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HISTOLOGICAL AND HISTOMETRICAL FEATURES OF THE INTESTINAL MORPHOLOGY OF BROILER CHICKENS TREATED WITH DIFFERENT DRUG FORMULATION, CONTAINING OREGANO OIL

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ABSTRACT

The study was conducted with 40 clinically healthy (equal in both sexes) broiler chickens. Biosamples were taken at the end of the finishing period (day 41) in each group, from each bird, from all parts of the intestinal tract. Durable histological preparations were made by classical methods with each biosample.

In the histological study, it was found that there was a microstructured change in all segments of the intestinal mucosa in the two experimental groups – first one, which received Ecodiar® liquid 5% to the drinking water, in dosage 0.5 ml of drug formulation/L water, and second one, which received Ecodiar® powder 5% to the feed, in dosage 0.5 g of drug formulation/kg of feed. In the third experimental group, which received 5 ml of 1% oregano oil/kg of feed and in the fourth (control) group, no histological changes in the intestinal wall were found.

In the histometric study, the highest intestinal villi were found in the ileum, followed by the duodenum and the jejunum. Intestinal crypts had the largest outer diameter in the caecum, followed by the ileum, jejunum and duodenum. These two parameters showed different histometric values in each of the test groups of broiler chickens and each of the intestinal segments examined.

Key words: histological study, chickens, oregano oil, gut.

Introduction

Since the 1990s, a number of authors (Buddle & Bolton, 1992; Uni et al., 1998; Laudadio et al., 2012) believe that the height of the intestinal villi and the outer diameter of the incisional histological surface of the intestinal crypts are the determinants of healthy status in broiler chickens as well as for the secretory and absorption capacity of their intestinal mucosa.

The aim in the present experimental research is to identify the morphological and histometric features of the different intestinal compartments in the intestinal wall at the end of their finishing period (day 41) of broiler chickens, which received different drug formulations, containing oregano oil.

Materials and methods

We have used material from 40 clinically healthy broilers Cobb-500, 41 day old. The chickens were divided by 10 chickens in 4 groups (5 males and 5 females per group). The birds of the test groups were treated with different drug formulations, containing oregano oil from the 1 to the last day of the finishing period (day 41) of the experiment.

The birds of the first group were treated 41 days ad libitum to the drinking water with Ecodiar® liquid 5% in dosage 0.5 ml of drug formulation/L.

The second group received ad libitum Ecodiar® powder 5%, well mixed with the daily ration of food, in dosage 0.5 g of drug formulation/1 kg of feed.

The third group (positive control), was treated ex tempore with 1% oregano essential oil (prepared by mixing 1 ml of 100% oregano oil with sunflower oil until reaching the volume of 100 ml) well mixed with food in dosage 5 ml of 1% oregano oil/kg of feed.

The fourth group (negative control) received water and food without oregano oil during the growing period.

Biosamples were taken from each bird, from all parts of the intestinal tract – from duodenum, jejunum, ileum, caecum and colon in the last day of the finishing period (day 41) of the experiment. The intestinal biosamples were put in 10% aqueous solution of formalin and in fixation mixtures of Carnua and Buen. After the paraffin histological sections (5–7 μm thickness) were coloured with hematoxylin by Erlich, eosin, durable histological preparations were made. The light microscopic and histometric study was performed on durable histological preparations using a light microscope "Ergaval". Accordance with Avtandilov's (1990) methods, histometric measurements were performed on durable histological preparations from 4 birds from each group, on 10 microscopic fields in each preparation.

For statistical analysis and determination of significant differences a computer program Statistica 6.0. was used. The values were determined by the nonparametric Mann Withney U-test and by the one-way ANOVA method.

The microphotodocumentation of the histological state in the studied segments of the intestine were performed with a universal light microscope NU – 2 (Carl Zeiss, Jena, Germany).

Results

It was found that the wall in all the segments of the intestine was preserved with the microarchitectonics in all groups of broilers and it contained all the layers of the intestinal wall. In two of the experimental groups of chickens, various microstructural changes in the intestinal mucosal layer were observed. It was found that the lumen in the intestinal segments was partially or totally filled with nutritional content in I and II group of broilers. Basically, in both groups of birds, the intestinal contents were filled with nutritional content, which in the duodenum were well mixed with desquamated enterocytes and lymphocytes. The last were more common in the case of chickens in the II group.

