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# HISTOPATHOLOGIC EVALUATION OF DIGESTIVE TRACT FROM HENS FED WITH RICH CELLULOSE DIET

# EVALUAREA HISTOPATOLOGICĂ A TRACTULUI DIGESTIV LA GĂINI HRĂNITE CU FURAJE BOGATE ÎN CELULOZĂ

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## ABSTRACT | REZUMAT

The present paper focused on monitoring morphologic changes in the digestive tract of experimental egg -laying hens fed with high cellulose diets. In total, 80 birds were examined, after different hours from feeding (1, 4, 8, 24). The diet types consisted of regular food (control group) and 6 or 7% enriched cellulose diets with or without enzymes added (experimental groups).

The proventriculus exhibited intact epithelium and glands, and well represented associated lymphoid tissue. The segments of the small intestine constantly showed an intact mucosa, along with diffuse disposal of associated lymphoid tissue, less developed within the jejunum. The intact mucosa of the cecum is rich in goblet cells, while the cecal tonsils consist of a diffuse component alternating with primary lymphoid nodules or germinating centers. Histopathology performed on pancreas samples revealed normal aspects of parenchyma and associated lymphoid tissue. In most cases, the liver architecture was preserved, except different degree changes within the portal tract (diffuse or nodular leukocyte infiltration). Three cases, two in the control groups and one in experimental group, presented a chronic - subacute hepatitis, with diffuse leukocyte infiltration and accumulation of protein, but apart from the affected regions, the general liver structure was normal. For these cases, the etiology of the lesions did not match any issue linked to the experiment effects, since two of them were from the control groups. The goblet cell count in the ileum revealed a significantly increased number for the experimental group, regardless of the added enzymes, compared to the control groups.

In conclusion, main digestive tract organs and the adjacent glands of hens fed with rich cellulose diets presented normal morphological aspects induced by the administered diets under the conditions of the present experiment.

**Keywords**: histopathology, cellulose, hens, digestive tract, goblet cells

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Prezenta lucrare s-a concentrat pe monitorizarea aspectelor morfologice ale tractului digestiv la găinile ouătoare din loturi experimentale hrănite cu diete bogate în celuloză. În total au fost examinate 80 de găini, la diferite ore de la momentul hrănirii (1, 4, 8, 24).

Dietele au constat în hrană uzuală (lotul martor) și hrană bogată în celuloză 6 sau 7% cu sau fără enzime adăugate (loturile experimentale).

Proventriculul a prezentat epiteliul și glandele intacte, precum și țesutul limfoid asociat bine reprezentat. Segmentele intestinului subțire au prezentat constant mucoasa intactă, alături de o dispunere difuză a țesutului limfoid asociat, mai puțin reprezentat în jejun. Mucoasa intactă a cecumurilor a fost bogată în celule caliciforme, iar amigdalele cecale prezintă o componentă limfoidă difuză ce alternează cu noduli limfoizi primari sau centri germinativi.

Histopatologia pancreasului a relevat aspecte normale ale parenchimului și ale țesutului limfoid asociat.

În majoritatea cazurilor, arhitectura ficatului a fost păstrată, cu excepția unor modificări ale spațiului port (infiltrat leucocitar difuz sau nodular). Trei cazuri, două din loturile martor și unul dintr-un lot experimental, au prezentat o hepatită subacută-cronică, cu infiltrare leucocitară difuză și acumulare de material proteic. Cu excepția acestor zone, arhitectura hepatică generală a fost fără modificări. Pentru aceste cazuri etiologia leziunilor nu are legătură directă experimentul, ipoteză susținută și de faptul că două cazuri aparțin loturilor martor.

Numărătoarea celulelor caliciforme din ileum a relevat un număr semnificativ mai mare pentru loturile experimentale, cu sau fără administrare de enzime, față de loturile martor.

În concluzie, segmentele și glandele adiacente principale ale tubului digestiv ale găinilor hrănite cu diete bogate în celuloză au prezentat aspecte morfologice normale induse de dietele administrate în condițiile prezentului experiment.

