

Comprehensive characteristics of rectal morphology in chickens with different types of autonomous regulation

A. M. Tybinka  

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Pekarska Str., 50, Lviv, 79010, Ukraine

Article info

Received 27.06.2023

Received in revised form

27.07.2023

Accepted 28.07.2023

Correspondence author

Andrii Tybinka

Tel.: +38-067-353-03-20

E-mail: a.m.tybinka@gmail.com

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Abstract

The influence of typological features of autonomous regulation on the dimensions, structure, and blood supply of the rectum in chickens has been studied. The experimental group consisted of clinically healthy adult chickens of the Isa-Brown breed, divided into two groups using electrocardiography and variation pulsometry methods: chickens with sympathetic-tonic regulation and chickens with sympathetic-normotonic regulation. In each group, the length and diameter of the rectum, the branching of its arterial vessels, and the microscopic structure of the intestinal wall were examined. The corresponding artery provides the blood supply to the rectum, the branching of which showed no significant differences between the poultry groups. The peculiarities of autonomous regulation significantly influence the morphological structure of the intestinal wall's mucous and muscular membranes. The serous membrane does not respond to these features. Chickens with sympathetic-tonic regulation (ST chickens) are characterized by a greater thickness of all indicated membranes and, consequently, the entire intestinal wall. Chickens with sympathetic-normotonic regulation (ST-NT chickens) have a greater thickness only in the muscular plate of the mucous membrane and its longitudinal layer. Regarding the quantity of collagen and elastic fibers, ST chickens dominate the mucous membrane's crypt area, while ST-NT chickens dominate the muscular membrane. However, the density of connective tissue fibers in the mucous membrane is much lower than in the muscular one. The quantity and distribution of plasma cells have similar patterns in both groups. Specific morphometric compensation between two functionally related indicators is also an important aspect. In this case, larger values of one indicator are combined with smaller values of another. For example, a smaller diameter of the rectum in ST chickens is associated with its greater length; a smaller number of goblet cells on one villus corresponds to a larger area of their secretory section; a larger area of the nerve node of the intramuscular nerve plexus is combined with a smaller number of these nodes per 1 cm² of the intestinal wall. Thus, the structure of the rectum in chickens, adapting to the corresponding type of autonomous regulation, maintains the balance at the level of individual membranes and the entire intestinal wall.

Keywords: sympathetic-tonic chickens; sympathetic-normotonic chickens; mucous membrane; muscular membrane; arterial channel.

Citation:

Tybinka, A. M. (2023). Comprehensive characteristics of rectal morphology in chickens with different types of autonomous regulation *Ukrainian Journal of Veterinary and Agricultural Sciences*, 6(2), 44–55.

1. Introduction

Industrial poultry farming, aimed at a constant increase in meat and egg production, often pays attention to the condition of the digestive system as a source of plastic and energy materials in the organism (Paraskeuas et al., 2017; Scanes, 2017). Critical stages of growth and development of these organs are established better to understand the morpho-functional characteristics of different digestive organs and identify their stimulation possibilities (Southwell, 2006). The small intestine, where the main digestion processes occur, is most frequently studied by researchers (Uni et al., 2003; Walton et al., 2016). In the early stages of embryogenesis (within the third day), the primary gut appears as a simple tube, elongated along the body and suspended on the dorsal surface (LeGuen et al., 2015). Through molecular and mechanical interactions of different tissues, its parts

quickly differentiate into distinct sections according to their functional purpose, and the wall is divided into three layers (Coulombre & Coulombre, 1958; Crosnier et al., 2006; Chin et al., 2017; Huycke & Tabin, 2018). The formation of the muscular layer gives rise to the motility of the intestine. The first signs of motility in the embryonic gut of chickens are observed on the fifth day of age. On the sixth day of age, with the appearance of circular smooth muscles, the first waves of contractions occur.

Interestingly, the initial stages of intestinal peristalsis development are highly dependent on calcium ions and unrelated to neurons in the intestinal wall (Chevalier et al., 2017; Chevalier, 2018). Neural control over peristalsis is established in 16-day-old embryos (Chevalier et al., 2019). In the mucous membrane, two types of villi are distinguished one day before hatching: taller and often pear-shaped villi and shorter and narrower villi (Uni et al., 2003).

Crypts begin to form on the day of hatching, and initially, one crypt corresponds to one villus. Crypts quickly branch out, increasing in size. Enterocytes in their walls also rapidly proliferate, changing their shape and migrating to the villi. The surface area and number of villi per 1 cm² of the mucous membrane constantly increase. These processes exhibit significant differences in different sections of the intestine (Uni et al., 2001; Geyra et al., 2001). During the first week after hatching, the weight of the intestine increases faster than the body weight (Sklan, 2001; Nasrin et al., 2012).