In the intestinal lumen of the jejunum, the nutritional contents contained fragments of intestinal villi, while in the ileum there were whole intestinal villi. In the intestinal contents of the jejunum, erythrocytes were common. In chickens of groups I and II at the tips of the intestinal villi significant number of microhemorrhages were present. They were in contact with the contents of the intestinal lumen. In the jejunum of group II of broilers there was a diffuse lymphoid cell infiltration in the propria, which reached periglandular of the Lieberkühn glands (Figure 1).

In the chickens' ileum from group II, there were desquamation processes reaching the deep parts of the propria, where only the bottom of the Lieberkühn glands were found. The surrounding loose connective tissue contained massive, diffuse lymphoid cell infiltrates (Figure 2).

In the layer of the mucosa in the caecum, extensive desquamation zones were established where only the bottom of the caecum glands were preserved. In the colon and caecum of the broiler chickens from II group the described microstructural changes were widely distributed and better expressed. In the desquamally altered intestinal sections the loose connective tissues of the mucosal propria were diffusely infiltrated with lymphocytes and lymphoid cell elements. They reached subepithelially to the top of the intestinal villi. In the depth of the mucosal propria (especially in II and

III group of birds), these infiltrates covered the loose connective tissue and reached the lamina muscularis mucosae and around the cecal tonsils (Figure 3).

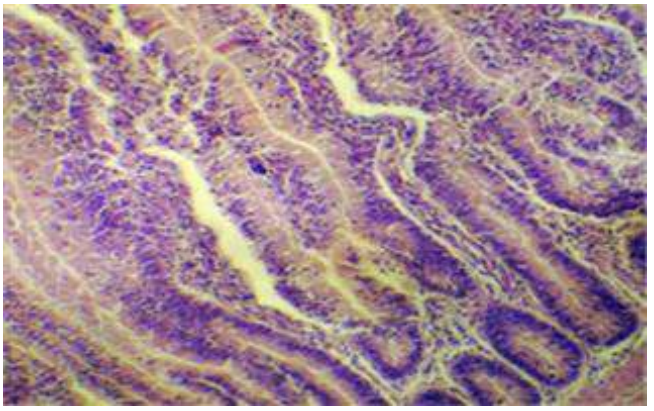

Figure 1: The broilers jejunum from group II; H & E; Increase – 125X.

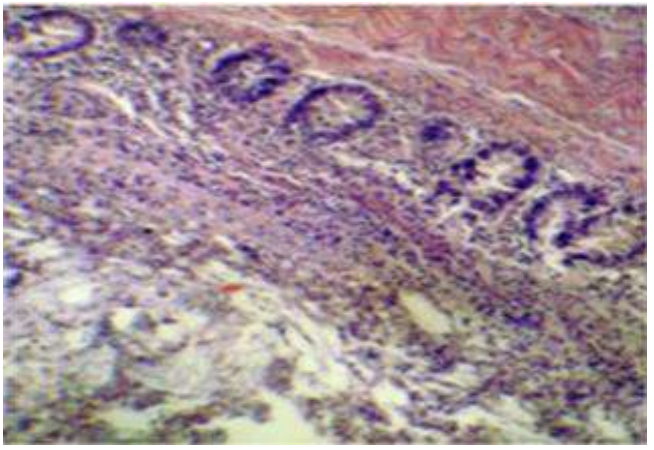

Figure 2: The broilers' ileum from group II; H & E; Increase – 125 X.

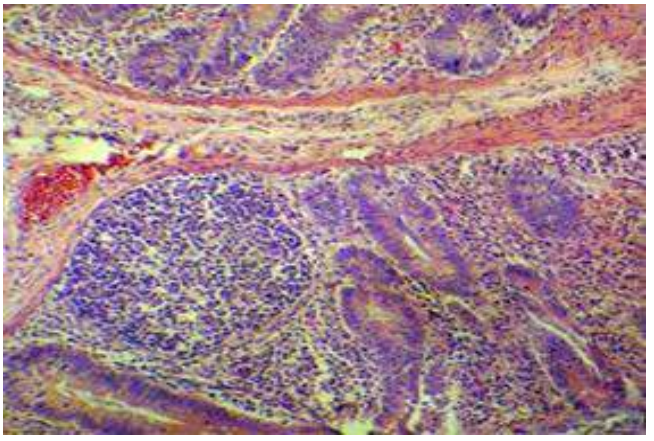

Figure 3: The broilers' caecum from group III; H & E; Increase – 250X.