**Cuvinte cheie**: histopatologie, celuloză, găini, tract digestiv, celule caliciforme

Digestive tract of domestic birds presents a simple, but efficient,overall structure, in comparison with other domestic animals, such as ruminants. Birds have developed the ability to fly, due to their capacity of easily digesting food along with other criteria. Moreover, the diets of avian species need to take in consideration the simplicity of the digestive tract, with the final purpose of obtaining all components needed for a balanced metabolism and indirect results of production parameters.

Therefore, in poultry industry, feeding was a great concern in the past 50 years, since new sources of protein and good amino acids and fatty acids are researched every day (1, 2, 3, 7, 21).

The goal is to obtain good quality of the final products (meat and/or eggs) with less financial costs along with other qualities (16).

One theory is that dietary fiber can increase the time needed for the digesta to pass the upper part of the digestive tract, improving gizzard function and in consequence, the inclusion of fiber can promote nutrient digestibility and growth performances in broilers (10, 12). Another study, by Rezaei et col., revealed that supplementation of micronized insoluble fibers enhanced the mucosal absorptive surface area resulting in greater nutrient absorption and superior performance (20). Wils-Plotz and Dilger launched another theory, in 2013, when they researched the effect on the growth performance and intestinal health of chicken. In particular, they observed the increase in the production and quality of the mucin by goblet cells, after addition of dietary fiber. Mucin protects the lining of the intestinal epithelium against acid and enzymes and acts as a barrier against foreign pathogens (24). Also, in a study regarding higher level of pure cellulose in broiler chickens diet resulted in increased body weight and higher content of protein in the pancreas and a higher activity of proteolytic enzymes of the pancreas glands (5).

In addition to these studies, other researches focused on assessing the effects of cellulose and fat diets on poultry industry on feed consumption in order to obtain a constant level of energy intake (6, 8).

Another aspect taken into consideration was that inclusion of inulin and cellulose in the diet of hens reduced feed intake and body weight and improved reproductive performance (19). A study from 2012 concerned obesity in broiler breeder hens, which is often linked to changes in hepatic fatty acid metabolism, leading to accumulation of triglycerides in the liver. The results revealed that a moderate increase in crude protein reduced fat content in the liver.

In change, insulin and cellulose reduced abdominal fat deposition (18). Another study concerning laying hens' diet revealed low fiber alfa-alfa (*Medicago sativa L.)* could be the most cost-effective suitable diet for laying performance to improve egg quality. Recent studies showed alfa-alfa could reduce the concentrations of cholesterol in the meat and egg yolk (16).

Another issue regarding poultry industry is feather pecking in laying hens. Some studies suggested that nutritional factors, such as the time spent for eating could affect positively this behavior. With nutrient dilution and nonstarch polysaccharide addition in diets along with introducing this food early, since the chicken growth phase, resulted in a good feather condition at the end of the laying period (13, 14, 15).

Studies showed that, along with modified fiber diets, supplementation with appropriate enzymes could result in reduced intestinal viscosity, better food efficiency and better growth in broiler chickens (4, 11, 17).

The present experiment concerned evaluating and obtaining samples from the digestive tract with key roles in the process of digestion: proventriculus, duodenum, jejunum, ileum, colon and caecum.

Microscopic evaluation of digestive tract organs provides important clues on the digestion process and the efficiency of it.

## MATERIALS AND METHODS

The experiment included 80 hens, fed either with usual diets (control group) or with rich cellulose diets, with or without enzyme addition. Foods used to increase the percentage of cellulose were alfalfa and sunflower meal.

Eight groups of chickens were formed, fed with 6% and 7% cellulose, with or without enzymatic addition.

Animals were selected and euthanized at week 36 of age and gross examination was performed at one hour, four hours, eight hours and 24 hours after feeding. The tissues of interest were proventriculus, duodenum, jejunum, ileum, ceca, colon, pancreas and liver, for which samples were taken to perform the histopathological examination.

All samples were fixed for 48 hours in 10% buffered formaldehyde and processed according to standard histological techniques.

After partial fixation (24 hours) in 10% buffered formaldehyde, the samples were modeled, sectioning 3 mm tissue fragments. For a fast and correct fixation, the cut fragments were again immersed in formaldehyde solution of the same concentration and left for a further 24 hours at room temperature.

Subsequently, the samples were subjected to a transfer in successive baths of different ethyl and butyl alcohol for dehydration. The next step was the pre-inclusion in successive paraffin baths at 58°C and the construction of the paraffin blocks, which were then sectioned at 5 μm. The paraffin blocks were made using the Tissue Tek Sakura paraffin inclusion station, and 5 μm sections were cut using the Microtec Cut 4055 microtome.

