromatography) plate, was performed for the determination and differentiation of the lipid composition of the serum and ovarian tissue. For this purpose, 1 ml of tissue homogenate or serum was added 1 ml of an n-hexane/isopropanol mixture (2:1 (v/v)). Once the cap was tightly closed, the tube was thoroughly shaken, kept still for 10 min, and shaken again. This procedure was repeated twice more [13]. Subsequently, the tubes were centrifuged at 8.000 rpm for 10 min, and the supernatant was loaded on the HPTLC plate. The plates were run on a hexane: diethyl ether: formic acid (80:20:2 (v/v/v) mixture for 15 cm and were dried after being developed. The spots on the dry plates were made visible by being burnt on hot plates with 3% $CuSO_4$ in 8% phosphoric acid [14]. The standard lipid mixture, which comprised of l-α-phosphatidylcholine, cholesterol, palmitic acid, triolein, squalene, and serum lipids, was classified as monoacylglycerol, diacylglycerol, triacylglycerol, free fatty acids, cholesterol and phospholipids.

Statistical Analyses

The statistical analysis of the findings obtained in this study was performed using the Statistical Package for the Social Sciences (SPSS) software [15]. One-way analysis of variance (ANOVA) was used for the analysis of the serum and ovarian tissue levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), triacylglycerol, free fatty acids, cholesterol, diacylglycerol, monoacylglycerol and phospholipids, whilst the significance of the differences between the study groups was determined with Duncan's test. The histopathological and immunohistochemical alterations in the ovaries were calculated by means of the Kruskal-Wallis test, a non-parametric test. The data were expressed as mean ± standard error of mean (SEM). A value of $P<0.05$ and $P<0.01$ were considered significant.

RESULTS

Histopathological Findings

Values obtained upon the histopathological examination of the ovarian tissues are shown in Table 2, whilst the statistical analysis results of these values are presented in Table 3. The histopathological analyses performed in the ovarian tissue samples of the animals at the end of the trial showed that primordial follicle, primary follicle, secondary follicle, graafian follicle, atrophic follicle and, corpora lutea parameters were no statistically significant difference between the study groups ($P>0.05$) (Table 3) (Figure 1).

Table 2. Values obtained with the hematoxylin-eosin and immunohistochemical staining of the rat ovarian tissue samples.

	Histopathological Parameters						Immunohistochemical Parameters					
n	**Pr.F.**	**Pm.F.**	**Sc.F.**	**Gr.F.**	**At.F.**	**CL**	**TGM2**	**Gld.**	**IgA**	**IgG**	**CD4**	**CD8**
Wheat Group												
1	+1	+1	+2	+1	+2	+1	+4	+2	+4	+3	+3	+4
2	+1	+1	+2	+1	0	+1	+3	+3	+4	+2	+3	+2
3	+1	+1	+2	+1	+2	+1	+3	+2	+3	+2	+2	+2
4	+1	+1	+2	+2	+1	+1	+3	+2	+3	+2	+2	+2
5	0	0	+1	+1	+2	+1	+2	+2	+2	+2	+2	+2
6	0	0	+1	+1	+1	+1	+2	+1	+2	+1	+1	+1
7	0	0	0	0	0	0	+1	+1	+2	+1	+1	+1
8	0	0	0	0	0	0	+1	+1	+1	+1	+1	+1
Corn Group												
1	+1	+1	+1	+1	+1	+1	+3	+2	+3	+2	+2	+3
2	+1	+1	+2	+1	+2	+1	+3	+2	+3	+2	+2	+2
3	0	+2	+2	+1	+2	+1	+2	+2	+2	+1	+1	+2
4	+1	+2	+2	+1	+1	+1	+2	+1	+2	+1	+1	+2
5	+1	+1	+1	+2	+1	+2	+2	+1	+2	+1	+2	+1
6	+1	+1	+3	+1	+1	+1	+1	+1	+1	+1	+1	+1
7	0	0	0	0	0	0	+1	+1	+1	+1	+1	+1
8	0	0	0	0	0	0	+1	0	+1	0	+1	0
Soybean Group												
1	+1	+1	+2	+1	+1	+1	+2	+2	+2	+2	+2	+2
2	+1	+1	+2	+1	+1	+2	+2	+1	+2	+2	+2	+2
3	+1	+1	+2	+1	+2	+2	+2	+1	+2	+1	+1	+2
4	+1	+2	+3	+1	+1	+2	+2	+1	+1	+1	+1	+2
5	+1	+1	+2	+1	0	+1	+1	+1	+2	+1	+2	+1
6	+1	+2	+2	+2	0	+1	+1	+1	+1	+1	+1	+1
7	0	0	0	0	0	0	+1	+1	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0

