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Histological Effects of Different Levels of Sorghum Grain on the Abomasum, Duodenum and Colon of *Ghezel×Arkhar-Merino* Crossbred Lambs

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Abstract: Sorghum grain is comparable to maize in feeding value and can reduce feed cost and dependency on maize. Sorghum grain containing reasonable amounts of tannin which have ability to bind dietary protein, carbohydrate and minerals. Consuming high amounts of tannins may cause bowel irritation on gastrointestinal tissues. The objective of this study was to assess the effect of replacement of different levels of barley with sorghum grain on histological changes of the abomasum, duodenum and colon. In this study sixteen male *Ghezel×Arkhar-merino* crossbred lambs were used. Dietary treatments had same amounts of alfalfa hay (20% total DM) and different levels of the barley grain substituted with sorghum grain in which sorghum grain was used in the levels of 0, 60, 70 and 80 percent of total ration. Lambs were randomly assigned to one of the four dietary treatments in a completely randomized design assignment. At the end of finishing period the lambs were slaughtered and samples were prepared. The gross examination of abomasum, duodenum and colon did not reveal any significant necropsy signs. Samples were preceded by routine histological techniques and H and E staining method. Histological results showed that, abomasum has not distinct histological damage; except increasing of parietal cells and lymphocytosis in lamina propria and tunica submucosa. The presence of lymphocytosis probably is not related to the sorghum usages. microscopic observations of duodenum and colon showed that, higher dietary sorghum grain resulting histological changes as increase of goblet cells, dilatation of lumen of Lyberkohn's glands in order to degeneration of apical parts and brush borders of secretory units surface cells and degeneration of apical parts of villi surface cell. Also hyperemia and sporadic hemorrhage was observed in lamina propria and tunica submucosa of duodenum and colon in high level sorghum diet.

Keywords: Abomasum, Histology, Colon, Duodenum, Sorghum Grain, Sheep

1. Introduction

Tannins are polyphenolic substances with various molecular weights and a variable complexity. They are tentatively classified into two classes: hydrolysable and condensed tannins and are considered to have both adverse and beneficial effects depending on their concentration and nature besides other factors such as animal species, physiological state of the animal and composition of the diet [12]. Sorghum grains are becoming an increasingly important source of energy in animal nutrition which contain considerable amount of tannins. Dietary tannins have ability to bind with dietary protein, carbohydrate and minerals [9] [13] [22]. Consuming high amounts of tannins may cause bowel and kidney irritation, liver damage, irritation of the stomach and gastrointestinal pain [10] [11] [20].Tannin can reduce intestinal enzymes realasing.it has been shown that high level of dietary tannin causes reduced density and length of duodenal microvilli. The number of duodenal mucosal and

goblet cell increases under influence of high dietary tannin [1] [14]. Reducion in the length of duodenal microvilli can decrease absorption. [14]. Karimi et al. (2014) reported histological changes in the liver and kidney of lambs when fed with high levels of sorghum grain. [8]. The aim of present study was to understand the effects of different level of dietary sorghum grain on of abomasum, duodenum and colon histology and structural changes on these organs.

2. Material and Methods

Sixteen male Ghezel×Arkhar-merino crossbred lambs with live weights ranging from 34 to 55 kg (46±5.8) were used in the experiments. Experimental animals were kept at research farm University of Tabriz. Lambs were randomly assigned to one of the four dietary treatments (Table 1) in a completely randomized design (CRD) assignment. Animals were adapted for 3 weeks before starting main experiment. Each pen was provided with a food trough and a water container, and food was offered two times daily. (Table 1)

Table 1. *Experimental rations compositions.*

Treatment	A	B	C	D
Alfalfa hay	20	20	20	20
Barley grain	80	20	10	0
Sorghum grain	0	60	70	80

