Control on the Wheat Non-Starch Polysaccharides (NSP)' Anti-Nutritional Effect on Intestinal Wall, by Introducing Xylanase in Broiler Feed

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Abstract
The experiment was performed in order to determine the protocol of xylanase utilization to fight against the anti-nutritional effect exerted by wheat non-starch polysaccharides (NSP) on intestinal wall in broilers. The experimental works were carried out on broilers, the hybrid Ross 308. We formed four experimental groups, as follows: the experimental group LE1, fed on forage without wheat in its structure, the experimental group LE2, fed on combined forage including wheat in a proportion of 40%, the experimental group LE3 including wheat in a proportion of 40% and xylanase, in an amount of 25 g / to, and the experimental group LE4, including wheat in a proportion of 40% and xylanase, in an amount of 100 g. At 3 and 6 weeks, successive to chicken killing, we sampled the intestinal wall and determined the main changes occurred. The histo-morpho-metric analysis of the four experimental groups led to the conclusions that: wheat administration in a proportion of 40% in the individuals in LE2 determines the development of vilositary muscle elements, decreased of intestinal villosities height, leucocitary migration, and also vascular ectasies and reduced hemorrhagic areas; xylanase addition in the wheat-based feed may be associated with the increase of intestinal villosities height, and especially at jejuna level the villosities seem slightly branched, a slight hypertrophy of the epithelial cells compared with the individuals in LE2, the increase of goblet cells frequency and hypertrophy of the capillary network. These microscopic aspects come together with more intens digestion and absorption processes, and especially in the experimental group 4.

Key words: broiler, intestinal wall, non-starch polysaccharides, xylanase, wheat.

1. Introduction

Wheat is the preferentially grown cereal in many European countries. However, different wheat cultivars vary with respect to their apparent metabolisable energy values [1], which is the reason why the quality of wheat for broiler chickens in some cases may questioned [1, 2]. Carbohydrates reaching the large intestine of pigs and poultry determine largely the type and the activity of the gut microflora [3]. This, in turn, is related to the dietary carbohydrates provided to the animal and their digestibility. Increasing dietary levels of „viscous" cereals cause an increase in weight of the total gut, indicating a close relationship between digesta viscosity and gut morphology. Furthermore, several authors reported that high NSP concentrations might stimulate the development of goblet cells on the epithelial gut surface [4]. [5] reported that the intensity of mucin secretion, as affected by addition of NSP degrading enzymes, may influence the thickness of the so-called „unstirred"

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water layer 2 which has a significant impact on nutrient absorption and utilization.

2. Material and methods

The objective of this experiment was to determine the protocol of xylanase utilization in the fighting against the anti-nutritional effect of wheat non-starch polysaccharides (NSP) on the intestinal wall in broiler chickens. Wheat's proportion of participation in the combined forage was 0, respectively 40 %. To this, we added the variants with incorporation of xylanase, in a quantity of 25 g/ t forage, respectively 100 g / t combined forage.
The experiments were performed on broiler chickens, the hybrid Ross 308, in the Department for Animal Nutrition and Alimentation, Didactic Station of BUASVM Timişoara.
To determine the nutritive value of the combined forage offered the broiler chickens in the four experiments, we applied the standard methods in concordance with the WEENDE scheme; the analyses were carried out in the laboratory of the Department for Animal Nutrition and Alimentation, Faculty of Animal Sciences and Biotechnologies Timişoara.
The NSP content of the raw matter was determined in a laboratory belonging to the University of Dublin [6].
To determine the morpho-physiological changes induced by the different NSP sources on the epithelial tissue, we took 1-2 cm^2 samples from duodenum and jejuna from each chicken.
The histological research of the intestinal wall was performed by sampling, washing, embedding in paraffin, sectioning and staining with haematoxyline-eosine, respectively with trichromic Mallory staining. The histo-morpho-metric study was performed with the research microscope Olympus CX41, endowed with software for image analysis and with digital photo camera. The tissue fragments taken from duodenum and jejuna, from the four groups, were fixed in neuter formalin 10%, and then dehydrated, cleared and embedded in histological paraffin, to form the paraffin blocks. Successively, these were sectioned at the thickness of 5 μ, with the manual rotating microtome Leyca, fixed on plates and stained with the trichromic Mallory method. The microscopic study of the stained sections was performed with the microscope Olympux CX41, endowed with digital photo camera and software (QuickPhoto Micro 2.2) for image acquisition and histo-morpho-metric analysis.