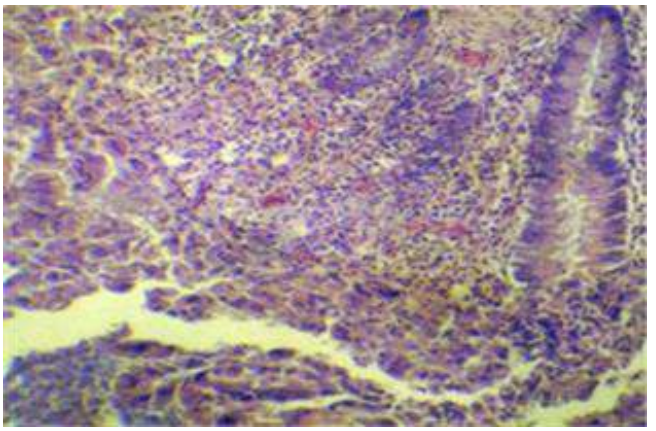

Figure 4: In the broilers' colon from group II; H & E; Increase – 250X.

In the caecum and colon in the intestinal content there were desquamated covering epithelium and glandular epithelium. The desquamation processes covered the ostia of the Lieberkühn glands (Figure 4).

In all compartments of the intestinal tract in III and IV group of broilers no microstructural changes were observed in the mucosal layers and sublayers of the intestinal wall.

The results of our light microscopy histometric study showed that in the last day of the finishing period of broiler chickens the highest number of intestinal villi were found in the ileum, followed by the duodenum and the jejunum. In the intestinal segments, in the duodenum the highest number of intestinal villi was found in the group I birds (1116.896 μm), while in the jejunum, the intestinal villi were highest in the group III of birds (1110.365 μm). In the ileum the intestinal villi were highest the group I of broiler chickens (1726.625 μm).

The results of the histometric study of the intestinal crypts showed that the crypts in the caecum were with the largest outer diameter of the incisional histological surface, followed by the dimples in the ileum, jejunum and duodenum. The control group broilers (33.699 μm) had the largest outer diameter of the incisional surface of the gland crypts in the duodenum, while in the jejunum, these crypts dominated in the birds of the third group (37.933 μm). In the ileum the broiler chickens in the control group (40.103 μm) had the largest outer diameter of the incisional histological surface of the intestinal crypts, while in the caecum the broiler chickens in the III group (49.897 μm) had the largest outer diameter of the incisional histological surface of the intestinal crypts.

Discussion

Aparecida da Silva et al. (2009) examined the structure of the intestinal mucosa in broiler chickens after their experimental infection with Eimeria tenella and their treatment with different combinations and dosages of oregano oil. Their histological studies did not detect histological changes in the duodenal mucosa of the birds 144 hours after the infection tested in 42-days broiler chickens.

In three publications Karadas et al. (2012; 2013; 2014) reported that nutritional diets for broiler chickens containing herbal extracts and oils (XT 6930 containing: 5% carvacrol, 3% cinnamaldehyde and 2% capsular oleoresin) do not cause microstructural changes in the duodenal mucosa of the birds.

In the scientific literature scientific reports were published providing data for the experimental uses of the oregano oil for broiler chickens during the past five years (Betancourt et al., 2012; Giannenas et al., 2016, et al., 2017; Fonseca-Garsia et al. al., 2017; Tzora et al., 2017; Sarica et al., 2017). Some scientifics selected pigs for their experimental biological model (Zou, 2016). The authors declare that treatment with oregano oil in different doses and forms does not cause structural changes in the wall of the jejunum and in the ileum in the experimental animals, irrespective what experimental performance they performed.

According to researchers of the normal histostructure in the caecum of domestic birds (Georgescu et al., 2007), of chickens of the White Leghorn breed (Rezaian et al., 2007), and of broiler chickens after experiments with feed additive containing plant extracts and oils (Karadas et al., 2013; Karadas et al., 2014), it was proved that their application does not affect the histostructure of the caecum and colon.

The cited results of our histological study proved that the wall has a relatively preserved microarchitectonics in all studied intestinal departments in the four groups of broiler chickens. Their wall has all the layers (mucosa, submucosa, muscularis mucosae and serosa). We found out that the group of birds treated with oregano oil (III group) mixed with the daily ration food (in dosage 5 ml of 1% oregano oil/kg of feed) did not show any microstructural changes did in the intestinal wall. We believe that the results of this group of broiler chickens match the results of the researchers cited above (Karadas et al. 2012; Betancourt et al., 2012; Karadas et al., 2013; Giannenas et al., 2016; He et al., 2017; Fonseca-Garsia et al., 2017; Tzora et al., 2017; Sarica et al., 2017).