Then the animals were slaughtered, the abomasum, duodenum and colon samples were collected. The samples were fixed in 10% formalin and passaged for dehydration, infiltration and immersion by autotechnicon (JUNG HISTOKINET 2000 Leica). The samples embedded in the solid paraffin. The paraffinized blocks were sectioned by microtome (JUNG HISTOCUT 820 Leica) in the 0.7 micrometer thickness. The sections were stained by hematoxylin and eosin method. Length of villi of duodenum and length of mucosal layer of colon measured with following methods by scaled objective lens under CX31 Olympus microscope (Fig.1 and 2). The images were captured with the digital Olympus camera (DP12) under light fluorescence microscope (BX60). Data of length of duodenal villi and colon mucosa compared by randomized complete block design in SPSS.

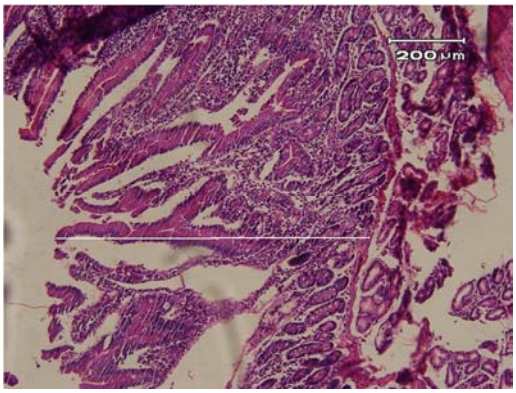

Figure. 1. *Method of measuring of duodenal villi. Height of villi was measured from tip of villous till muscularis mucosae layer.*

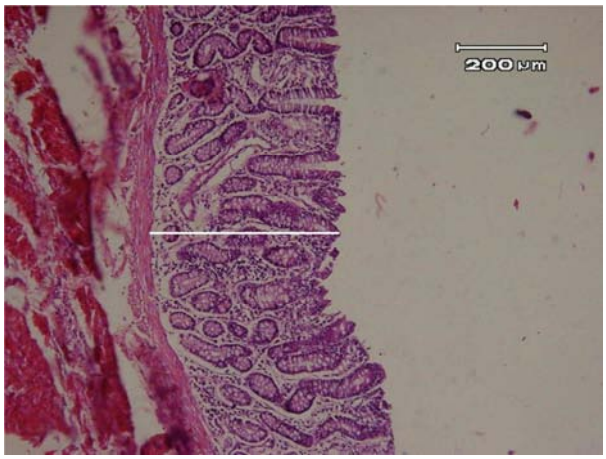

Figure. 2. *Method of measuring of colonal mucosa. Height of Mucosa was from tip of surface epithelial tissue cells till muscularis mucosae layer.*

3. Results

Due to the histological features of abomasum, duodenum and colon tissues are different and sorghum grain had different effects on these tissues, so the results will be presented in 3 part.

Part one: Histological effects of sorghum grain on the abomasal tissues.

Treatment of A: Significant disorders were not observed in the mucosa. lymphocytotsis was observed in lamina propria and tunica submucosa and congestion and hyperemia was observed in vessels of lamina propria and tunica submucosa. The length of gastric pit obviously was decreased (Fig. 3).

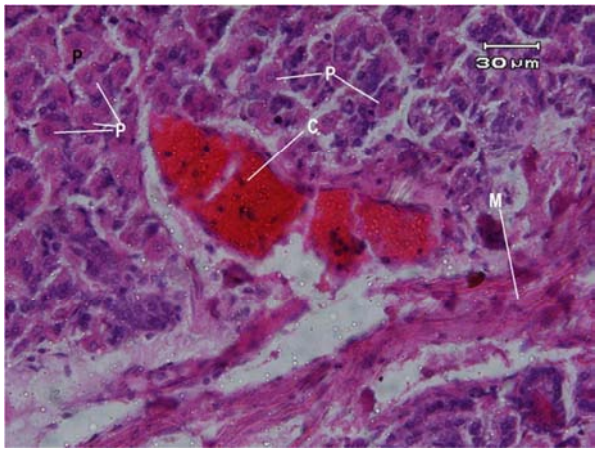

Figure 3. *Histological section of gastric glands of abomasum. P,parietal cells. C, congestion in vessels of lamina propria and tunica submucosa of abumasum. M, Muscularis mucosa. (Magnification x 400, H&E staining.).*