3. Results and discussions

The experiment was performed for six weeks on a number of 120 chickens, distributed in four experimental variants (LE1, LE2 and LE3, LE4). The hybrid used was Ross 308. The chickens in the four experimental groups were fed as follows: during the first growth period, from eclosion to 3 weeks, the combined forage provided 3157-3204 kcal ME and a CP content of 22.91-22.94 %. In the second growth period, from 3 to 6 weeks, the combined forage provided 3201.75-3244 kcal ME and 19.92-20.16 % CP [4].
We formed four experimental groups, as follows: the experimental group LE1, fed on forage without wheat in its structure, the experimental group LE2, fed on combined forage including wheat in a proportion of 40%, the experimental group LE3 including wheat in a proportion of 40% and xylanase, in an amount of 25g /t, and the experimental group LE4, including wheat in a proportion of 40% and xylanase, in an amount of 100 g / t. At 3 and 6 weeks, successive to chicken killing, we sampled the intestinal wall and determined the main changes occurred. The combined forage's NSP content was determined in relationship with the available data [7] and with the determinations performed at the laboratory from the University of Dublin.
The experimental organization scheme is presented in table 2, and the nutritional features of the combined forage used in this experiment.

Table 2. Experimental organization scheme

Period 0-3 weeks			
LE1	LE2	LE3	LE4
CF (0 % wheat) ME (kcal/kg) 3204 CP 22.91%	CF (40 % wheat) ME (kcal/kg) 3157 CP 22.94%	CF (40 % wheat plus xylanase 25 g / t) ME (kcal/kg) 3157 CP 22.94%	CF (40 % wheat plus xylanase 100 g / t) ME (kcal/kg) 3157 CP 22.94%
Period 3-6 weeks			
LE1	LE2	LE3	LE4
CF (0 % wheat) ME (kcal/kg) 3244 CP 20.16%	CF (40 % wheat) ME (kcal/kg) 3201.75 CP 19.92 %	CF (40 % wheat plus xylanase 25 g / t) ME (kcal/kg) 3201.75 CP 19.92 %	CF (40 % wheat plus xylanase 100 g / t) ME (kcal/kg) 3201.75 CP 19.92 %

CP – crude protein
ME - metabolizable energy
CF- combined feed

Combined forage content in non-starch polysaccharides (NSP)

According to our determinations and table data, we obtained the content in soluble, insoluble NSP and also total NSP of the combined forage.

The NSPs content in the growth period 0-3 weeks has a bigger value in the forage including 40% wheat with 0.965 percentage points. As regards NSPi, they are bigger with 0.046 percentage points in the same groups. During the growth period 3-6 weeks, the combined forage including 40% wheat presented a NSPs content bigger with 1.18487 percentage points than in the group fed forage without wheat. As regards the NSPi content, it decreases with 0.117 percentage points in the same groups. The NSPt value presents an increase with 0.951 percentage points in the first growth period and with 0.972 in the second, in the favour of the groups fed forage including wheat. Among NSP, the wheat contains a bigger amount of arabinoxylans. We may observe that the combined forage including 40% wheat in its structure presented an arabinoxylan content bigger with 1.17 percentage points than LE1 in the period 0-3 weeks and with 1.19 percentage points in the period 3-6 weeks.

The most of the soybean grit oligosaccharides belong to the alpha-galactosidase family (raffinose, stachyose and verbascose), which cannot be hydrolyzed by host's digestive enzymes. The non-cellulosic components of the soybean grit contain various monomers: uronic acid, galactose, manose, arabinose, xylose, ramnose and glucose as predominant monosaccharides and they differ considerably from cereals in terms of composition [8].