Pr.F.: Primordial Follicle, Pm.F.: Primary Follicle, Sc.F.: Secondary Follicle, Gr.F.: Graafian Follicle, At.F.: Atrophic Follicle, CL: Corpora Lutea , TGM2: Transglutaminase 2, Gld.: Gliadin, IgA: Immunoglobulin A, IgG: Immunoglobulin G, n=8.

Table 3. Statistical values of the histopathological and immunohistochemical parameters of the ovarian tissue samples taken at the end of the trial.

Parameters		Groups			P values
		Wheat	Corn	Soybean	
Histopathological					
Pr.F.	X±SEM	0.50±0.188	0.63±0.182	0.75±0.163	0.600
	Median	0.50	1.00	1.00	
Pm.F.	X±SEM	0.50±0.188	1.00±0.267	1.33±0.210	0.274
	Median	0.50	1.00	1.00	
Sc.F.	X±SEM	1.25±0.313	1.38±0.307	1.63±0.375	0.666
	Median	1.50	1.50	2.00	
Gr.F.	X±SEM	0.88±0.226	0.88±0.226	1.17±0.166	1.000
	Median	1.00	1.00	1.00	
At.F.	X±SEM	1.00±0.327	1.00±0.267	0.63±0.263	0.559
	Median	1.00	1.00	0.50	
CL	X±SEM	0.75±0.163	0.88±0.226	1.125±0.295	0.547
	Median	1.00	1.00	1.10	
Immunohistochemical					
TGM2	X±SEM	2.38±0.375	1.88±0.295	1.67±0.210	0.258
	Median	2.50	2.00	2.00	
Gld.	X±SEM	1.75±0.250	1.25±0.250	1.67±0.166	0.124
	Median	2.00	1.00	1.00	
IgA	X±SEM	2.63±0.375	1.88±0.295	1.67±0.210	0.103
	Median	2.50	2.00	2.00	
IgG	X±SEM	1.75±0.250	1.125±0.226	1.33±0.210	0.166
	Median	2.00	1.00	1.00	
CD4	X±SEM	1.88±0.295	1.38±0.182	1.50±0.223	0.330
	Median	2.00	1.00	1.50	
CD8	X±SEM	1.88±0.350	1.50±0.327	1.67±0.210	0.762
	Median	2.00	1.50	2.00	

The values are given as mean ± standard error of the mean (SEM), n=8. Pr.F.: Primordial Follicle, Pm.F.: Primary Follicle, Sc.F.: Secondary Follicle, Gr.F.: Graafian Follicle, At.F.: Atrophic Follicle, CL: Corpora Lutea , TGM2: Transglutaminase 2, Gld.: Gliadin, IgA: Immunoglobulin A, IgG: Immunoglobulin G.