Treatment B: No significant disorders were found in the mucosa. Penetration of lymphocytes in lamina propria and tunica submucosa was observed. Congestion and hyperemia was observed in vessels of lamina propria and tunica submucosa.

Treatment C: Influx of lymphocytes in lamina propria, increased mucosa secreting cells in gastric gland, the parietal cells had normal distribution.

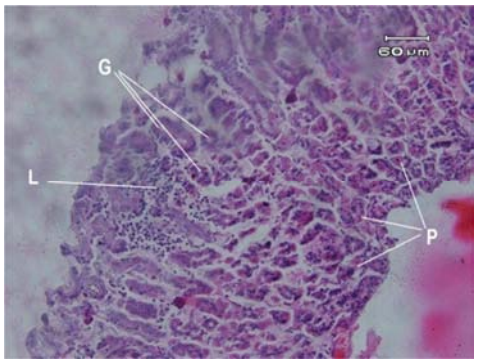

Figure 4. *Histological section of gastric glands of abomasum. G, Gasteric galnds, L, lymphocytosis. P, Parietal cells. (Magnification x 200, H&E staining).*

Treatment D: Decreased length of gastric pit decreased gastric gland and replacement of them by connective tissue, decrease of parietal cells and increased of mucosa cells.

Part two: Histologocal effects of sorghum grain on the duodenom.

Treatment of A: Lymphocytosis in limana propria and tunica submucosa, Dilutation of secretory units of Broner's glands.

Treatment of B: Lymphocytosis in limana propria and tunica submucosa, Hyperemia in lamina propria (Fig.5).

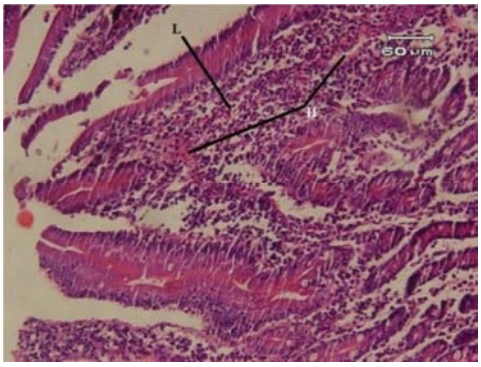

Figure 5. *Microscopic section of duodenum of lamb with 60% sorghum grain diet. H, hyperemia. L, Lymphocytosis. (H&E Staining, Magnification ×400).*

Treatment C: Lymphocytosis in limana propria and tunica submucosa, Increase of goblet cells (Fig. 6).

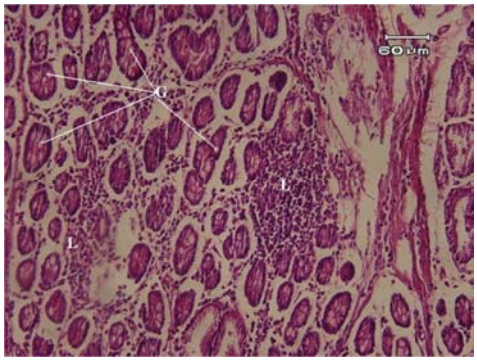

Figure 6. *Microscopic section of duodenum of lamb with 70% sorghum grain diet. G, Goblet cells which increased. L, lymphocytosis. (H&E Staining, Magnification ×400).*

Treatment D: Lymphocytosis in limana propria and tunica submucosa, Dilatation of secretory units of lieberkün glands, Dilatation of secretory units of Brunner's glands, Decrease of distribution of villi and height of absorptive columnar cell due to degeneration of these cell apical brush borders (Fig.7).

