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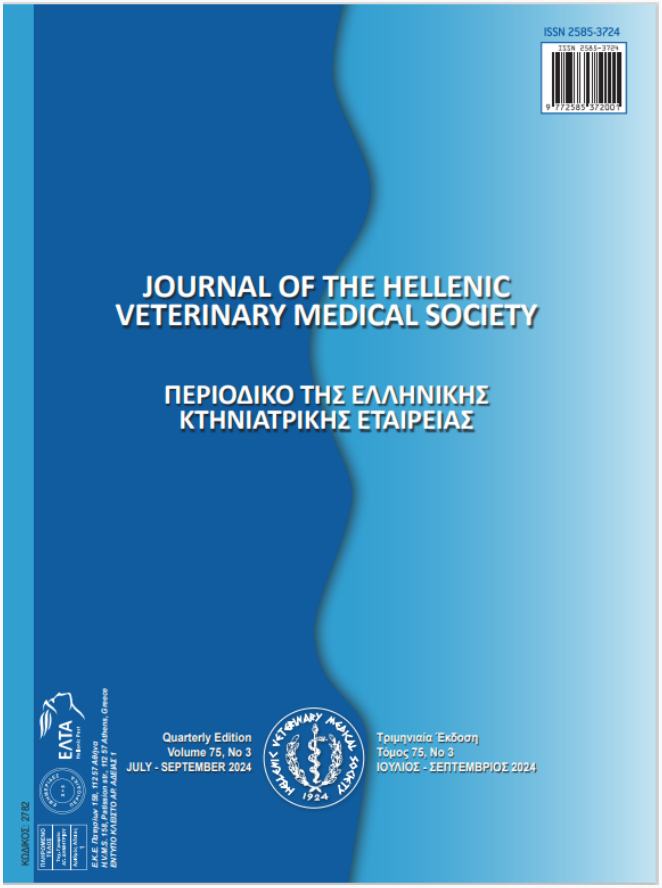


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# Effects of wheat and corn gluten on growth performance, histopathologic and autoimmune metabolism of entero-hepatictissue in lambs

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**ABSTRACT:** This study investigated the effects of wheat gluten and corn gluten, alone as a protein source intorations as protein sources, on fattening performance and the histopathological and autoimmune metabolism of the small intestinal and hepatictissues in lambs. The animals enrolled in the study were fed on isonitrogenous and isoenergetic diets. The protein sources provided to the animals were soybean meal and safflower meal in the control group, wheat gluten in GroupWheat, and corn gluten in Group Corn. The study animals included 24 male Morkaraman (Red Karaman) lambs, which were of a meanage of 9 months and assigned to three groups, each of 8 animals. Meandaily body weight gain (0-56 day) were observed to be significantly lower in Group Wheat ($p<0.05$). Mean daily feed in take were observed to be significantly lower in Group Wheat and Group Corn ($p<0.05$). At the end of the study, when compared to the control group, Group Wheat displayed significantly higher levels of villousatrophy, inflammation, crypthyperplasia and transglutaminase immunopositivity in the small intestinal tissue as well as necrosis, inflammation, bile ducthyperplasia and transglutaminase immunopositivity in the hepatictissue, and a lower level of degeneration in the liver ($p<0.05$). The findings detected in Group Corn were variable. In result, wheat gluten significantly affected both performance parameters and the histopathological and autoimmune metabolism of the intestinal and hepatictissues in lambs.

***Keywords*:** Corn; gluten; lamb; wheat; histopathological, small ruminant

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## INTRODUCTION

Corn and wheat plants are widely produced in the world geography, while the production area of soya bean plant is quite limited. Safflower plant, which is among the oilseed plants, is resistant to arid climatic conditions as well as adapting to different soil structures (Kara, 2020). Although these plants are produced as human food, by-products obtained directly or after processing in industry are also used in animal nutrition. In most of the countries where livestock breeding activities are carried out, the production of soya bean plants is either very limited or almost non-existent. As a result, soya meal, which is used as a protein source in animal feeding, causes an increase in feed cost. Therefore, alternative protein sources are needed (Tufarelli et al., 2013).