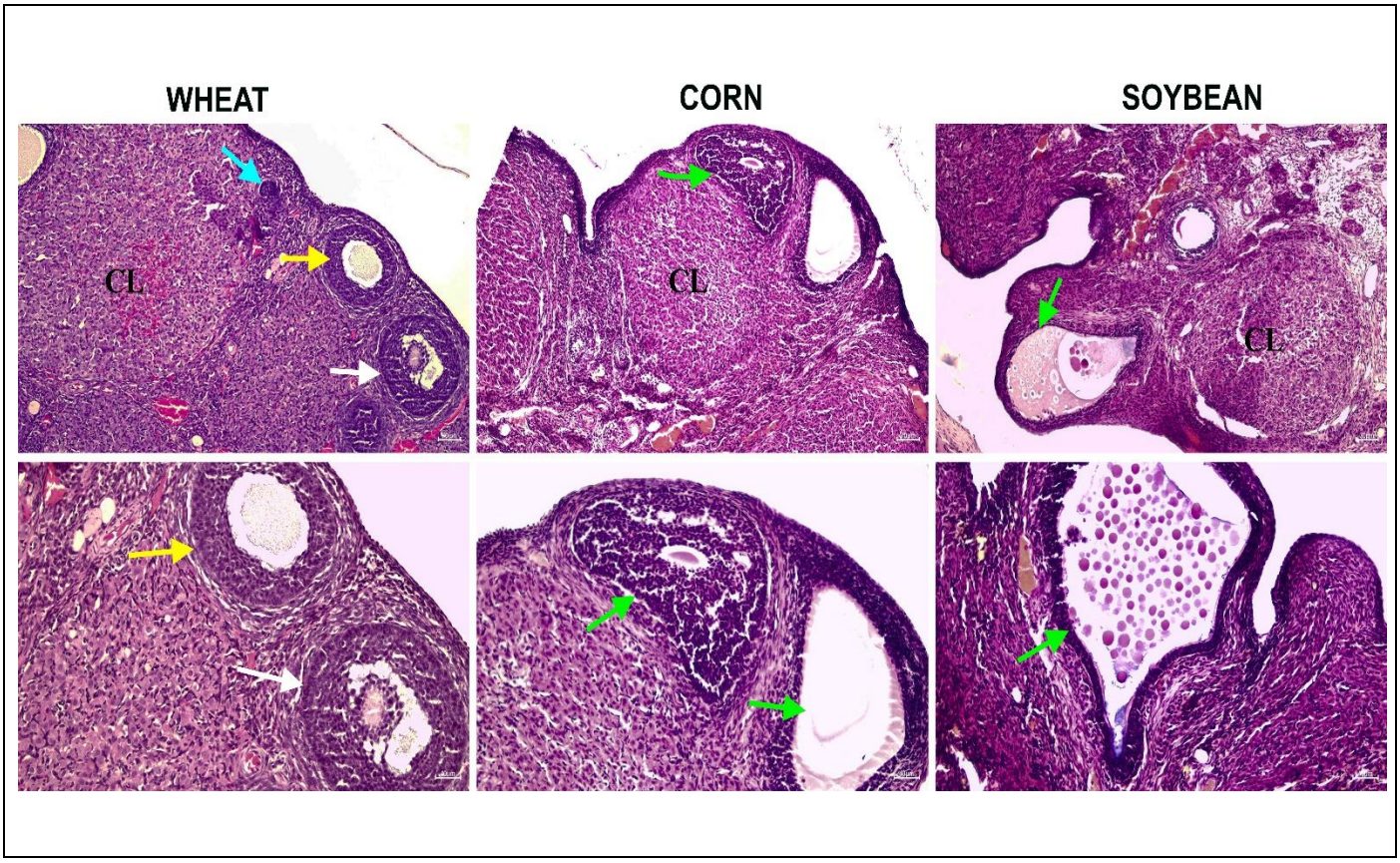

Figure 1. Hematoxylin-eosin staining of the ovarian tissue of experimental groups. Appearence of primordial (blue arrow), primary (yellow arrow), secondary (green arrow), Graafian (white arrow) and, corpora lutea (CL); Bar: 70 µm, 40 µm.

Immunohistochemical Findings

Values obtained upon the immunohistochemical examination of the ovarian tissues are shown in Table 2, whilst the statistical analysis results of these values are presented in Table 3. The immunohistochemical analyses performed in the ovarian tissue samples of the animals at the end of the trial showed that for TGM2, gliadin, IgA, IgG, CD4 and CD8 parameters were no statistically significant difference between the study groups ($P>0.05$) (Table 3). However, immunopositivity for expressions TGM2, IgA, IgG, gliadin, CD4 and CD8 was observed to be high in Wheat Group (Figure 2).