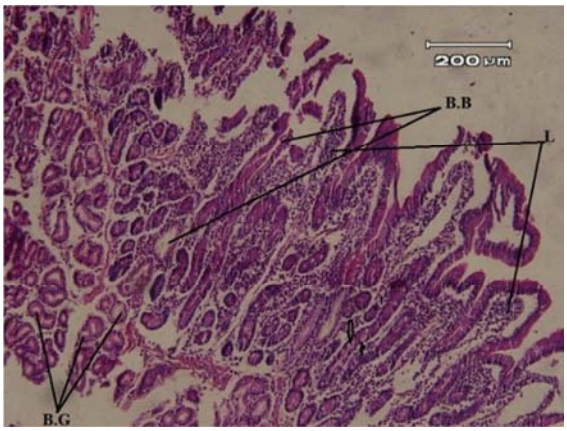

Figure 7. *Microscopic section of duodenum of lamb with 80% sorghum grain diet. B.B, Decrease brush borders, L, lymphocytosis, B.G, Dilatation in Brunners' glands lumen. (H&E staining, magnification ×200).*

Part three: Histological effects of sorghum grain on the colon.

Treatment A: Lymphocytosis in limana propria and tunica submucosa,

Treatment B: Lymphocytosis in limana propria and tunica submucosa.

Treatment C: Degeneration of Surface epithelium and lymphocytosis in limana propria and tunica submucosa, increase of number of goblet cell (Fig.8 and 10).

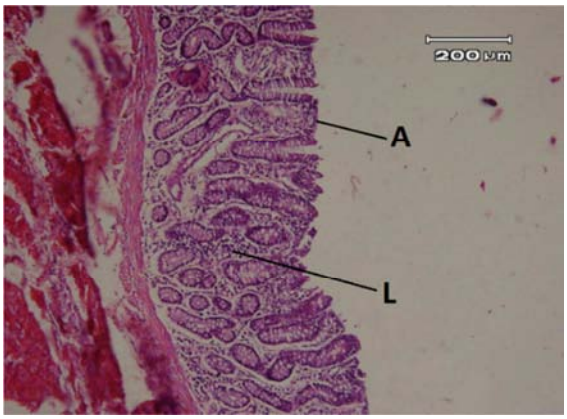

Figure 8. *Microscopic section of colon of lamb with 70% Sorghum grain diet. A, degeneration and flux of apical cells. L, Lymphocytosis. (H&E Staining, Magnification ×100).*

Treatment D: Degeneration of Surface epithelium and lymphocytosis in limana propria and tunica submucosa, Dilatation of secretory units of lieberkün glands and increase of goblet cells.

Lymphocytosis was observed in all samples abomasum, duodenum and colon and it doesn't have any relation to sorghum grain level in diet. It can be cause by an unknown inflammation or infection factor (Fig.8, 9 and 10).

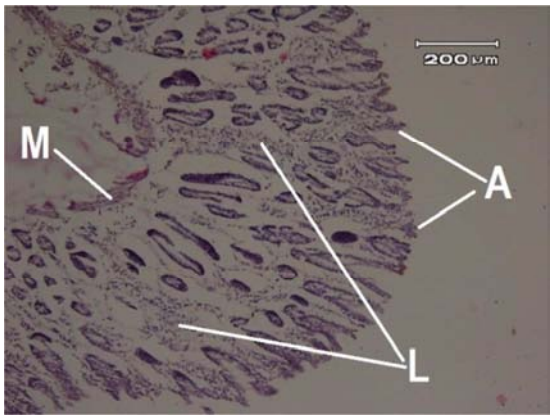

Figure 9. *Microscopic section of duodenum of lamb with 80% sorghum grain diet. A, degeneration and flux of apical cells. (H&E Staining, Magnification ×100).*