Gluten is the protein-rich part remaining after grains such as wheat, corn, barley, rye and oats are processed, and their starch is removed (Gumus et al., 2020). Nearly 5.4% of a wheat grain and almost 3-3.5% of a corn grain are composed of gluten (Schroeder, 1997; Kaushik et al., 2015). Wheat gluten is composed of glutenin and gliadins, which are found as storage proteins in endosperm cells (Wang et al., 2014). Gluten proteins are a subclass of prolamins, which constitute 70% of total wheat protein (Hudacko et al., 2015). These are poor in the amino acids lysine, tryptophan and methionine (Shewry, 2007). Corn gluten contains nearly 60% of protein, a significant amount of which is bypass protein (Hardwick and Glatz, 1989). The chemical composition of gluten shows that while it is rich in sulphur-containing amino acids such as methionine and cysteine, it is poor in lysine and tryptophan (St-Hilaire et al., 2007; Adamidou et al., 2009). On the other hand, gluten is rich in glutamic acid and vitamins B and E. Soybean meal contains nearly 44-50% of protein. This protein is poor in methionine, but rich in lysine and has a good amino acid balance (Banaszkiewicz, 2011). Depending on the level of husk in the structure of safflower meal, the crude protein rate can be up to 46% (Kellems and Church, 2002). Safflower meal protein has low biological value in terms of sulphurous amino acids and lysine. Therefore, its use in poultry nutrition is limited (Ehsani et al., 2014). In ruminants, the prominence of microbial protein synthesis and the fact that safflower meal is a relatively cheaper source of vegetable protein have enabled it to be used more. In a study, it was reported that when 15% safflower seed was used instead of soybean meal, it could be used as an alternative because it positively affected feed consumption and rumen fermentation (Rodríguez et al., 2015). On the other hand, there are studies showing that the use of corn gluten instead of soya meal in ruminant diets increases performance parameters (feed intake, body weight gain and feed conversion ratio) (Schrage et al., 1991; Siverson et al., 2014).

Soybean meal, safflower meal and corn gluten are commonly used in animal nutrition. Wheat gluten is used in the food industry and not in animal nutrition. Protein digestion is enzymatic and localized to the small intestine in monogastric animals, whilst in ruminants, proteins largely undergo microbial digestion in the rumen, and to a less extent, enzymatic digestion in the small intestine. Proteins that pass from the rumen to the small intestine undigested are mostly bypass proteins. Despite their gastrointestinal digestibility, gluten proteins cause several gastrointestinal, autoimmune and allergic health problems, including celiac disease, wheat allergy, gluten ataxia and dermatitis herpetiformis in certain organisms, primarily those carrying the genes HLA-DQ2 and HLA DQ-8 (Sapone et al., 2012).

This study was aimed at investigating the metabolic effects of dietary wheat gluten and corn gluten on the fattening performance and small intestinal and hepatic tissues of lambs. Histopathological evaluation was based on the analysis of villous atrophy, inflammation and crypt hyperplasia in the small intestine and degeneration, inflammation, necrosis and bile duct hyperplasia in the liver. Autoimmune sensitivity was detected by measuring transglutaminase enzyme activity.

## MATERIAL AND METHODS

### Animal material, study groups and diet

Twenty-four male Morkaraman (Red Karaman) lambs, of a mean age of 9 months, were included in the study. Lambs with similar live weight and body condition scores were selected. The animals were assigned to three study groups, including a control group, Group Wheat and Group Corn. The lambs were housed indoors on a farm, in compartments measuring 150×150×100 cm each. The rations provided to the animals were formulated to be isonitrogenous and isoenergetic. While the control group received soybean meal and safflower meal, Group Wheat received wheat gluten and Group Corn received corn gluten (Table 1). After undergoing a 21-day acclimatization period, the animals were fattened for a period of 56 days. All lambs were provided ad libitum access to

**Table 1.** Composition of lamb fattening feed used in the study

| Item | Control | Wheat | Corn |
|---|---|---|---|
| *Ingredients, %* | | | |
| Barley | 60.00 | 52.50 | 60.00 |
| Soybean meal | 15.93 | | |
| Rice bran | 10.00 | | |
| Safflower meal | 7.47 | | |
| Wheat | | 30.00 | |
| Corn | | | 18.22 |
| Corn gluten | | | 14.78 |
| Wheat gluten | | 10.30 | |
| Molasses | 3.00 | 3.00 | 3.00 |
| Marbledust | 2.40 | 1.65 | 2.35 |
| Soy oil | 0.60 | 0.33 | |
| Salt | 0.30 | 0.30 | 0.31 |
| Ammoniumchloride | 0.20 | 0.30 | 0.28 |
| Dicalciumphosphate | | 1.51 | 0.96 |
| Vitamin-Mineral premix | 0.10 | 0.10 | 0.10 |
| Total | 100 | 100 | 100 |
| *Nutrientcomposition* | | | |
| Crude protein, % | 17.00 | 17.00 | 17.00 |
| Metabolisableenergy, (kcal/kg) | 2.700 | 2.700 | 2.700 |