The intestine size, as well as its wall structure, is quite sensitive to changes in feeding conditions, housing, microclimate, vaccinations, and other factors (Lilburn & Loeffler, 2015). This primarily affects the histochemical indicators of the intestinal mucosa (Tsirtsikos et al., 2012; Biasato et al., 2018). Including animal-derived feed additives in broiler diets leads to a decrease in villus height and an increase in crypt depth (Biasato et al., 2018). The addition of propolis and bee pollen, natural or modified zeolites, causes an increase in villus height and crypt depth, as well as improves the activity of protease and amylase enzymes (Wu et al., 2013; Prakatur et al., 2019). Similar effects on villus and crypt sizes are observed using synbiotics, probiotics, and prebiotics. In addition, the intestine's mass increases, but the density of villi remains unchanged (Pelicano et al., 2005; Awad et al., 2009).

The intestine plays a crucial role in the formation of the organism's protective barrier, as its wall contains a significant amount of lymphoid tissue, the function of which is activated by the intestinal microbiota (Dyshlyuk, 2012; Dyshlyuk, 2013; Guralaska, 2015; Willson et al., 2018; Zhao & Elson, 2018). The intestinal microbiota composition also depends on the diet structure (Mountzouris et al., 2015; Palamidi & Mountzouris, 2018; Paraskeuas & Mountzouris, 2019). The formation of lymphoid structures associated with the small intestine wall occurs in three main stages (Gavrylin et al., 2014).

The nervous system of the intestine as a whole, and the rectum in particular, is complex, well-developed, and consists of several interconnected nerve plexuses. During embryogenesis, the "vagal" part of the neural crest and the sacral area of the neural tube serves as the source of neurons for the rectum (Goldstein & Nagy, 2008; Espinosa-Medina et al., 2017). The cells of these two neural primordia undergo complex processes of proliferation and differentiation, ultimately transforming into various types of neurons and glial cells (Sasselli et al., 2012; Nagy & Goldstein, 2017). Neural cells primarily colonize the muscular layer of the intestine, forming the myenteric (Auerbach's) nerve plexus. Subsequently, the internal migration of neurons begins, forming the submucosal plexus and the submucous nerve network with the nervous network of the mucous membrane (Uesaka et al., 2016). The proper progression of this process and the subsequent optimal interaction between neurons and surrounding tissues is essential for the normal development of all intestinal wall layers and the formation of their functional characteristics. This enables the newborn organism to adapt to new diets and colonization by the gut microbiota (Uesaka et al., 2015; Hao et al., 2016). The development of the enteric nervous system is influenced by numerous genetic and environmental factors, which affect the structure of nerve plexuses and interneuronal connections. This determines functional changes and sometimes the occurrence of pathological processes in the intestinal wall (Lake & Heuck-

eroth, 2013; Rao & Gershon, 2018). Overall, intestinal nerve cells secrete up to 50 biologically active substances that regulate various aspects of the gastrointestinal tract (Schemann, 2005).

The presented data demonstrate that the intestine is a dynamic structure that rapidly responds to external influences and changes in the organism's internal environment.

The **study aims** to establish the relationship between the type of autonomous regulation in chickens' organisms and the structural characteristics of their rectum.

2. Materials and methods

The experimental group comprised 24 clinically healthy Isa-Brown crossbred chickens aged 1.5 years. Electrocardiograms were recorded for all chickens and analyzed using the method of variational pulsometry. At least 100 R-R intervals were examined in each chicken (Baevsky & Chernikova, 2017). Based on the obtained results, the poultry was divided into two groups: 1) chickens with sympathetic tone dominance (ST), characterized by pronounced dominance of sympathetic tone over the parasympathetic one; 2) chickens with sympathetic-normotonic (ST-NT) dominance, where the dominance of sympathetic tone over the parasympathetic one was minimal. Each group consisted of 12 chickens. Euthanasia was performed using an inhalation overdose of chloroform. Ethical requirements for working with experimental animals were followed during the research (Directive 2010/63/EU, 2010). A 10 % gelatin solution stained with red pigment was injected to study the blood vessels and arterial lumen. The absolute and relative length of the rectum and its diameter were measured simultaneously. The number of villi was determined in 1 cm² of the mucous membrane. Staining of the myenteric nerve plexus was performed by combining Dogel's method (staining technique) and Shabadash's method (composition of the staining solution) (Suvarna et al., 2019). The area of the nerve ganglion and nerve loop and the number of these structures per 1 mm² of the area were determined on the obtained film preparations.