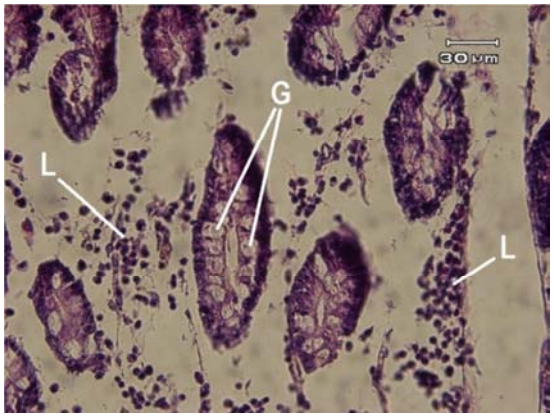

Figure 10. *Microscopic section of colon of lamb with 80% sorghum grain diet. G, Gublet cells increased.* ***L,*** *Lumphocytes. (H&E staining, magnification ×400).*

4. Discussion

Ruminants that graze or browse on toxic plants have adapted rumen organisms that detoxify many, but not all secondary metabolites [3] [4]. However, the detoxification process may cause adverse effects in ruminants as a consequence of increased enzymatic demand in the liver, kidney, gut mucosa and other tissues [29]. Sorghum plant is belonging to the grass family. This plant is native to warm areas, areas with poor soils and low annual precipitation produces a good product. Sorghum because of strong roots and the cuticle layer of wax on their leaves has the power tolerance to the dehydration stress. This plant has many varieties such as grain, forage, sugar, sweep and wax that seed type is used in human and animal nutrition. Sorghum grain in terms of nutrient composition very close to corn and wheat, but despite this, little attention is placed by producers. Tannin in sorghum grain as anti-nutritional factors creates a strong bond with protein at high levels and carbohydrates and minerals in the lower levels that's why the plant is resistant against digestive enzymes. Generally, sorghum compared with corn contains more protein and less oil [16]. Sorghum grain tannin has a negative effect on protein digestibility [5] [6]. High levels of tannins (6%) have a negative impact on the growth and production of ruminants. Diets rich in tannin reduce the intake of nutrients, growth, palatability, enzyme activities, microbial attack food particles, gas production, indigestibility, organic matter disappearance from the rumen and abnormal epithelial ulcers in digestive tract [2] [21] [25] [28]. The results of the high tannin usage are secreted endogenous proteins such as saliva and mucosal glycoproteins, digestive enzymes and increase intestinal cells shedding [16] [31]. Many believe that tannins can have a negative effect on the absorption of nutrients from the small intestine. This could be due to intestinal stability tannin-protein complex that it is not able to disintegrate in abomasum. Formation of tannin-digestive enzymes complex or new complex of tannin-protein in ration or changes in intestinal absorption is the result of the interaction between tannins and intestinal mucosa [15] [24].

Mbatha et al. (2002) reported that, the width of epithelial layer of the abomasum increased significantly with tannin ($P< 0.05$). Abomasal epithelial width was significantly different between the control diet and that containing 150 g/kg of tannin ($P<0.05$). Villous height in the duodenum differed significantly among the diets ($P< 0·05$), whereas the crypt depth was not affected ($P > 0.05$). Degree of keratinization of the epithelium increased with tannin level in the reticulum ($P < 0.01$), rumen ($P<0.01$), omasum ($P< 0·05$) and abomasum ($P< 0.001$) [14]. Results of this study on abumasum showed that, increasing of sorghum grain in diets cause the decreases of parietal cells. Also the increasing of sorghum grains resulted increasing of mucosa cells in gastric gland and mucosa layer of abomasum. These changes in the foregut epithelial tissue are unlikely to affect absorptive processes; however increased keratinization of the abomasum might jeopardize the secretary function and limit eventual protein digestion. In contrast, condensed tannins have been shown not to have adverse effects on the morphology of the intestinal tract of rats as opposed to tannic acid [17] [22]. Also the results of this research showed that, the length of gastric pit was decreased during the consuming of high levels of sorghum grain diets. High level of sorghum grain caused hyperemia and congestion in lamina propria and tunica submucosa in abomasum. It is interesting to note that some regions of the GIT did not have thickened epithelium (reticulum and omasum) which is indicative of normal function and, the color of the small intestines darkened with increased dietary tannin levels, probably indicative of either the consequence of its interaction with CT or its role in the absorption and detoxification of unidentified components of the commercial tannin mixture. Because of the toxic properties of the tannins and effects of it on the abomasum mucosa, the mucus cells of abomasum were increased. Also these properties caused destroying of gastric glands and its affected inflammation, congestion and hyperemia in lamina propria and tunica submucosa of abomasum [2]. Lyphocytosis is an important sign for inflammation, but it was observed in either high level or low level treatment of this research, therefore it has any relation to level of surghum grain diet.