However, in contrast to what was published in these scientific teams, our experimental research found that in two of the trial formulations (Ecodiar® liquid 5% in dosage 0.5 ml/L water and Ecodiar® 5% powder in dosage 0.5g/kg feed) administered to the drinking water and to the feed in two of the groups of broilers, microstructural changes in different form and degree of manifestation were manifested in the mucosa of all intestinal studied segments.

If we refer to the experimental scientific results during the last five years, we should note that Betancourt et al. (2012) achieved the highest increase in the height and the area of the intestine villi as well as in the depth of the intestinal crypts after the addition of 200 ppm of oregano oil to the ration of 3-days broiler chickens. Karadas et al. (2012), Karadas et al. (2013), Karadas et al. (2014) observed stimulation of the extension of intestinal villi and crypts as a result of food diets enriched with plant extracts and oils.

In an experiment with 256 broiler chickens, which received Ecodiar® powder 5% in dosage 500 g/t of feed, Giannenas et al. (2016), observed the highest intestinal villi in the duodenum (1899,4 μm), followed by those in the jejunum (1665.3 μm) and the ileum (1034.5 μm). The deepest intestinal crypts were observed in the duodenum (185.0 μm), followed by the jejunum (171.8 μm) and the ileum (129.6 μm).

Tzora et al. (2017) measured the highest intestinal villi in the duodenum, followed by the ileum and the jejunum respectively. In the duodenum, the highest villi were observed in the group of broiler chickens, which received benzoic acid (Vevovital® 1 g/kg of feed, containing 100% benzoic acid) (1796.7 μm), followed by these treated with Ecodiar® powder 5% (1754.2 μm). The

deepest intestinal crypts were measured in the duodenum, followed by these in the jejunum and the ileum respectively. In the duodenum, the crypts were most shallow in the birds, which received Ecodiar® powder 5% (184.3 μm). In the jejunum the crypts were with the smallest depth in the broilers, which were treated with benzoic acid (131.6 μm), followed by the birds, which were treated with Ecodiar® powder 5% (145.0 μm). In the ileum the crypts were most shallow in the birds treated with benzoic acid (86.9 μm), followed by broilers, which received atapulgite (105.5 μm) and Ecodiar® powder 5% (106.9 μm).

Fonseca-Garsia et al. (2017) found that in the last day of the finishing period the broiler chickens, which received oregano oil in dosage 400 mg/kg of feed, had the highest intestinal villi, followed by these treated with oregano oil in dosage 200 mg/ kg of feed, then the broiler chickens in the control group and these birds, which received Ecodiar® powder 5%.

During the last five years the cited experimental results showed that despite the same experimental biological model of broilers chickens (laying hens) treated with oregano oil in different forms and dosages, the results of the different scientific teams differed and very often had contradictory character.

The analysis of the results in our histometric study does not fully confirm the statement of Fonseca-Garsia et al. (2017) that the increase in the dose of oregano oil leads to increase in the height of the intestinal villi in broiler chickens. Our results proved that in the last day of the finishing period (day 41), the height of the intestinal villi of the broiler chickens dominated in the duodenum, followed by the jejunum and the ileum. Our histometric results are in line with these achieved by Giannenas et al. (2016) in relation to the fact that the longest intestinal villi are found in the duodenum, followed by the jejunum and the ileum, compared to the control (IV) group of broiler chickens. This may mean that, due to the application of drug formulations, containing oregano oil to the birds, the number of the pathogenic bacteria in their digestive tract has decreased, suggesting the presence of well-structured and functional intestinal mucosa with preserved absorption and secretory abilities.

Conclusion

Based on our results we state that the use of different drug formulations, containing oregano oil for broiler chickens could be accepted as an alternative to those drugs that improve health status, absorption and secretion in the intestinal tract of the birds.

The results of our experimental research allow us to suggest the alternative 1% oregano oil (prepared by mixing of the 100% oregano oil with sunflower oil, of the formulation and the dosage, as previously described) as a component of the daily ration for broiler chickens Cobb-500 from the hatch (day 1) to the last day of their finishing period.

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