Histological study of intestinal wall in broiler chickens

In the case of the individuals from the experimental group 1 (LE1), the microscopic sections in duodenum reveal the organization of the wall in the four superposed layers: mucous, submucous, musculous and serous. The mucous presents villosities with a mean height of approx. 1905.3 μ, slightly widen at the base (figure 1) and coated with a high mono-layered epithelium, with a mean height of 26.6μ, endowed with a ribbed plateau. The interglandular areas are reduced, occupied with lax connnectival tissue consisted of collagen fibres, fibroblasts, mono- and poly-morphonuclear leukocytes and sanguine capillaries. Numerous infiltrative cells may be noticed in the interepithelial glandular and vilositary (figure 2) regions, and also on epithelium surface. The connective tissue within the submucous coat structure contains numerous vascular packages and the muscular tunic, consisted of two superposed layers of smooth muscular fibres, internal circular and external longitudinal, has a mean width of approx. 174.3 μ. At jejunal mucous level, villosities present different sizes, but their mean height is 974 μ. The intestinal glands begin at vilosity base (fig. 2), with wide lumen, whose epithelium is crossed by infiltrative cells, also present in the glandular lumen. The lax connectival tissue from the

vilositary and interglandular chorion structure is reduced, it covers the slightly ectasied capillary and lymphatic network. The mean muscular tunic width at this level is approx. 150.0 μ.

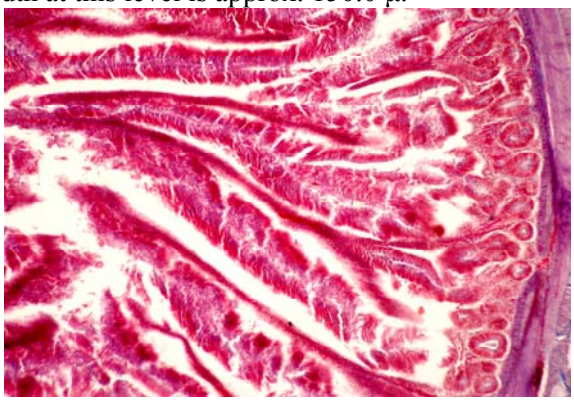

Figure 1. Experimental group (LE1), section through duodenum, assembly (100x; trichromic Mallory st.), (original)

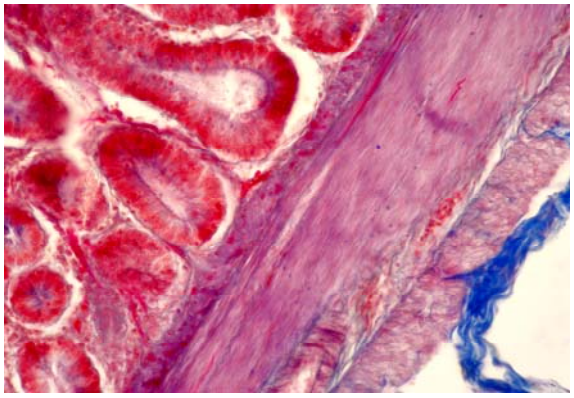

Figure 2. Experimental group (LE1), section through duodenum – intestinal glands and interglandular chorion with sanguine infiltrates and capillaries (400x; trichromic Mallory st.), (original)

In the case of the individuals from the experimental group LE2, the duodenum mucous presents villosities with a mean height of approx. 1090.8 μ, slightly branched (figure 3), coated with a slightly hypertrophic epithelium, predominantly consisted of absorbent prismatic cells, with a mean height of approx. 32.6μ. Among the absorbent cells, we may notice a reduced number of goblet cells, which are a bit more numerous at glandular epithelium level, and migrating leukocytes (figure 5).

Also, in the interglandular chorion, we may notice disparate smooth muscle fascicles, originating in mucous muscular (figure 5).

The periglandular areas are reduced, occupied with lax connectival tissue, the support of an easily-noticeable capillary network and of an abundant leucocitary infiltrate. In the vilositary chorion, there is a strong leucocitary infiltrate, and the capillary network becomes slightly hypertrophic. The smooth muscle fascicle or the Brüke muscle represents a well developed structural element of the vilositary chorion (figure 3, 4). The muscular tunic has a mean width of approx. 132.1 μ.