feed and water. Following their transport to the farm, the animals were treated for endo-and ectoparasites (Albendazole, Ivomec). The lambs were vaccinated twice with the subkutan injection of Coglavax® against enterotoxemia. Feed was provided in preweighed quantities, twice a day, at 8.00 am and 4.00 pm. Two separate areas with a size of 50×50×15 cm was made for the consumption of concentrate and roughage in each compartment. Concentrate and roughage intakes were calculated separately.

**Determination of the performance parameters**

Body weight measurements were performed at the onset of the study and on days 14, 28, 42 and 56, in the morning, before the animals were provided with feed. Live weight measurement was made with a special cage scale with an accuracy of ± 50 grams and a maximum measurement of 300 kg. Daily feed intake was calculated by weighing the quantities of concentrate and roughage remaining in the feed troughs before the renewal of feed in the morning. Daily body weight gain was calculated by dividing the difference between two body weight measurements by the time elapsed between the two measurements. The feed conversion rate was calculated based on the amount of feed consumed for 1 kg of body weight gain and the increase in body weight.

**Collection of the enteric and hepatic tissue samples**

At the end of the study period, the animals were slaughtered and tissue samples were taken from the small intestine and liver of each lamb for histopathological and immunohistochemical examinations. Lesions were scored semi-quantitatively, based on the microscopic examination of 10 different regions at 40x magnification, as follows: 0 (negative), +1 (mild), +2 (moderate), +3 (severe) and +4 (very severe). Analyses were carried out at the Pathology Department of Atatürk University, Faculty of Veterinary Medicine.

**Histopathologic analyses**

The tissue samples taken from the small intestine and liver of each animal were fixed in 10% formaldehyde solution for 48-72 h. After being was he dunder running tap water for 6-8 h, the tissue sunder went routine tissue processing, and were passed through gradedalcohol (70°, 80°, 90°, 96°, 100°) and xylolseriesto be embedded in paraffin. Four-micron-thick section scut from the paraffinblocks were deparaffinized by being passed through xylolandalcohol series, each for 3 minutes. Next, the tissues were rehydrated in distilled water for 5 min, and stained with hematoxylin for 3 min. After being stained with hematoxylin, the tissue samples were placed in eosinsolution for 10-15 seconds and passed through gradedalcohol (100°, 96°, 90°, 80°, 70°) and xylolseries (Taylor and

Cote, 2006). Finally, the sections were mounted in Entellanand examined under a light microscope. Histopathological findings were classified for severity as follows: nofinding (-), mild (+), moderate (++) and severe (+++). Samples displaying histopathological findings were imaged (Olympus BX51 with DP72).

### Immunohistochemical analyses

The small intestinal and hepatic tissue samples were embedded in paraffin. Four-micron-thick sections were cut from the paraffinblocks and mounted on to polylysine-coatedglass slides. After being deparaffinized through alcoholandxylolseries, the sections were rehydrated in distilled water for 5 min. Subsequently, tissue endogenous peroxidase activity was blocked by incubating the sections in hydrogenperoxide ($H_2O_2$) solution for 10 min. Following two consecutive washes in phosphate-bufferedsaline (PBS), antigen retrieval was performed by heating the sections in a microwave oven at 800 watt in 0.01 mol/L citrate buffer (pH 6) solution for 10 min. After three more washes in PBS, the sections were outlined with a PAPpen, and a protein block solution was applied for 10 min. The tissues were treated with the primary antibody Transaminase (ab236658) and incubated in a humiditychamber for 1 h. At the end of the incubation period, the tissues were washed 3 times in PBS and treated with secondary antibodies for 15 min. At the final stage, a DAB solution was applied as a chromogento visualize the binding of the primary and secondary antibodies (ab64264). Next, the tissues were counter stained withMayer'shematoxylin, mounted in Entellan, and covered with a coverslip (Dokumacioglu*et al.*, 2018). By lightmicroscopic examination, immuno positivities were scored as follows: absent (-), mild (+), moderate (++), intense (+++).