The sections thus obtained were displayed on histological slides, Hematoxylin-Eosin stained, the classic method for differentiating cell types, and subsequently fixed with Canada balsam.

The histopathological slides were examined using the Olympus BX 41 microscope, with a digital camera attached (Olympus U-TV1X-2) for imaging. For the full and detailed examination of the preparations, the x4, x10, x20, x40 and x100 objectives were successively used. The evaluation of histopathological slides aimed at examining the proventriculus, duodenum, jejunum, ileum, ceca, colon, pancreas and liver.

For the uniform and objective interpretation of the histopathological aspects, we used the same criteria to be followed in each section, and the quantification of the morphological transformations according to the severity of the lesions. Regarding the cell count in the ileum, goblet cells were counted per 100 μm of linear epithelium in every case. Statistics were performed using Student's T-Test.

## RESULTS AND DISCUSSION

The proventriculus, using his developed glandular system, ensures digestion using secretion of hydrochloric acid needed to lower the pH and intervention of pepsin and gastrin, responsible of gastric and pancreatic secretion. Among with specific structures, the proventriculus presents a lymphoid tissue associated to the mucosa, with a remarkable concentration of lymphocytes as a border at the limit of proventriculus and esophagus and a disseminated distribution, as diffusely and solitary nodules in the rest of the mucosa. The subjects of the present study did not present any modifications to surpass normal limits (Fig. 1). The simple columnar epithelium of the mucosa and the tubular glands responsible for hydrochloric acid secretion were intact, similar to the hisological data found in the literature (22). The subepithelial layer contained lymphoid nodules composed of small lymphocytes, without signs of local stimulation. Proventricular glands (responsible of digestive enzyme synthesis) presented a normal development, without notice of desquamation, erosion or other type of alteration of the cubic simple secretory epithelium. In addition, one subject (experimental group, 6% cellulose from alfalfa + enzymes, 1h from feeding) presented in the chorion of the proventricular mucosa aspects of extramedullary granulocytopoiesis (situation in which animals produce myelopoiesis in other organs than the hematogenous marrow).

The duodenum exhibited normal histologic representation of overall layers in all subjects submitted in the present study. The mucosa was represented by an intact, simple columnar epithelium, crossed by numerous intraepithelial lymphocytes (IEL). Associated lymphoid tissue of the duodenum is present in high number, without aspects that could be linked to an specific immunologic stimulation. Four experimental cases, fed with 6% cellulose at 4 hours, with and without enzymes, presented evident lymphatic vessels, specific for digestion period of the intestine, without pathologic substrate (Fig. 2).

The jejunum and ileum both presented normal histological aspects. The integrity of the mucosa was constantly observed, along with an associated diffuse lymphoid tissue, with a smaller distribution in jejunum than duodenum and ileum. Transepithelial lymphocytes are less numerous comparing to the duodenal segment (Fig. 3 and 4).

The goblet cell count in the ileum revealed significant differences between the control groups and the experimental ones (Fig. 11-14), as every experimental group, regardless of feed type and cellulose concentration, presented more goblet cells than the control groups. Concerning the added enzymes, there were no significant differences between the groups fed only with rich cellulose diets than the corresponding ones with added enzymes. Also, no significant differences were seen between corresponding groups of 6% or 7% cellulose diets, regardless the feed type or the enzymes. The last comparison involved the different cellulose plant sources. As a result, the goblet cell number was similar for both alfalfa and sun flower diets (Table 1).

The final part of the process of digestion takes place in two appended blind sacs: the ceca and the colon. Histologic examination of the cecum and the colon presented normal microscopic aspects in the present experiment (Fig. 5 and 6), as the literature cites (22).

The epithelial, digestive and absorption component were studied in detail, all represented by the simple columnar epithelium, rich in goblet cells. The number of intraepithelial lymphocytes is less abundant than in other segments. The most important lymphoid component of the ceca is represented by the cecal tonsils, comprising both a diffuse part and numerous, primary and secondary lymphoid nodules/germinal centers. The immunologic response of the cecal tonsils is not exclusively the result of antigens crossing the digestive tract. The literature suggests that the reaction of the secondary lymphoid tissue can also be generated by the uric acid reflux from the cloaca that presents suction movements or can be the result of differentiation of local stem cells in B lymphocytes, process that takes place after involution of the cloacal bursa (23).