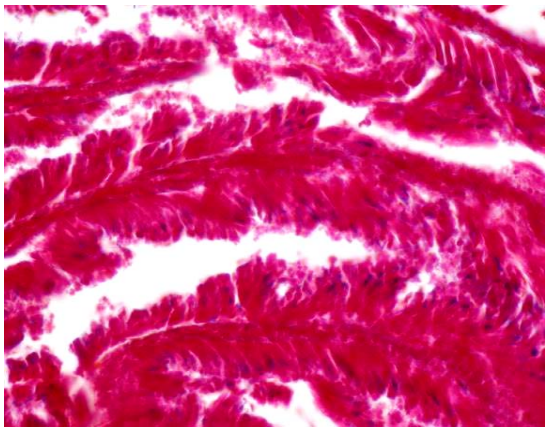

Fig. 3. Duodenum LE2, slightly branched intestinal villosities (400x; trichromic Mallory st.), (original)

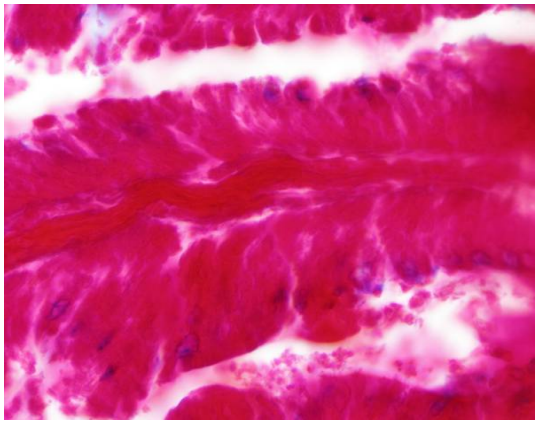

Fig. 4. Duodenum LE2, vilosity in whose chorion we may notice the Brüke muscle (1000x; trichromic Mallory st.), (original)

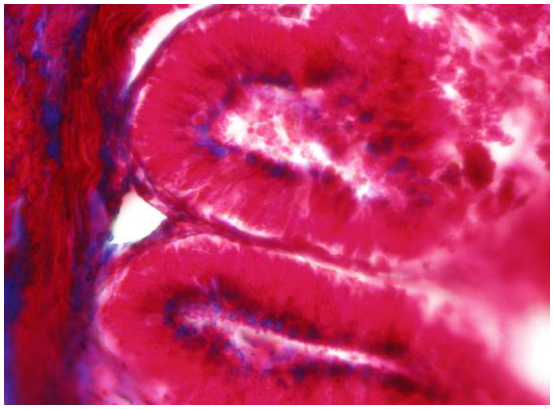

Fig. 5. Duodenum LE2, intestinal glands and interglandular chorion; glandular lumen with infiltrative cells (1000x; trichromic Mallory st.), (original)

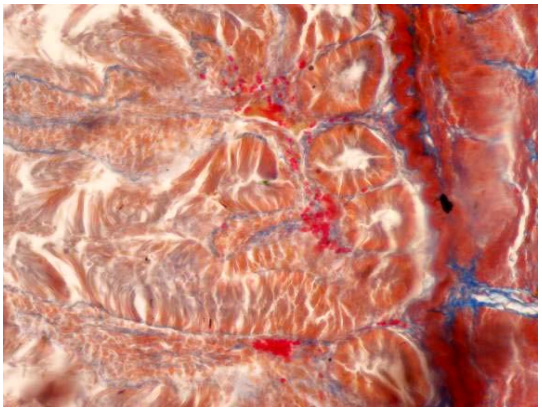

Fig. 6. Jejunum LE2, ectasied capillaries and hemorrhagic regions in mucous chorion (400x; trichromic Mallory st.), (original)

The jejunal mucous villosities, in the case of the individuals from the experimental group LE2, present a widen aspect, with short lateral ramifications, coated, like the ones from duodenum, with a slightly hypertrophic epithelium; their mean height is approx. 32.4 μ.

The mean vilosity height is approx. 684.6μ. The interglandular chorion structure includes the connnectival stroma rich in collagen fibres and dispersed smooth muscle fibre fascicles.

Periglandular and in submucous, the capillary network is slightly hypertrophic. In certain regions, capillaries are ectasied and we may notice hemorrhagic areas (figure 6). The vilositary chorion comprises abundant leucocitary infiltrate and the Brüke muscle fascicle.

The analysis of the histo-morpho-metric data proves that, in the case of the experimental variant LE2, the mean jejunal mucous vilosity height is smaller (684.6μ) than in LE1 (974 μ) and that villosities are coated with a slightly hypertrophic epithelium (32.4 μ).