### Statistical analysis

The statistical analyses of the study data were performed using the Statistical Package for the Social Sciences (SPSS) software. Data obtained for feed in take, feed conversion rate, and body weight gain were analyzed with one-way analysis of variance (ANOVA), and the statistical significance of the differences between the study groups was determined with Duncan's test. Histopathological and immunohisto chemical alterations in the small intestinal and hepatictissues were calculated with the Kruskal-Wallis test.

## RESULTS

### Findings on the Performance Parameters

Parameters related to the fattening performance of the lambs are presented in Table 2. No statistically significant difference was determined between the study groups for the final mean body weights of the lambs measured at the end of the fattening period ($p>0.05$). The mean daily body weight gain was highest in the control group (264.73 g/day), and lowest in the study group that received dietary wheat gluten (213.84 g/day) ($p<0.05$). The average daily consumption of concentrated feed was the highest in the control group, while the Group Wheat and Group Corn were similar ($p<0.01$). No statistically significant difference between the study groups in terms of mean daily roughage consumption ($p>0.05$). No statistically significant difference between the study groups in terms of mean daily roughage consumption ($p>0.05$). The feed conversion rate was lowest in the group fed on wheat gluten and highest in the group fed on corn gluten ($p<0.05$).

### Histopathological Findings

Statistical analysise valuations of samples taken from intestinal and liver tissues are shown in Table 3, and microscopic examinations are shown in Figure 1. Based on the histopathological evaluation of the intestinal tissues amples of the study groups, the highest score for villousatrophy was noted in Group Wheat, and the lowest villousatrophyscore was recorded in

**Table 2.** Effects of gluten on growth performance parameters in the experimental lambs.

| Parameters | Control | Wheat | Corn | P-value |
|---|---|---|---|---|
| Initial BW, kg | 28.98±0.82 | 29.69±0.80 | 28.91±0.55 | 0,731 |
| Final BW, kg | 43.81±1.24 | 41.66±0.91 | 42.63±1.36 | 0,414 |
| Average Daily gain (g/d) | 264,73±15,04$^{a}$ | 213,84±9,17$^{b}$ | 244,94±16,26$^{ab}$ | 0,037 |
| Roughage intake, g/day | 115,44±15,46 | 153,48±9,58 | 146,9±9,46 | 0,080 |
| Concentrated feed intake, g/day | 1372,5±33,86$^{a}$ | 1235,5±32,72$^{b}$ | 1209,66±24,14$^{b}$ | 0,004 |
| Total feed intake, g/day | 1487,5±33,85$^{a}$ | 1389±25,76$^{b}$ | 1356,66±29,88$^{b}$ | 0,018 |
| Feed efficiency, kg/kg | 5,62±0,12 | 6,49±0,26 | 5,54±0,22 | 0,065 |

$^{a, b, ab}$Means with different superscript letters in rows are significantly different, $p<0.05$.
BW: Body weight.

the control group ($p<0.01$). Based on the examination of the intestinal tissue samples, the highest level of inflammation was detected in Group Wheat, and the lowest level of inflammation was detected in Group Corn ($p<0.01$). Scores for crypthyperplasia were equal in the control group and Group Wheat, and lowest in Group Corn ($p<0.01$).

Based on the examination of the hepatic tissue samples, degeneration scores were found to be equal in the control group and Group Corn, and lowest in Group Wheat ($p<0.01$). The highest level of necrosis was detected in Group Wheat, and the lowest level was detected in the control group ($p<0.01$). Likewise, the level of inflammation was highest in Group Wheat and lowest in the control group ($p<0.01$). Scores for bile ducthyperplasia were equal in the control group and Group Wheat, and lowest in Group Corn ($p<0.05$).

## Immunohistochemical Findings

Statistical analysis evaluations of intestinal and liver tissues are shown in Table 3 and microscopic examinations are shown in Figure 2. Small intestinal transglutaminase values were highest in Group Wheat and lowest in Group Corn ($p<0.01$). Similarly, hepatic tissue transglutaminase values were highest in Group Wheat and lowest in Group Corn ($p<0.05$).