The pancreas, with an important role in the physiology of digestion, did not reveal lesions associated to the present experiment (Fig. 7). The exocrine pancreas is represented by compound tubuloalveolar adenomeres with intact epithelium (22). The pancreatic parenchyma is disrupted only by associated lymphoid nodules. These structures are part of the normal histology of associated disseminated lymphoid tissue specific to avian species. Histologic slides revealed in some cases aspects of extramedullary granulocytopoiesis, expressed by perivascular infiltrates of myeloid cells. Langerhans islets did not present any morphologic changes. A single case, from this study (experimental group, 6% cellulose from alfalfa, at 1h), presented aspects resembling amyloidosis, in the form of amorphous, oxyphilic material that disrupts cells from the pancreatic islets. In this case, we consider that this pathology, observed in a single case of the entire group and moreover, the entire experiment, resulted from a chronic evolution, from transforming in amyloid from polypeptide hormones synthetized by pancreatic cells, with no correlation between the conditions in the present study.

The liver, as the largest gland in the body, has vital roles in maintaining homeostasis. Same as the cases of mammals, the liver of birds has a central role in digestion, being directly involved in lipid, proteic and glucidic metabolism (22). In a study concerning dietary nanocrystalline cellulose supplementation in meat ducks feeding, lipid metabolism was of great interest. Beside measurements and serum investigations, liver histology was performed. Results resulted in a positive effect on growth performance, reduction of abdominal fat and serum triglyceride concentration and lipid deposition on liver by histologic examination (9).

The liver also has a particular characteristic, the ability to regenerate, which has to be considered functional. The hepatic lesions must be extensive and of great intensity in order to be functionally and morphologically expressed, otherwise they can go unnoticed. Generally, the liver reaction to pathogens can manifest either by affecting hepatocytes (degenerative processes, hepatocyte death or cellular karyomegaly) or by reactions in the portal space (inflammation, biliary duct hyperplasia and fibrosis). Regarding morphologic parameters of liver integrity in the present study, the following aspects were taken in consideration: hepatocyte integrity, portal space integrity (presence / absence of leukocyte infiltrate), including the integrity of biliary ducts. In most cases submitted to examination, normal hepatic cell pattern was observed (Fig. 8). In some cases, few changes were observed in the portal space, reflecting leukocyte, sometimes lymphocytic, infiltrates with a diffuse or nodular pattern (Fig. 9). These inflammatory reactions were of minor or medium intensity and affected isolated portal spaces. Also, it is important to mention the fact that these changes were observed both in experimental and control groups, which stands for a reaction to general bird housing and management, rather than a pathologic reaction related directly to the experiment. These reactions are also noticed in healthy broiler chickens submitted to slaughter. Another hypothesis is that these areas of inflammatory infiltrates are related to extramedullary hematopoiesis. For normal and pathogenic substances that come from the intestinal tract and can interfere with the liver cells, it is expected that the areas around the centrolobular vein to react. In the present study, although alterations of the hepatocytes were observed, none had this type of distribution. All in all, no correlation was observed between the diet administered to the experimental groups and alterations of the liver morphology. In three cases, a chronic - subacute hepatitis was noticed, associated with diffuse leukocyte infiltrate and proteic-like deposits that replace altered hepatocytes (Fig. 10). For these cases, gross examination revealed lesions of hepatitis and necrosis, confirmed later by histopathologic examinations of those samples. In the present experiment, only three cases out of 80 (3.75%) exhibited the lesions, which excludes a direct correlation between the feeding diet and the hepatitis. Moreover, two cases belonged to the control groups.

Due to the fact that the morphological aspects were very similar in exhibiting no microscopic lesions for every digestive tract segment, a few images were selected to emphasize the aforementioned features.