Also, in the case of the variant LE2, periglandular and in submucous, the capillary network is slightly hypertrophic; in some regions, the capillaries are ectasied and we may notice hemorrhagic areas.

The vilositary chorion includes an abundant leucocitary infiltrate and the Brüke muscle fascicle, and, in the glandular epithelium structure, we may notice a few goblet cells and numerous infiltrative cells.

A number of authors have reported that dietary NSPs have a considerable impact on the gut anatomy and gut development. A prolonged consumption of soluble NSPs is associated with increased size and length of the digestive organs in pigs [9], chickens [10] and fish [11] accompanied by a decrease in nutrient digestion. Similarly, administration of an oral dose of cellulose to preweaning pigs reduced villus length by approximately15% in the small intestine [12]. Furthermore, [11] reported that addition of sodium carboxymethylcellulose (CMC) to a highly digestible cooked rice-based weaner diet (40 g/kg diet dry matter) for 13 days significantly increased the intestinal viscosity of digesta within the small and large intestine, which led to decreased villus length and increased crypt depth. Similar results have been reported by [13] and [14].

The microscopic sections through duodenum wall show, in the case of the individuals from the experimental group LE3, the presence of tall villosities (figure 7), with a mean dimension of approx. 1257.8 μ. The epithelium coating these villosities has a dimension of approx. 29.1 μ and presents a developed ribbed plateau at the apical pole.

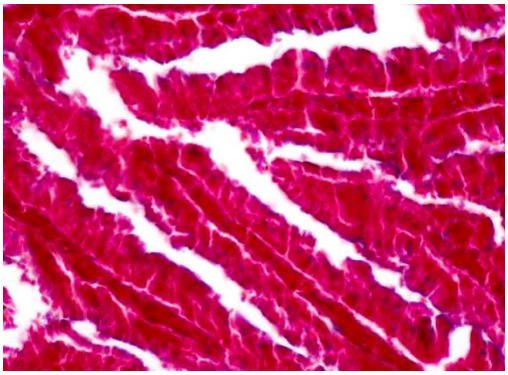

Figure 7. Duodenum LE3, intestinal villosities (400x; trichromic Mallory st.), (original)

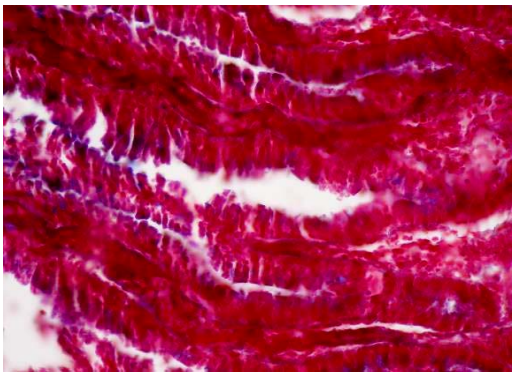

Figure 8. Duodenum LE3, basal chorion and villosities (400x; trichromic Mallory st.), (original)

The glandular apparatus is developed, and the glandular epithelium includes numerous goblet cells. The interglandular chorion is reduced, loaded with numerous infiltrative cells, and the best developed structural element in the vilositary chorion is the smooth muscle fascicle (figure 8), which centres the vilosity from its base and up to its top. The mean muscular tunic width is approx. 144.0 μ.

Jejunal mucous villosities present a mean height of approx. 718.0 μ, they are slightly branched and the epithelium that covers them is hypertrophic (33.0 μ), with a developed ribbed plateau (figure 9). In the vilositary epithelium structure, and also in the glandular epithelium (figure 10), we may observe numerous goblet cells. Also, these two structures comprise smooth muscle fascicles. The mean muscular tunic width is 147.7 μ. Analyzing the microscopic aspects of the duodenal mucous in the individuals from the experimental group LE3, we may observe that, although the intestinal villosities have a reduced mean height compared with LE1, the epithelium that covers them presents a developed ribbed plateau, and smooth muscle fascicles develop in the mucous chorion, loaded with leucocitary infiltrative cells. In the case of jejunum, we may notice that intestinal villosities are smaller in LE3 than in the individuals from LE1, and the epithelium that covers them is hypertrophic and includes numerous goblet cells. Also, in LE3, we may notice the hypertrophy of the vascular network from mucous chorion. These histological aspects represent intense processes of absorption and local defence.