For histological investigations, samples of the rectal wall were collected, fixed in Bouin's fluid, embedded in paraffin, and histological sections were prepared with a thickness of 7 µm. The sections were stained according to the requirements of further research. Overview preparations were stained with Mayer's hematoxylin and eosin (Mulisch & Welsch, 2010). Van Gieson's and Pachini's methods were used for the simultaneous detection of connective tissue fibers, while Weigert's method was used for the selective detection of elastic fibers (Mulisch & Welsch, 2010). Cup-shaped cells were detected using the PAS reaction (Mulisch & Welsch, 2010). Plasma cells were stained using the Brachet method (Pearse, 1985). This method, along with the Einarson method (Pearse, 1985), was used to detect DNA and RNA simultaneously. The Feulgen and Rossenbeck methods were used for selective detection of DNA (Pearse, 1985).

A Leica DM-2500 microscope with a Leica DFC450C camera and computer programs such as Image-Tool 3 and WCIF ImageJ were used to examine prepared histological slides. Thickness measurements were taken for individual layers and the overall thickness of the rectal wall. Within the mucous membrane, the dimensions of its structural components were determined, including the villi height, crypts' depth, and epithelial and muscular layer thickness. Similar-

ly, the thickness of the muscle layers (circular and longitudinal) within the muscular coat was measured. The number of goblet cells within a single villus and the average area of the secretory part of a goblet cell were determined within the mucous membrane epithelium. The relative area (in %) of all connective tissue fibers and specifically elastic fibers, was measured in the area of the mucous membrane crypts and within the muscular coat. The relative area (in %) of nucleic acids, both total and precisely that of DNA, was determined in the nuclei of columnar epithelial cells. Based on the obtained morphometric measurements, specific derived values were calculated, including the ratio of villus height to crypt depth and villus height to the number of goblet cells.

The mean values of the experimental groups were compared using a one-way analysis of variance (ANOVA) with Bonferroni correction. The numerical data in tables and text are presented as $x \pm SE$ (where x represents the sample mean, and SE represents the standard error). Calculations were performed using the StatPlus software (AnalystSoft Inc., USA). A significant difference between the parameters was indicated: * – $P < 0.05$, ** – $P < 0.01$.

3. Results and discussion

3.1 Results

Firstly, it should be noted that no single type of autonomous regulation completely dominates all investigated parameters of the rectum. Sometimes, in functionally related parameters, there is an effect of morphological compensation, where larger values of one parameter are always associated with smaller values of an adjacent parameter. For example, this pattern is observed in the dimensions of the rectum, which has a longer length but a smaller diameter in the ST chickens (Table 1). The rectum is the shortest part of the intestine but has the largest diameter. The absolute length of the rectum differs by 0.3 cm, the relative length by 0.3 %, and the diameter by 1.2 mm between the experimental groups ($P < 0.05$).

Table 1

Morphometric parameters of the rectum in chickens ($x \pm SE$)

Indicators	Groups of poultry	
	ST chickens n = 12	ST-NT chickens n = 12
Intestine length (absolute), cm	7.0 ± 0.49	6.7 ± 0.63
Intestine length (relative), %	4.0 ± 0.29	3.7 ± 0.26
Diameter of the intestine, mm	11.1 ± 0.53	$12.3 \pm 0.49^*$
Rectal artery length, cm	$8.1 \pm 0.54^*$	7.3 ± 0.49

Note: * – $P < 0.05$

The blood supply to the rectum is provided by the rectal artery, which branches off from the caudal abdominal artery and runs dorsocaudally concerning the intestinal wall. Therefore, the length of the rectal artery slightly exceeds the rectum size. Accordingly, compared to ST-NT ones, this vessel is 0.8 cm ($P < 0.05$) longer in ST chickens. There are no significant differences in the branching of the rectal artery between ST and ST-NT chickens. The rectal artery gives off five paired straight arteries of different sizes (Fig. 1) that supply the intestinal wall. Accordingly, the first straight artery is the shortest, with a length of approximately

0.65 cm in both groups. The last and most extended straight artery length ranges from 3–4 cm. In ST chickens, its size is more significant. In the caudal direction, the size of the straight arteries and the distance between them increase. The first vessels are relatively closely spaced, approximately 0.7–1.5 cm apart. However, the distance between the last two straight arteries increases to 2–3 cm.