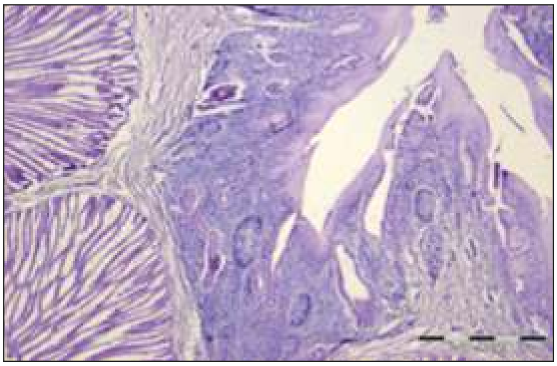

**Fig. 1**. Proventriculus: intact mucosa and submucosa, well represented lymphoid tissue. (HE, 40x)

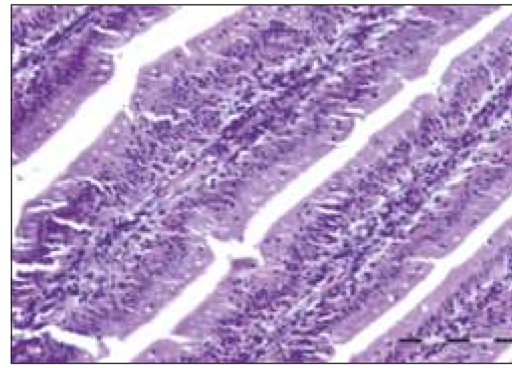

**Fig. 2**. Duodenum: intact epithelium, with intraepithelial lymphocytes. (HE, 200x)

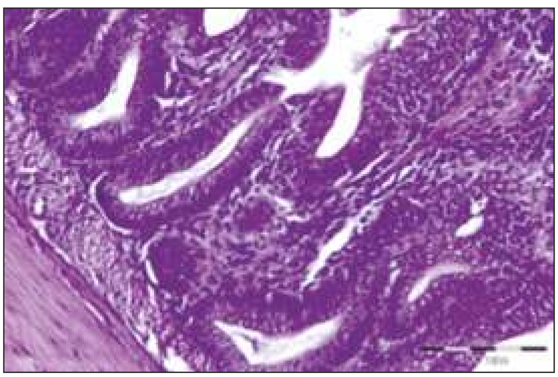

**Fig. 3**. Jejunum: both, functional and proliferative compartments are intact. (HE, 200x)

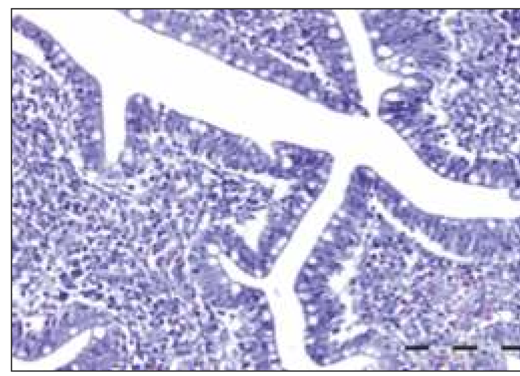

**Fig. 4**. Ileum: intact epithelium, visible lymphatic vessels, discrete leukocyte infiltrate. (HE, 200x)

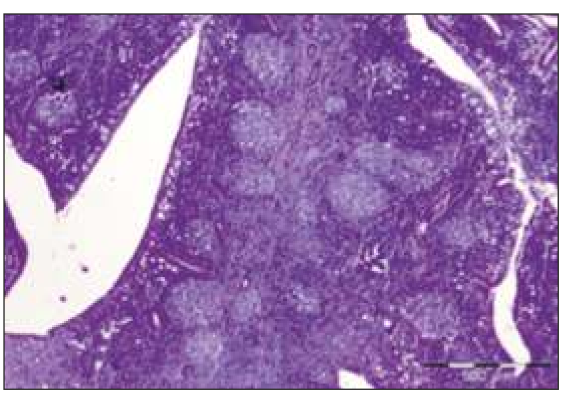

**Fig. 5**. Ceca: intact epithelial component, tonsil with activated lymphoid tissue. (HE, 40x)

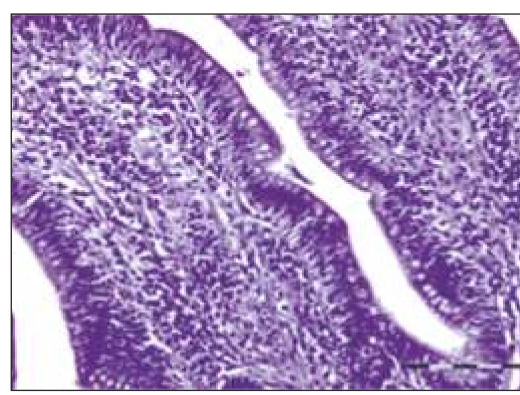

**Fig. 6**. Colon: intact mucosa, with numerous goblet cells. (HE, 200x)

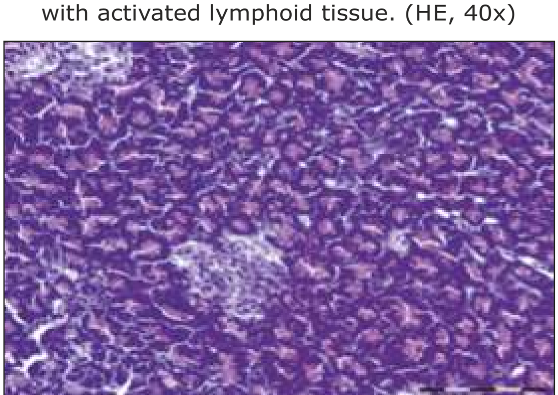

**Fig. 7**. Pancreas: endocrine and exocrine compartments with no lesions. (HE, 200x)

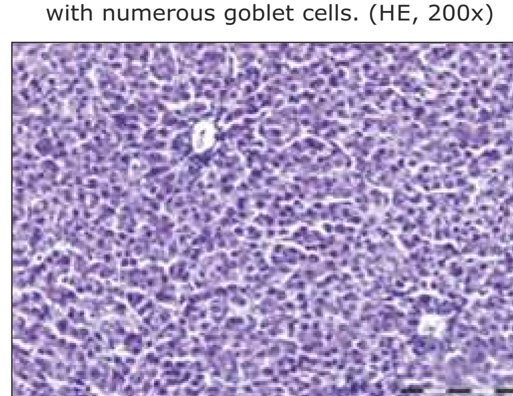