Compared with LE2, the intestinal mucous of the individuals from LE3 present taller villosities at duodenum level and at jejunum level as well, with hypertrophic epithelium, endowed with an obvious ribbed plateau at the apical pole and numerous goblet cells. In the mucous chorion, we may observe the capillary network hypertrophy, without the hemorrhagic processes met in the case of the group LE2.

The microscopic study of the duodenal mucous reveal a hypertrophic aspect in LE4, compared with the other groups. The histo-morpho-metric analysis indicates a mean vilosity height of approx. 1358.4 μ. The mean muscular tunic width is approx. 127.1 μ.

The jejunal mucous villosities, in the individuals from this experimental variant, are wider and slightly branched (figure 11), with a mean height of approx. 772.0 μ, and the epithelium that covers them has a mean height of approx. 32.9 μ. Among the absorbent cells of the vilositary and glandular epithelium, we may frequently notice the presence of the goblet cells (figure 12). The mucous chorion seems "dense" because of the dense leucocitary infiltrate (figure 11), this one being represented by lymphoid follicles in some regions, and also because of the smooth muscle fascicles (figure 12). Also, in mucous chorion and submucous, we may notice vascular hypertrophies (figure 11, 12). The mean muscular tunic width, determined successive to the histo-morpho-metric study, is approx. 105.7 μ.

Compared with LE1, although the duodenal villosities are a bit smaller, at duodenal segment level and at jejunal segment level as well, we may observe epithelial hypertrophic processes, a bigger frequency of goblet cells, the vascular network hypertrophy in mucous and submucous, leucocitary infiltrate development and, in some regions, the presence of lymphoid follicles. The histo-morpho-metric analysis shows big differences between the vilosity heights in LE4 and LE1, and the intestinal mucous' morphologic

aspect reveals intense morpho-metabolic processes in the case of LE4.

Enzyme supplementation of diets based on rye, wheat, barley or oats can reduce the mentioned adverse effects [15, 16]. Enzymes decrease the viscosity of gut contents, resulting in improvements in nutrient digestibility and performance when added to poultry diets). The mode of action of NSP-hydrolyzing enzymes is not completely understood, and is being debated [17, 18]. Enzymes hydrolyse part of NSP reducing digesta viscosity in the small intestine [19], improving nutrient digestibility and modifying the gut microflora [18, 19, 21, 16, 20].

At the groups that received xylanase in CF, already suggested a relaxation of the lining of the intestinal villi development in the sense that (they are high and slightly branched), epithelial hypertrophy, high frequency goblet cells, hypertrophied capillaries suggests digestion and absorption processes good, probably due to decrease in intestinal content viscosity.These microscopic processes interfere with the digestion and absorption more intense, deeper processes of individuals in experimental group 4.

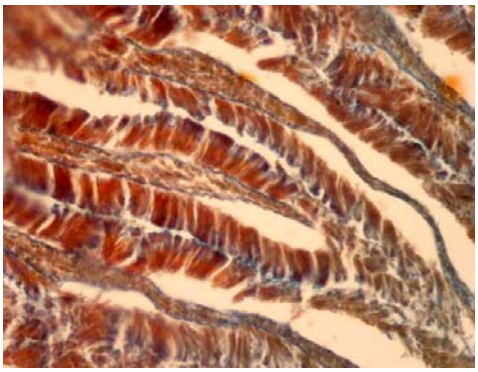

Figure 9. Jejunum LE3, vilositary epithelium with ribbed plateau at the apical pole (400x; trichromic Mallory st.), (original)

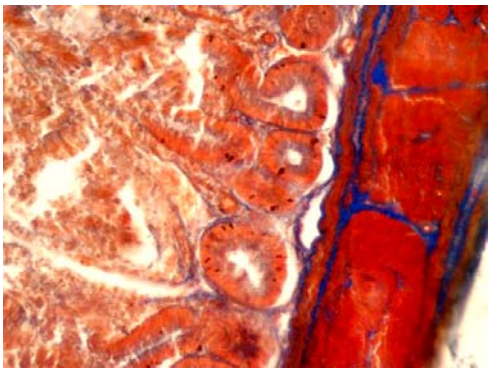

Figure 10. Jejunum LE3, intestinal glands (400x; trichromic Mallory st.), (original)