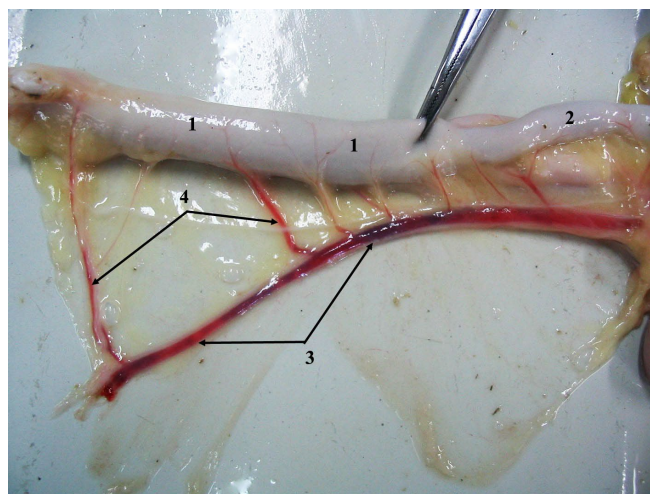


Fig. 1. The arterial bed of the rectum in ST chickens: 1 – rectum; 2 – cecum; 3 – rectal artery; 4 – straight arteries. Natural specimen.

In addition to influencing the rectum's overall size and blood supply, the typological features of autonomous regulation also manifest in the thickness and structure of its wall (Table 2). Since the inner surface of the intestine is not smooth and is characterized by longitudinal folds, the individual layers' thickness (mucosal, muscular, serosal) was measured in the areas between the folds.

Some specific parameters within the mucosal and muscular layers are more sensitive to changes in autonomous regulation, and the differences between ST chickens (Fig. 2) and ST-NT chickens (Fig. 3) are often statistically significant. However, the overall thickness of these layers and the entire intestinal wall does not show significant differences between the two groups. The serosal layer is independent of the autonomous regulation differences. It has nearly the same thickness in both groups, slightly increasing to only 0.1 μm in ST chickens. ST chickens' dominance in the other layers' thickness is slightly more pronounced. The muscular layer in ST chickens differs from ST-NT ones by 18.1 μm , while the mucosal layer differs by 36.3 μm . Based on the measurements of individual layers, it is evident that the overall thickness of the rectal wall is also more prominent in ST chickens, surpassing the other group by 54.5 μm .

Preserving the morphological balance between its layers is essential to the intestinal wall's integrity and stability. It has been found that the ratio between the components within the mucosal and muscular layers can significantly differ in each group. However, at the level of the entire intestinal wall, the ratio between the layers remains relatively stable. The proportion of the serosal layer in the intestinal wall of ST and ST-NT chickens is 1.8 % and 1.9 %, respectively; the muscular layer is 49.6 % and 50.2 %, and the mucosal layer is 48.6 % and 47.9 %. Thus, the typological features of autonomous regulation contribute to complex changes in the rectal wall.

Table 2

Morphometric parameters of the mucosal and muscular layers of the rectum in chickens (mean ± SE)

Indicators	Groups of poultry	
	ST chickens n = 12	ST-NT chickens n = 12
Total intestinal wall thickness, μm	1354.9 ± 97.02	1300.4 ± 89.35
Mucous membrane thickness, μm	659.1 ± 41.49	622.8 ± 38.61
Muscle membrane thickness, μm	671.3 ± 65.72	653.2 ± 63.87
Serous membrane thickness, μm	24.5 ± 1.58	24.4 ± 1.98
Number of mucous membrane villi, units/cm ²	912.2 ± 23.27	847.3 ± 31.05*
Height of the mucous membrane villi, μm	478.4 ± 28.36	447.1 ± 22.80
Depth of the mucous membrane crypts, μm	142.9 ± 10.52	134.6 ± 9.94
The ratio of the villi height to the crypt depth, units	3.3 ± 0.19	3.3 ± 0.15
The thickness of the mucous membrane epithelium, μm	36.5 ± 1.04**	33.0 ± 0.89
The thickness of the mucous membrane's muscular plate, μm	37.8 ± 0.52	41.1 ± 0.43**
Number of goblet cells on one villi of the mucous membrane, units	84.0 ± 4.32	89.1 ± 5.29
Area of the goblet cell's secretory department, μm^2	55.2 ± 2.26*	48.2 ± 1.93
The thickness of the muscle membrane's circular layer, μm	541.8 ± 58.29	512.4 ± 57.63
The thickness of the muscle membrane's longitudinal layer, μm	129.5 ± 7.74	140.8 ± 8.08
The relative area of connective tissue fibers in the crypt area, %	9.6 ± 0.34*	8.7 ± 0.39
The relative area of elastic fibers in the crypt area, %	3.1 ± 0.14	2.9 ± 0.09
The relative area of connective tissue fibers in the composition of the muscle sheath, %	12.9 ± 0.64	15.3 ± 0.88*
The relative area of elastic fibers in the composition of the muscle sheath, %	7.9 ± 0.38	9.8 ± 0.45*
The relative area of nucleic acids in the nuclei of intestinal mucosa's columnar epitheliocytes, %	21.4 ± 0.62	22.6 ± 0.46*
The relative area of deoxyribonucleic acid in the nuclei of intestinal mucosa's columnar epitheliocytes, %	10.3 ± 0.32	11.2 ± 0.39*