The processes of digestion are completed in the small intestine and the products of digestion are absorbed. Under microscope, intestinal villi are seen in mucosae layer of small intestine which they increase absorptive surface. Also microvilli are seen in apical border of simple columnar cell of small intestine epithelium, which they are called brush border. Between the villi are small opening tubular gland called intestinal gland or lieberkün and a series mucosal gland (Brunner's gland) were observed in tunica submucosa of duodenum [7]. Colon is a part of large intestine. It has 3 portions as ascending, transvers and descending. Histological structure is same in all of the parts of colon. Mucus layer of colon doesn't have any villus. It has very low digestion but it has considerable absorption function, especially liquid absorption. Colon has intestinal long glands which characterized by a great abundance of goblet cells and a small number of enteroendocrine cells. Absorptive cells are columnar and have short, irregular microvilli [7].

Tannins are increasingly obvious that even the modified definition of a tannin as "a compound that binds to proteins" is imprecise and misleading and no longer serves any useful purpose [26]. Ortiz et al. (1994) found histological lesions in the ileum and liver of chicks and rats fed diets high in tannins extracted from faba bean (*Vicia faba*) suggesting a loss of digestive capacity [19]. Karimi et all. reported, which high dose of tannin has histological effect on kidney and liver [8]. In present survey, degeneration of surface epithelium and lymphocytosis in lamina propria and tunica submucosa was observed in diet with 70% sorghum grain. Also degeneration of surface epithelium and lymphocytosis in lamina propria and tunica submucosa, dilatation of secretory units of lieberkün glands and increase of goblet cells were observed in diet with 80% sorghum grain. Lymphocytosis was observed in all of the samples and it doesn't have any relation to sorghum grain level in diet. It can be cause by an unknown inflammation or other infectious factors. Degeneration of surface epithelium can be related to effect of tannin on plasma membrane proteins. These changes in the foregut epithelial tissue are unlikely to affect absorptive processes. In contrast, condensed tannins have been shown not to have adverse effects on the morphology of the intestinal tract of rats as opposed to tannic acid [17] [22]. Our survey appeared which goblet cells increased in duodenum and colon intestinal gland during high level sorghum grain diet. Also microscopic observation showed which lumen of Lieberkün glands was diluted. It was caused by degeneration of apical border of secretory units cells of these glands under influence of high level of sorghum grain diet. Mbatha et all. (2002) were reported which distribution of villi were decreased under high level tannin diet. They also showed which height of absorptive columnar cells decreased due to degeneration of this cell apical brush borders under high level tannin diet [14]. This research results confirmed last researches results about brush border and goblets cells but any changes was not observed in height villi of duodenum in this research results. Also it appeared hyperemia in lamina properia that it probably can be cause by inflammation under tannin toxicity.

In generally our study was appeared which high dose of diet tannin have important histological damage and effects, therefore feeding with tannin have to performed in ruminants carefully.

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