**Fig. 8**. Liver: normal histologic structure. (HE, 200x)

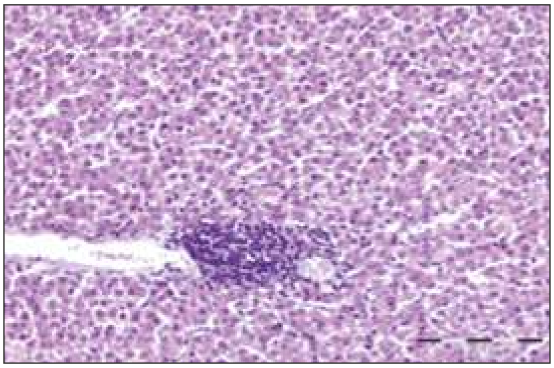

**Fig. 9**. Liver: intact hepatocytes and focal leukocyte infiltrate within portal area. (HE, 200x)

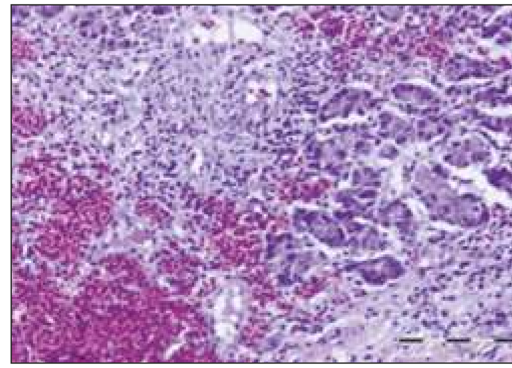

**Fig. 10**. Liver: focally altered architecture, hyperemia, chronic-subacute hepatitis. (HE, 200x)

Group 1

11 11 11 10

Group 2

12 15 12 13 14 11 13 16
14 13 11 12 11 12 13 14

The means of Group 1 and Group 2 are significantly different at $p < 0.05$.

Summary

| | Group 1 | Group 2 |
|---|---|---|
| Mean | 10.75 | 12.875 |
| Variance | 0.25 | 2.1167 |
| Stand. Dev. | 0.5 | 1.4549 |
| n | 4 | 16 |
| t | -2.829 | |
| degrees of freedom | 18 | |
| critical value | 2.101 | |

**Fig. 11**. Student's T-Test for the control and experimental groups at 1 hour.

Group 1

10 10 10 10

Group 2

14 11 10 12 11 13 10 10
11 14 14 10 13 11 13 14

The means of Group 1 and Group 2 are significantly different at $p < 0.05$.