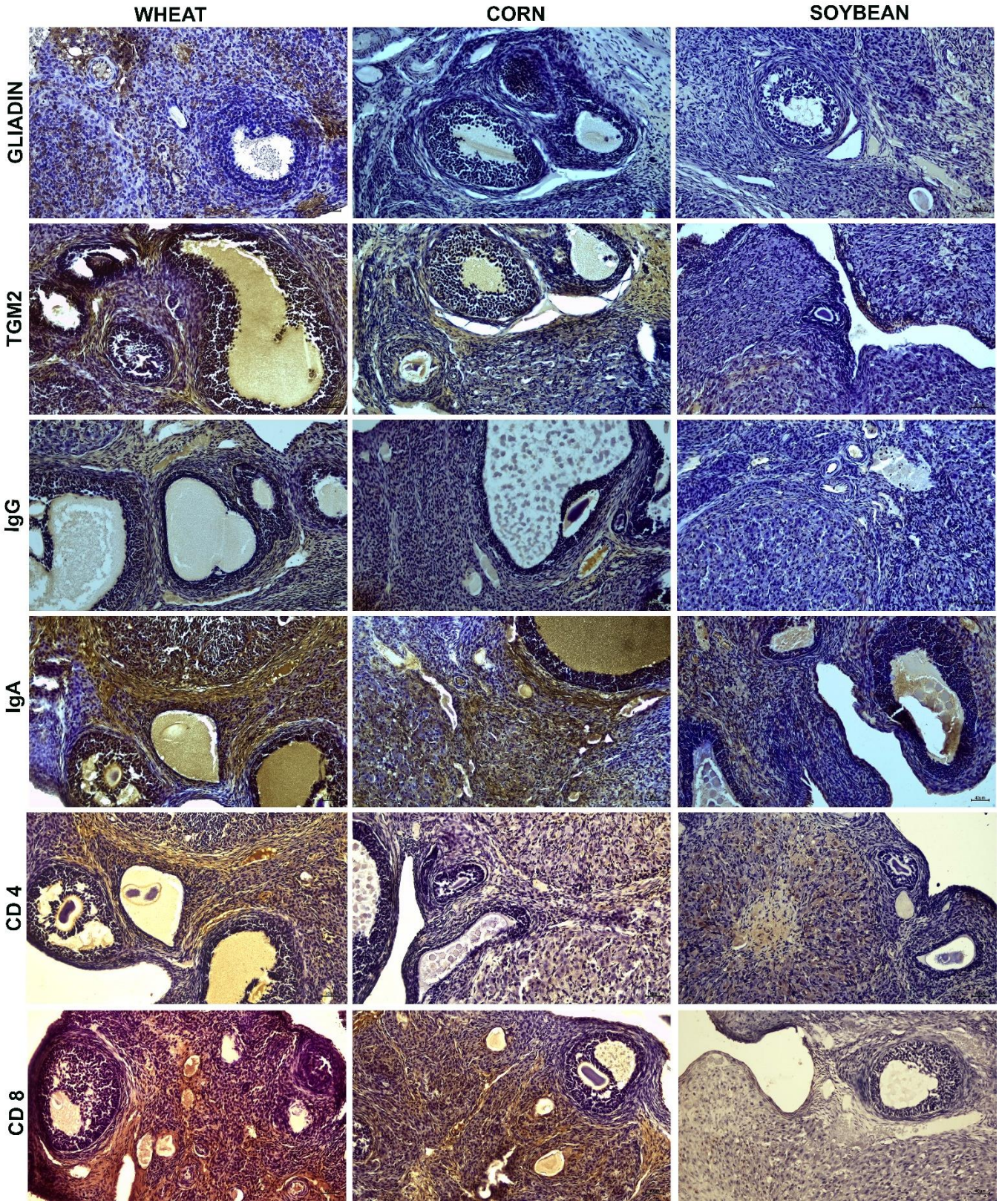

Figure 2. Immunohistochemistry of gliadin, transglutaminase 2 (TGM2), IgG, IgA, CD4 and CD8, expressions in the ovarian tissue of experimental groups, Bar: 40 µm.

The FSH and LH Findings of Serum and Ovarian Tissue

The LH and FSH levels measured in the serum and ovarian tissue samples are shown in Table 4. The ovarian tissue LH and serum FSH levels determined in Wheat Group were found to be statistically different from those measured in the other groups (P<0.05) (Table 4). While the ovarian tissue LH level of Wheat Group was significantly lower than the levels of Corn Group and Soybean Group, the serum FSH levels of Wheat Group and Corn Group were significantly higher than the level of Soybean Group (P<0.05) (Table 4).

Table 4. The levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) measured in the ovarian tissue and serum samples of the study groups.

Groups	Ovarian LH	Ovarian FSH	Serum LH	Serum FSH
Wheat Group	2.128 ± 0.098^{b}	3.513 ± 0.105	2.497 ± 0.097	4.531 ± 0.233^{a}
Corn Group	2.582 ± 0.049^{a}	3.877 ± 0.086	2.418 ± 0.212	4.035 ± 0.170^{a}
Soybean Group	2.424 ± 0.073^{a}	3.748 ± 0.158	2.781 ± 0.153	3.052 ± 0.217^{b}
P values	0.004	0.137	0.295	0.002

The values are given as mean ± standard error of the mean (SEM), n=8.