Note: * – $P < 0.05$; ** – $P < 0.01$

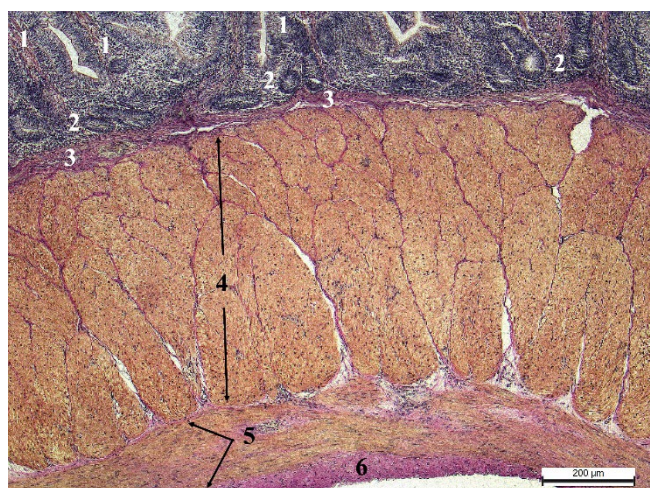


Fig. 2. Structure of the rectum wall of the ST chicken: 1 – villi; 2 – crypts; 3 – mucous membrane's muscular plate; 4 – muscular shell's circular layer; 5 – muscular membrane's longitudinal layer; 6 – serous membrane. Van Gieson

Regarding the mucosal layer, it has been observed that the number of villi per 1 cm² is higher in ST chickens than in ST-NT. The difference between the two groups is 64.9 villi ($P < 0.05$). A similar pattern is observed in the villi height, where ST chickens exceed ST-NT chickens by 31.3 μm .

The most minor and statistically insignificant differences between the experimental groups are observed in the crypt depth. The advantage of ST chickens over ST-NT chickens in this parameter is only 8.3 μm .

Since the villi height and the crypt depth have a similar dependence on autonomous regulation, the ratio between them is the same in both groups, amounting to 3.3 units. Thus, the villi height and the crypt depth jointly respond to the influence of the autonomous nervous system.

In contrast to the previous two parameters, the mucosal membrane's epithelial layer is more influenced by the type of autonomous regulation, with ST chickens showing a



Fig. 3. Structure of the rectum wall in ST-NT chicken: 1 – villi; 2 – villi epithelial layer; 3 – crypts; 4 – mucous membrane's muscular plate. Hematoxylin and eosin

significant advantage over ST-NT ones of 3.5 μm ($P < 0.01$).

Significant differences between the groups are also observed in the thickness of the mucosal membrane's muscular layer. However, in this case, ST-NT chickens surpass ST chickens by 3.3 μm ($P < 0.01$).

For a more objective characterization of the mucous membrane dimensions under different types of autonomous regulation, the percentage ratio between the indicators determining its thickness was investigated. Thus, the proportion of villi, crypts, and muscular plate in the total mucous membrane thickness of ST chickens is 72.6 %, 21.7 %, and 5.7 %, respectively. In ST-NT chickens, the corresponding ratio is 71.8 %, 21.6 %, and 6.6 %. From the presented indicators, it is clear that the percentage share of crypts is almost independent of the characteristics of autonomous regulation. In contrast, the shares of villi and muscular plate are moder-

ately dependent. An increase in sympathetic tone leads to an increase in the villi proportion. In contrast, an increase in parasympathetic tone increases the proportion of the mucous membrane's muscular plate.