**Table 3.** Effects of gluten on histopathological and immunohistochemical indices of entero-hepatic tissue in the experimental lambs.

| Histopathological and Immunohistochemical Parameters | Control | | Wheat | | Corn | | P-value |
|---|---|---|---|---|---|---|---|
| | Mean±SEM | Median | Mean±SEM | Median | Mean±SEM | Median | |
| Small IntestinalTissue | | | | | | | |
| Villous atrophy | 1.12±0.12 | 1,000 | 3.62±0.18 | 4,000 | 2.87±0.12 | 3,000 | 0,001 |
| Inflammation | 3.12±0.12 | 3,000 | 3.62±0.18 | 4,000 | 2.37±0.18 | 2,000 | 0,001 |
| Crypt hyperplasia | 4.00±0.00 | 4,000 | 4.00±0.00 | 4,000 | 2.50±0.18 | 2,500 | 0,001 |
| Transglutaminase | 1.25±0.16 | 1,000 | 2.87±0.12 | 3,000 | 1.00±0.00 | 1,000 | 0,001 |
| LiverTissue | | | | | | | |
| Degeneration | 4.00±0.00 | 4,000 | 2.62±0.18 | 3,000 | 4.00±0.00 | 4,000 | 0,001 |
| Necrosis | 1.75±0.16 | 2,000 | 3.75±0.16 | 4,000 | 2.75±0.16 | 3,000 | 0,001 |
| Inflammation | 1.37±0.26 | 1,000 | 3.62±0.18 | 4,000 | 3.00±0.00 | 3,000 | 0,001 |
| Bileduct hyperplasia | 4.00±0.00 | 4,000 | 4.00±0.00 | 4,000 | 3.18±0.51 | 4,000 | 0,037 |
| Transglutaminase | 1.37±0.18 | 1,000 | 1.87±0.12 | 2,000 | 1.00±0.00 | 1,000 | 0,002 |

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

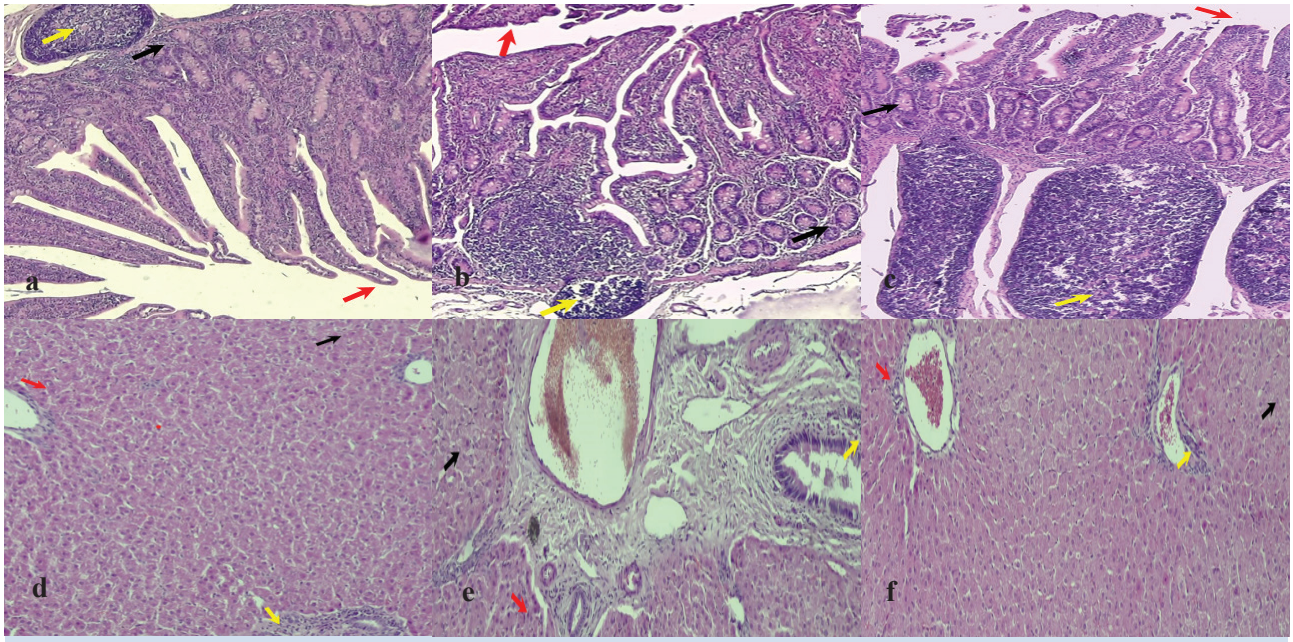


**Figure 1.** Hematoxylin-eosin staining of the intestinal and liver tissue of experimental groups. İntestinal tissue; Villus atrophy (Red Arrow), Necrosis (Yellow Arrow), Crypt hyperplasia (Black Arrow), a: Control, b: Wheat, c: Corn, Bar; 600 µm. Liver tissue; Bile Duct Hyperplasia (Red Arrow), Inflammation (Yellow Arrow), Degeneration (Black Arrow), d: Control, e: Wheat, f: Corn, Bar; 200 µm.