Findings of Serum and Ovarian Tissue Lipid Profiles

The fatty acid profiles determined for the ovarian tissue and serum samples in the present study are shown in Table 5. Accordingly, it was ascertained that the ovarian tissue cholesterol levels of Wheat Group were significantly lower than those of Corn Group and Soybean Group; whilst the serum monoacylglycerol levels of Corn Group were found to be significantly lower than those of Wheat Group (P<0.05).

Table 5. The fatty acid profiles of the ovarian tissue and serum samples.

Parameters	Groups			P values
	Wheat	Corn	Soybean	
Ovarian				
TAG	34.516±1.541	33.668±1.855	31.078±1.837	0.381
DAG	9.638 ± 0.791^{a}	7.780 ± 0.467^{b}	7.806 ± 0.443^{b}	0.073
MAG	7.984 ± 0.342^{a}	6.644 ± 0.331^{b}	7.470 ± 0.432^{ab}	0.071
FFA	14.060±1.781	13.770±0.632	12.862±1.489	0.819
CHOL	21.438 ± 1.649^{b}	24.844 ± 1.243^{ab}	27.270 ± 1.519^{a}	0.049
PL	12.430±0.944	13.294±0.550	13.492±0.428	0.518
Serum				
TAG	47.992±2.170	49.968±3.027	48.072±2.522	0.833
DAG	8.492±0.409	6.708±1.312	7.006±0.893	0.390
MAG	7.190 ± 0.432^{a}	5.396 ± 0.336^{b}	6.624 ± 0.562^{ab}	0.044
FFA	17.760±1.327	22.926±2.388	20.594±1.747	0.191
CHOL	7.510 ± 0.437^{a}	5.236 ± 1.050^{b}	7.086 ± 0.294^{ab}	0.078
PL	11.056±1.173	9.760±0.369	10.618±0.659	0.530

The values are given as mean ± standard error of the mean (SEM), n=8. TAG: Triacylglycerol, DAG: Diacylglycerol, MAG: Monoacylglycerol, FFA: Free fatty acids, CHOL: Cholesterol, PL: Phospholipids.

DISCUSSION

Animal experiments are used as models to develop solutions to health problems, such that findings obtained in these experiments enable the design and development of prophylactic and therapeutic methods in the medical sector. The present study was aimed at determining the effects of wheat and corn glutens on the histopathological and immunohistochemical structure of the ovaries, as well as on LH and FSH levels, and the lipid profile.

It is well known that balanced and regular nutrition is critical to the development of the reproductive system and the regularity of sexual activity. Healthy and normal ovarian activity depends not only on the amount of nutrient intake, but also on the chemical composition of the dietary nutrients. The main difference of the present study from previous research is the study groups having been fed on rations, which had equal energy and protein levels, but contained different protein sources. Of the protein sources included in the diets of the study groups, soybean protein is rich in essential amino acids, including among others lysine, methionine and tryptophan, whilst wheat proteins are rich in gliadins (prolamins), and corn protein is rich in zeins [16-18]. In terms of the essential amino acid content, soybean protein is considered particularly valuable and has found common use in animal nutrition [17-19], whilst the dietary use of wheat and corn glutens is generally limited. Thus, the use of high levels of wheat and corn glutens is another important aspect of the present study.

Some active substances found in feedstuff are known to either induce or inhibit the secretion of ovarian hormones [20-25]. It has been reported that, in the event of a negative energy balance, plasma insulin and glucose levels decrease, GnRH secretion and LH release are reduced, and in result, both follicular development and estrogenic activities are adversely affected and ovulation is prolonged [20,22]. When the level of protein in the diet is high, the proteins absorbed by the body are metabolically converted to urea in the liver. A very high urea level in the blood reduces the pH level of the uterus, which eventually decreases progesterone release and threatens the viability of the embryo and ovum [20,26]. It has been reported that the consumed protein sources are important in the formation of the normal activity of the ovary, in the release of hormones and in the preparation of the uterus for pregnancy [22]. The study, ovarian tissue LH and serum FSH levels determined in Wheat Group were found to be statistically different from those measured in the other groups. It is known that nutrition has an effect on FSH and LH levels [27]. Ajuogu and coauthors [28] has reported that low protein diet caused significant reductions in serum FSH concentration in rats. Similarly, in a previous study by Polkowska and coauthors [29] has raported that the low protein diet influenced the exerted an inhibitory effect on the synthesis and the release of LH in the pituitary gonadotrophs. Also, Cassidy and coauthors [30] showed that the midcycle peaks of LH and FSH were significantly suppressed by soy-protein ingestion. However, studies showing the effects of gluten on FSH and LH levels were not found in the literature.