In the study of goblet cells, two indicators were determined: the number of cells on a single villus and the area of the secretory section of a single cell. Along the intestine, an inverse relationship is observed between these indicators,

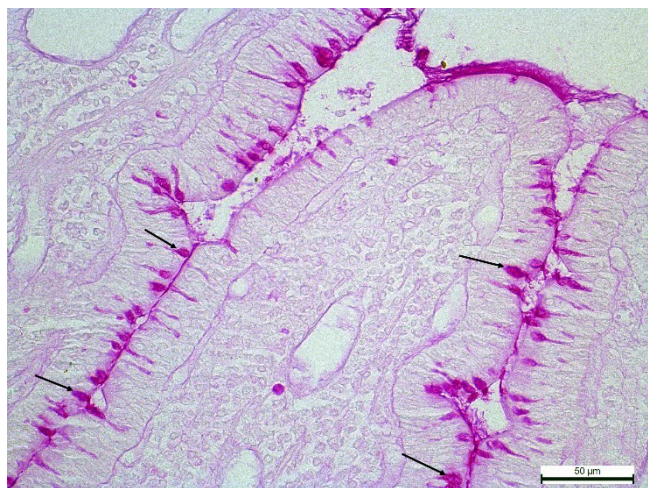


Fig. 4. Goblet cells (shown by arrows) of the mucous membrane villi in ST-NT chicken. PAS-reaction

meaning that a higher value of one indicator corresponds to a smaller value of the other. This is characteristic of both groups. Thus, in the rectum of ST-NT chickens (Fig. 4), the number of goblet cells on a single villus of the mucous membrane is 5.1 cells higher compared to the other group (Fig. 5). As for the area of the secretory section of the goblet cell, the advantage of ST chickens is 7.0 μm² (P < 0.05).

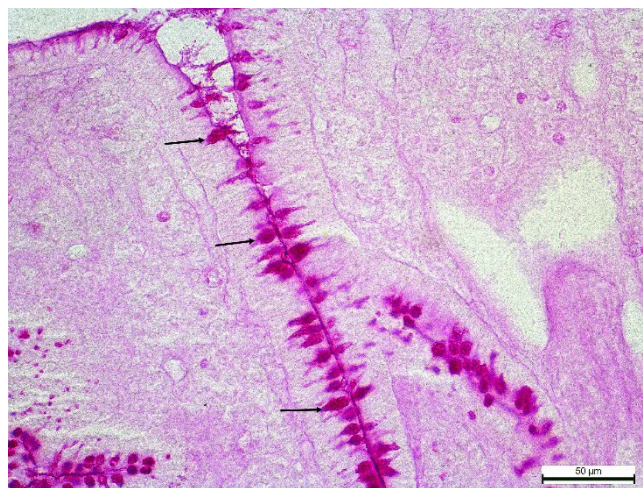


Fig. 5. Goblet cells (shown by arrows) of the mucous membrane villi in ST chicken. PAS-reaction

Alongside the mucous membrane, the influence of autonomous regulation's typological features is also manifested in the morphological characteristics of the rectum muscular layer. In the thickness of the circular layer, ST chickens dominate, surpassing ST-NT ones by 29.4 μm. However, in the thickness of the longitudinal layer, the advantage shifts to ST-NT chickens, and the other group lags by 11.3 μm.

For a more detailed characterization, the proportion of each muscle layer in the total thickness of the muscular layer was also investigated. Thus, in the muscular layer of ST chickens, the circular and longitudinal layers account for 80.7 % and 19.3 %, respectively. In ST-NT chickens, these layers account for 78.4 % and 21.6 %, respectively. The results indicate that an increase in sympathetic tone determines the increase in the proportion of the muscular mem-

brane's circular layer. In contrast, the strengthening of the parasympathetic tone influences the longitudinal layer. It is also noticeable that the percentage ratio between the structural parts of the muscular layer is more dependent on the type of autonomous regulation than the mucous membrane.

Connective tissue, including collagen and elastic fibers, is essential to all intestinal wall layers. In the crypt area of the mucous membrane, the relative area (expressed as a percentage) of both total connective tissue fibers and elastic fibers was investigated. The relative area of all fibers (Fig. 6) differs by 0.9 % (P < 0.05) among the studied groups, with dominance in ST chickens. Elastic fibers (Fig. 7) comprise approximately one-third of the previous indicator, and the advantage of ST chickens over ST-NT ones is relatively small, i.e., only 0.2 %.