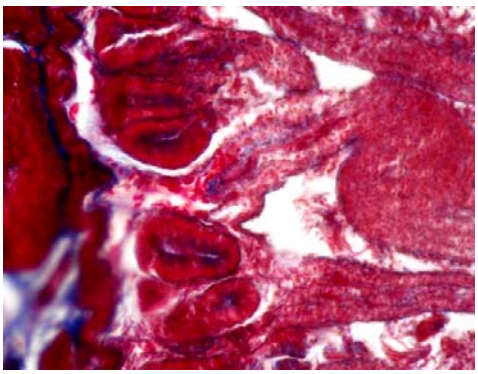

Figure 11. Jejunum LE4, leucocitary infiltrate and vascular hypertrophies (400x; trichromic Mallory st.), (original)

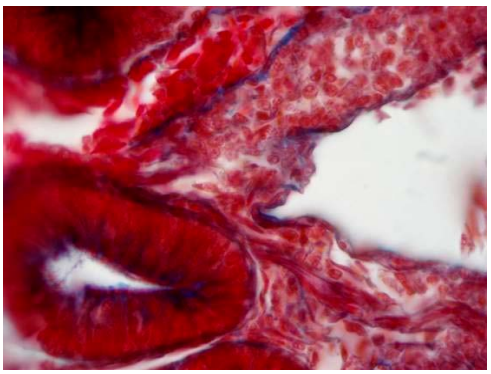

Figure 12. Jejunum LE4, vascular hypertrophies, infiltrate and smooth muscle fascicles in corion (1000x; trichromic Mallory st.), (original)

Conclusions

- in the case of the experimental variant LE2, the mean duodenal and jejunal mucous villosities height are smaller (1090,8, respectively 684.6μ) than in LE1 (1900, respectively 974 μ) and these are coated with a slightly hypertrophic epithelium (32.4 μ). Also, in the case of LE2, periglandular and in submucous, the capillary network is slightly hypertrophic, the capillaries are ectasied in some areas and we may observe hemorrhagic regions, a reduced number of goblet cells and the leucocitary infiltrate; the best developed structural

element of the vilositary chorion is represented by the smooth muscle fascicle or the Brüke muscle.
- the individuals from the experimental variant LE3 present, at jejunal segment level, villosities that are smaller than in LE1, and the epithelium that covers them is hypertrophic and includes numerous goblet cells. Also, in the individuals from LE3, we may notice the hypertrophy of the vascular network from mucous chorion. These histological aspects suggest intense processes of absorption and local defence at this level.
- compared with the experimental variant LE2, the intestinal mucous of the chickens in the variant LE3 presents taller villosities at duodenum and jejunum level, with hypertrophic epithelium, endowed with obviously-ribbed plateau at the apical pole and numerous goblet cells. In mucous chorion, we may notice the capillary network hypertrophy, without the hemorrhagic processes met in the case of the individuals in LE2;
- in LE4, although the intestinal villosities have a reduced height compared with LE1, we may observe epithelial hypertrophic processes, a big frequency of goblet cells, the hypertrophy of the vascular network in mucous and submucous, leucocitary infiltrate development and the presence, on some territories, of lymphoid follicles;
- the duodenal and jejunal mucous in LE4 presents a hypertrophic aspect compared with the experimental groups LE2 and LE3; the histo-morphometric analysis indicates a mean duodenal vilosity height of approx. 1358.4 μ, respectively a jejunal vilosity of approx. 772.0 μ. This process is associated with a bigger frequency of goblet cells, a dense leucocitary infiltrate – in some regions, this is represented by lymphoid follicles, and also of vascular hypertrophies.

The histo-morphometric analysis of the intestinal mucous in the four experimental groups shows that:

wheat administration, in a proportion of 40%, to the individuals from the experimental group LE2, determines decrease of intestinal villosities height, determines the development of vilositary muscular elements (the Bruke muscle), leucocitary migration, and also vascular ectasies and, on reduced regions, haemorrhages.

xylanase addition in the wheat-based feed may be associated with the increase of intestinal vilosity height, the villosities being slightly branched, a slight hypertrophy of epithelial cells compared with LE2, the increase of goblet cells frequency and capillary network hypertrophy. These microscopic aspects interfere with more intense processes of digestion and and absorption, which are more evident in the individuals from the experimental group 4.

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