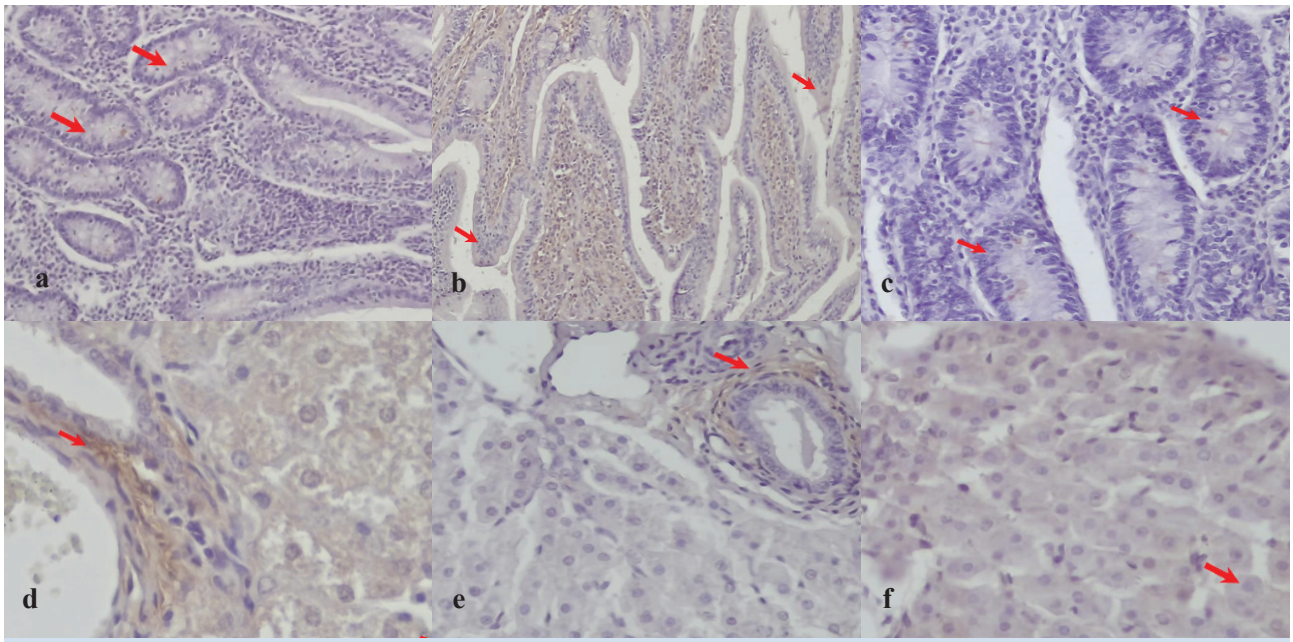


**Figure 2.** Transglutaminase immunopositivity in intestinal and liver tissue (Red Arrow), IHC. İntestinal tissue; a: Control, b: Wheat, c: Corn, Bar; 200 µm. Liver tissue; d: Control, e: Wheat, f: Corn, Bar; 100 µm.

## DISCUSSION

Meeting the protein requirements of animals correctly is very important both for their balanced and healthy nutrition and for increasing efficiency in protecting against immunity and immune system diseases (Zervas and Tsiplakou, 2011). The fattening performance of animals is affected by multiple factors. These include breed, sex, age, as well as farming and feeding conditions. While soybean meal, safflower meal and corn gluten are commonly used in animal nutrition, wheat gluten is mostly used in the food industry. This study was designed to investigate the effects of soybean meal, wheat gluten and corn gluten on the fattening performance of the lambs, and to determine the mechanism of action on the histopathological and immunohistochemical parameters of the small intestinal and hepatic tissues. In the study, the performance and metabolic effects of using soybean meal + safflower meal, corn gluten and wheat gluten in one way or at the highest ratios of protein requirements of lambs were comparatively revealed.