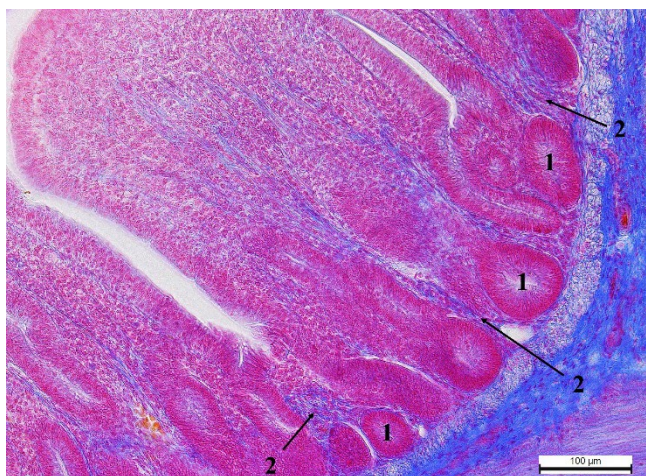


Fig. 6. Connective tissue fibers of the crypt area of the mucous membrane in ST-NT chicken: 1 – crypts; 2 – connective tissue fibers. Pacini



Fig. 7. Elastic fibers of the mucous membrane's crypt area in ST chicken: 1 – elastic fibers between the crypts; 2 – elastic fibers' layer between the muscular plate of the mucous membrane and the circular layer of the muscular membrane. Weigert

Furthermore, both connective tissue fibers and elastic fibers form a well-defined layer between the mucous membrane's muscular lamina and the circular layer. Its thickness in both groups is 40–45 μm .

The relative area of connective tissue fibers within the muscular layer is related to its thickness. Unlike the mucous membrane, a larger area of all fibers (Fig. 8) corresponds to ST-NT chickens, which surpasses the other group by 2.4 % ($P < 0.05$). As for elastic fibers (Fig. 9), their quantity significantly increases compared to the crypt area, and they occupy more than half of the total area of connective tissue fibers. In this case, the higher values of the area also belong to ST-NT chickens, while ST chickens lag by 1.9 % ($P < 0.05$).

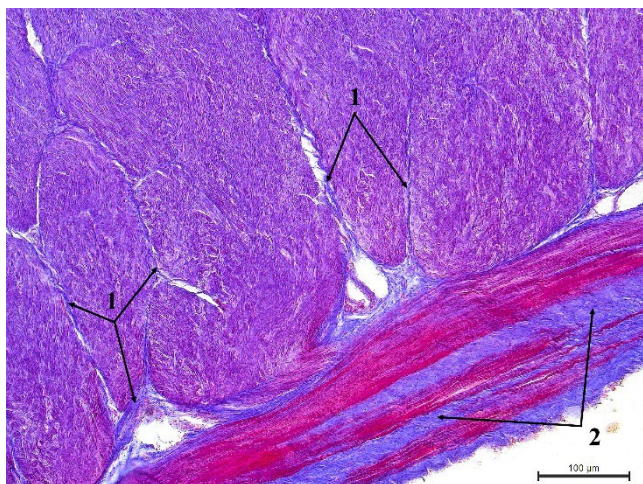


Fig. 8. Connective tissue fibers of the muscle sheath in ST-NT chicken: 1 – connective tissue fibers in the circular layer; 2 – connective tissue fibers in the longitudinal layer. Pacini

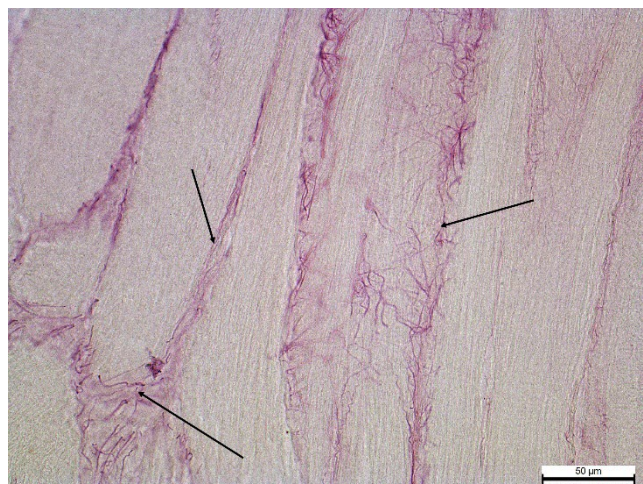


Fig. 9. Elastic fibers (shown by arrows) in the circular layer of the muscle sheath in ST-NT chicken. Weigert

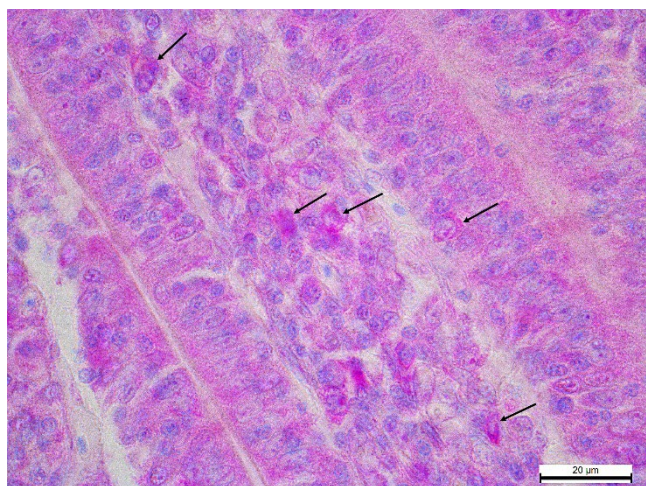


Fig. 10. Plasma cells (shown by arrows) in the composition of the mucous membrane villi in ST chicken. Brachet