In the present study, the histopathological analyses performed in the ovarian tissue samples of the animals at the end of the trial showed that primordial follicle, primary follicle, secondary follicle, graafian follicle, atrophic follicle and, corpora lutea parameters were no statistically significant difference between the study groups (Table 3). Although literature reports are available on the effects of nutrients on fertility [21-25], to the authors' knowledge, there is no previous study specific to the effects of glutens on follicular development. Therefore, a comparison of the findings obtained in the present study was not able to be made. A general interpretation of the findings is presented.

Hormones and enzymes play an important role in metabolic regulation and sustainability throughout the lifespan of living beings. However, the secretion and mode of action of these hormones and enzymes differ greatly. While animals depend on the secretion of hormones such as FSH, LH and estrogen to show estrus, they require hormones such as human chorionic gonadotropin (HCG), progesterone, progestogen, and prolactin to conceive and maintain the continuity of gestation [24,25,31]. On the other hand, there is a multitude of factors affecting the secretion of hormones, and one of the most influential factors is known to be nutrition. When speaking of nutrition, a balanced and adequate diet does not suffice; the quality of the foodstuffs is also important [21,22,24,25].

The mechanism of immunogenic peptides and their binding to human leukocyte antigen-DQ molecules (HLA-DQ2/8) involve many subcellular chemical alterations and biochemical reactions. In fact, celiac disease is induced by undigested gluten peptides being absorbed from the intestinal mucosa and adhering to dendritic cells specific to HLA-DQ2/8. Mature dendritic cells containing deamidated gluten react with CD4+ T cells, specific to HLA-DQ2/8, in the B cell zone. This reaction results in the formation of both gluten-specific B cells and B cells specific to transglutaminase 2. Gluten-reactive T cells produce several cytokines, and this favors the development of intestinal auto-inflammation [32,33]. In the present study, CD4- and CD8-immunopositivity levels were found to be higher in the group, which received wheat gluten. Similarly, in a previous study by Gümüş and coauthors [34], it was determined that male rats fed on wheat and corn glutens displayed higher CD8 levels in the small intestine.

The tissue enzyme transglutaminase acts as an autoantigen in the etiology of celiac disease [35]. Likewise, the diagnosis of gluten neuropathy, which is the second most common neurological sign of gluten sensitivity, is based on serological markers, including one or more anti-gliadin IgG and/or IgA, anti-endomysial and anti-transglutaminase-2 antibodies [36]. Via a T cell receptor, gliadin is presented to gliadin-

reactive CD4 + T cells, which leads to the production of cytokines that cause tissue damage [37]. Anti-gliadin IgA and IgG are among the parameters used for the diagnosis of celiac disease [38].

In the present study, it was determined that the group, which received dietary wheat gluten, displayed higher immunopositivity levels for expression anti-transglutaminase and anti-gliadin IgA and IgG. Similar to these results, previous studies have also reported elevated transglutaminase [39,40] and gliadin levels in the hepatic tissue of celiac disease patients [37]. It has also been suggested that gliadin and transglutaminase show a synergistic effect in the occurrence and advance of celiac disease [41].

The serum lipid profile is critical to the development of multiple diseases, including primarily those of the cardiovascular system. Cholesterol is not only a primary component of the plasma membrane and lipoproteins, but also a precursor to corticosteroids, sex hormones and bile acids [42]. The results of the present study suggest that dietary supplementation with high levels of glutens did not produce any adverse effect on the lipid profile of the serum and ovarian tissue.

CONCLUSION

In conclusion, it was determined that high dietary levels of wheat and corn glutens showed only a limited effect on the histopathological and immunohistochemical structure of rats with normal genes that did not carry the HLA-DQ2 and HLA-DQ8 genes. On the other hand, glutens were observed to show a distinct effect on ovarian tissue LH and serum FSH levels. The results of the present study offer novel literature data that may guide future research on glutens.

Funding: The present study was granted financial support, as an approved Basic Research Project, by the Coordination Centre for Scientific Research Projects (BAP) of Atatürk University, under the code number “TSA-2017-6279”.
Conflicts of Interest: The authors declare no conflict of interest.

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