In the gastrointestinal system, the digestion of protein varies under the influence of several factors (Mackie and Macierzanka, 2010). In monogastric animals, protein digestion occurs enzymatically in the small intestine. However, the digestive system of ruminants is anatomically and physiologically different from that of other animals. In ruminants, proteins largely undergo microbial digestion in the rumen, and to a small extent, enzymatic digestion in the small intestine. The occurrence of protein digestion in the rumen or small intestine varies with multiple factors. Zhang et al. (2023) reported that a certain amount of wheat gluten can be added instead of soya meal and fish meal in the diets of Japanese sea bass without affecting performance parameters. Lin et al. (2023) reported that the use of 20% corn gluten instead of fishmeal in the diet of juvenile white shrimps significantly increased dry matter consumption and energy digestion. At the end of the study, no statistically significant difference was detected between the study groups for the final mean body weights ($p>0.05$). On the other hand, throughout the study period, the study groups significantly differed for mean daily body weight gain ($p<0.05$). The daily body weight gain of Group Wheat was similar to that of Group Corn, but significantly lower than that of the control group ($p<0.05$). The feed intake of the group fed on soybean meal was significantly higher than the feed intake of the groups fed on wheat gluten and corn gluten (Table 2) ($p<0,05$). The feed conversion rate was lowest in Group Wheat and highest in the group that received soybean meal ($p<0.05$). In previous studies carried out with isonitrogenous and isoenergetic diets containing different feedstuffs, the performance of the animals was determined to vary. It has been reported that the feed consumption, feed conversion rate and live weight gain of animals fed with feed containing various amounts of corn gluten are positively affected (Jaster et al.,

1984; Richards et al., 1998; Beauchemin and Koenig, 2005; Siverson et al., 2014; Jiang et al., 2019; Baldassiniet al., 2021). At the same time, the addition of corn gluten to ruminant rations contributes positively to rumen metabolism (Allen and Grant, 2000; Parsons et al., 2007). In this study, in terms of feed efficiency and live weight gain of animals consuming corn gluten feed, it is in similar with the literature. Studies on how wheat gluten consumption alone and at high levels affect fattening performance in ruminants are limited. However, it has been reported that the addition of wheat gluten as a second protein source to the ration in different amounts has a positive effect on live weight gain (Fiemset al., 1995). These studies further demonstrated that feed additives also influenced performance parameters (Kumar et al., 2014). In the present study, the differences determined between the groups for the fattening parameters were attributed to differences in the metabolism of the protein sources in the digestive system. This was related to differences in metabolism and feed conversion, depending on the protein source incorporated into the ration.

Protein metabolism varies among organisms under the influence of multiple factors. In conditions where nutrition is inadequate or unbalanced, the immune system is adversely affected because the protein-energy balance is disrupted. In order to create a strong immune system and protect the organism against pathogens, sources such as amino acids and fatty acids used in energy production should be balanced in the ration. Nutritional style ensures the development of metabolic, nutritional and immunological functions in the intestinal microbiota. Once nutrients are digested and absorbed from the digestive tract, they are metabolized in the liver. Approximately 60% of the immune reactions take place in intestinal cells. As the metabolic center of the body, the liver hosts not only the metabolism of digested nutrients, but also many other metabolic reactions. Therefore, in the present study, the histopathological and immunohistochemical responses of the intestinal and hepatic tissues were investigated. Gluten proteins are easily digested by healthy people through the gastrointestinal tract. However, gluten proteins can cause various health problems in some people. Especially in individuals carrying the HLA-DQ2 and HLA DQ8 genes, gluten with a large molecular structure is difficult to digest, which can cause immune, digestive system problems and damage to some tissues and organs (Rubio-Tapia et al., 2013). Many health problems occur because the immune system is suppressed. However, when the studies on gluten consumption are examined, there is not enough information about the negative effects that occur in individuals who do not have the HLA-DQ2 and HLA DQ8 genes. Gliadin peptides, generated by the breakdown of gluten in monogastric species, bind to “Human Leukocyte Antigen” (HLA) molecules, and trigger a series of immunological events, resulting in the appearance of clinical symptoms specific to various diseases (Sapone et al., 2012). Peptides found in the structure of gliadin are known to induce cellular, humoral and inflammatory responses in tissues (Marsh, 1992; Molberg et al., 2001). Gliadin is highly resistant to its proteolytic digestion in the stomach, pancreas and intestines in the gastrointestinal tract. It also contains peptide sequences that are located in the human intestine and escape intact. The difficulty of digestive activity is caused by the high amino acid content of gliadin, which proline, glutamine and many proteases cannot break down. While corn gluten and soybean meal are protein sources commonly used in the feeding of ruminants, to the authors’ knowledge, there is no previous study on the use of wheat gluten in ruminant nutrition. In the present study, the histopathological examination of small intestinal tissue samples demonstrated that villous atrophy, inflammation and crypt hyperplasia were most severe in the group fed on wheat gluten ($p<0.01$). It can be stated that wheat gluten has a negative effect on the absorption and secretion of digested nutrients. Gluten-containing food products have been reported to cause damage to various organs and tissues, primarily the digestive system, in monogastric species (Sollid and Jabri, 2013). Gümüş et al. (2024) reported that the use of soybean meal compared to corn gluten and wheat gluten in the diets of rats significantly decreased IgA level among immunohistochemical parameters in liver tissue, but did not affect transglutaminase, IgG, CD4 and CD8 parameters. In the present study, dietary wheat gluten caused significant increase in necrosis, inflammation and bile duct hyperplasia in the hepatic tissue, but was associated with a significantly lower level of hepatic degeneration, compared to dietary corn gluten and soybean meal ($p<0.01$). These findings detected in the hepatic tissue are also observed in cases of intoxication, infection and stress.