Summary

| | Group 1 | Group 2 |
|---|---|---|
| Mean | 10 | 11.9375 |
| Variance | 0 | 2.5958 |
| Stand. Dev. | 0 | 1.6111 |
| n | 4 | 16 |
| t | -2.3065 | |
| degrees of freedom | 18 | |
| critical value | 2.101 | |

**Fig. 12**. Student's T-Test for the control and experimental groups at 4 hours.

Group 1

10 11 10 10

Group 2

13 11 12 12 13 11 10 14
13 13 14 11 12 10 11 12

The means of Group 1 and Group 2 are significantly different at $p < 0.05$.

Summary

| | Group 1 | Group 2 |
|---|---|---|
| Mean | 10.25 | 12 |
| Variance | 0.25 | 1.6 |
| Stand. Dev. | 0.5 | 1.2649 |
| n | 4 | 16 |
| t | -2.6497 | |
| degrees of freedom | 18 | |
| critical value | 2.101 | |

**Fig. 13**. Student's T-Test for the control and experimental groups at 8 hours.

Group 1

11 10 10 11

Group 2

12 10 12 14 11 11 13 12
12 11 12 13 12 14 11 10

The means of Group 1 and Group 2 are significantly different at $p < 0.05$.

Summary

| | Group 1 | Group 2 |
|---|---|---|
| Mean | 10.5 | 11.875 |
| Variance | 0.3333 | 1.45 |
| Stand. Dev. | 0.5773 | 1.2042 |
| n | 4 | 16 |
| t | -2.1879 | |
| degrees of freedom | 18 | |
| critical value | 2.101 | |

**Fig. 14**. Student's T-Test for the control and experimental groups at 24 hours.

## CONCLUSIONS

In conclusion, the controlled administration of 6 and 7% cellulose diets obtained from alfalfa and sunflower meals, with or without added enzymes, for laying hens, revealed normal morphologic aspects of the digestive segments.

The number of ileum goblet cells significantly increases for cellulose rich diets, with no pathological implication, due to a high density digesta.

The pancreas samples did not express signs of lesions in the cases of examined birds.

The liver morphology was not affected by conditions of the present study concerning fiber diet.

Microscopic morphology of alimentary tract and glands did not manifest alterations in correlation to the moment of obtaining the samples.

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Table 1

**Goblet cell count in the ileum for each individual**

| Diet type | | Number of goblet cells/100µm | | | |
|---|---|---|---|---|---|
| Control group, usual diet, room 13 | Group 1 | 11 | 10 | 10 | 11 |
| | | 11 | 10 | 11 | 10 |
| Control group, usual diet, room 17 | | 11 | 10 | 10 | 10 |
| | | 10 | 10 | 10 | 11 |
| Alfalfa, 6% cellulose | Group 2 | 12 | 14 | 13 | 12 |
| | | 15 | 11 | 11 | 10 |
| Alfalfa, 6% cellulose + enzymes | | 12 | 10 | 12 | 12 |
| | | 13 | 12 | 12 | 14 |
| Alfalfa, 7% cellulose | | 14 | 11 | 13 | 11 |
| | | 11 | 13 | 11 | 11 |
| Alfalfa, 7% cellulose + enzymes | | 13 | 10 | 10 | 13 |
| | | 16 | 10 | 14 | 12 |
| Alfalfa + sunflower, 6% cellulose | | 14 | 11 | 13 | 12 |
| | | 13 | 14 | 13 | 11 |
| Alfalfa + sunflower, 6% cellulose + enzymes | | 11 | 14 | 14 | 12 |
| | | 12 | 10 | 11 | 13 |
| Alfalfa + sunflower, 7% cellulose | | 11 | 13 | 12 | 12 |
| | | 12 | 11 | 10 | 14 |
| Alfalfa + sunflower, 7% cellulose + enzymes | | 13 | 13 | 11 | 11 |
| | | 14 | 14 | 12 | 10 |
| **Hours from feeding** | | **1** | **4** | **8** | **24** |

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