Each body tissue has a distinctive immune system of its own. These systems enable a healthy metabolism in the organism. The production of tissue transglutaminase antibodies occurs by a complex mechanism. Gluten (mainly gliadins) in cereal proteins is perceived by tissues as an antigen and stimulates the

development of T-cells to produce antibodies against antigens (Sulkanen et al., 1998; Korponay-Szabó et al., 2000; Kalliokoski et al., 2017). For this reason, an increase in transglutaminase antibody is observed. It is very important to examine the immunohistochemical parameters in order to support the histopathological findings that occur as a result of gluten sensitivity in the tissues. Antibodies are used to detect gluten sensitivity immunohistochemically (Maiuri et al., 2003). Gumus et al. (2024) reported that the use of soybean meal in the diet of rats, compared to corn gluten and wheat gluten, significantly reduced the level of CD4, one of the immunohistochemical parameters, in the intestinal tissue, but did not affect the transglutaminase, IgG, IgA and CD8 parameters. Imik et al. (2024) reported that if male rats were fed different diets containing soybean meal, corn gluten and wheat gluten, the values in the intestinal tissue were villous atrophy 1.42, 2.14 and 1.5, lymphocyte plasma neutrophil 2.57, 1.57 and 1.66, and crypt hyperplasia 1.71, 0.71 and 0.33, respectively. Although crypt hyperplasia among these parameters was found to be statistically significant between the groups, no significant difference was found between the groups in other parameters. On the other hand, transglutaminase enzyme activity values were 0.57, 0.42 and 0.16; gliadin values were 1.85, 1.42 and 0.83; IgA values were 0.85, 1.57 and 1.50, respectively (soya meal, corn gluten and wheat gluten). Among these parameters, although gliadin values were found to be statistically significant between the groups, no significant difference was found between the groups in terms of transglutaminase enzyme activity and IgA. In this study, Transglutaminase antibody test analysis was performed in order to determine the sensitivity of animals to gluten. In the present study, transglutaminase levels, one of the most important autoimmune parameters, being highest in the group fed on wheat gluten, demonstrated that the ruminant small intestine and liver were more sensitive to wheat gluten. Histopathological and immunohistochemical values in different studies show similarity with the literature information (Gümüş et al., 2021; İmik et al., 2022).

## CONCLUSION

The present study was aimed at determining the effects of dietary wheat gluten and corn gluten on fattening performance and the histopathological and autoimmune metabolism of the small intestinal and hepatic tissues in lambs. Performance parameters in the group containing wheat gluten in the feed were found to be significantly lower compared to the group fed with soybean meal (Control Group). The results showed that the lambs evaluated the different protein sources used in the study in different ways. The performance parameter values were determined to be the highest in the group given soybean meal (Control group) and the lowest in the Group Wheat. It was determined that wheat gluten significantly increased histopathological lesions in the small intestinal and hepatic tissues. Furthermore, wheat gluten was also associated with more severe responses to the transglutaminase enzyme, a major autoimmune parameter. The effects of corn gluten were observed to be variable. Further detailed research is required to fully elucidate these variable effects. The intestinal and liver tissues of lambs were sensitive to high levels of wheat gluten in the diet. Further studies including diets containing different proportions of wheat gluten are needed to utilize wheat gluten as an alternative protein source in lamb diets.

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## CONFLICT OF INTEREST

The authors declare no competing interests.

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