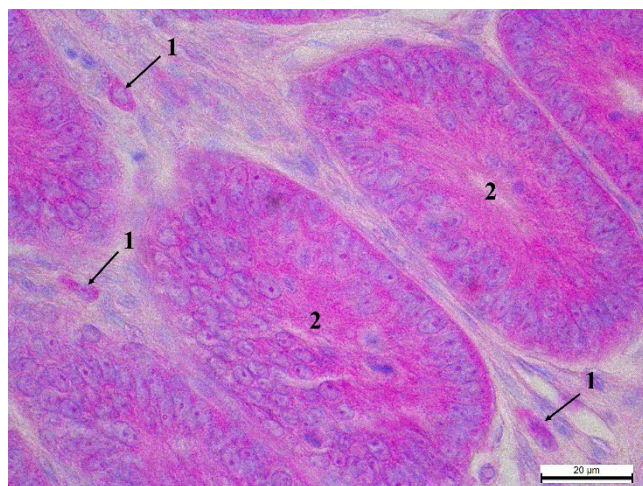


Fig. 11. Plasma cells in the crypt area of the mucous membrane in ST chicken: 1 – plasma cells; 2 – crypts. Brachet

The morphological study of deoxyribonucleic acid (DNA) allows us to detect chromatin condensation and, based on this, describe the functional state of the cell nucleus. The influence of typological features of autonomous regulation on the relative area (expressed as a percentage) of both the total amount of nucleic acids (DNA + RNA, Fig. 12) and DNA alone (Fig. 13) in the nuclei of columnar epithelial cells of the mucous membrane was investigated. The relative area of the total amount of nucleic acids in ST-

To characterize the intestine's defensive mechanisms, plasma cells in its wall were investigated. These cells and their precursors (plasmoblasts and others) were found in the mucous membrane, particularly in the main lamina propria of the villi and the connective tissue between the crypts. In some intestinal wall areas, the distribution of plasma cells is relatively uniform. In contrast, in others, it exhibits focal patterns, where neighboring villi or crypts contain significantly different amounts of cells. Generally, the density of plasma cells in the villi (Fig. 10) is higher than in the crypts (Fig. 11). Based on the obtained results, it is not possible to conclude a significant correlation between the quantity and topography of the investigated cells and the typological features of autonomous regulation.

NT chickens is 1.2 % ($P < 0.05$), more significant than in ST chickens. As for the relative area of DNA alone, its dependence on autonomous regulation is similar to the previous indicator, and ST-NT chickens surpass ST ones by 0.9 % ($P < 0.05$). The area of DNA accounts for approximately 50 % of the total nucleic acids area. Therefore, a shift in autonomous tone towards normotonia promotes the transition of euchromatin to heterochromatin, thus indicating a lower level of synthesis processes inside the cell.

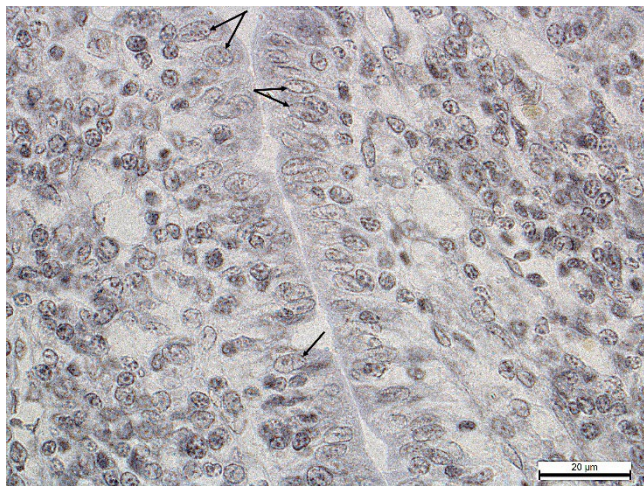


Fig. 12. Nucleic acids (DNA+RNA) in the nuclei of columnar epitheliocytes of the mucosa in ST chicken (shown by arrows). Einarson

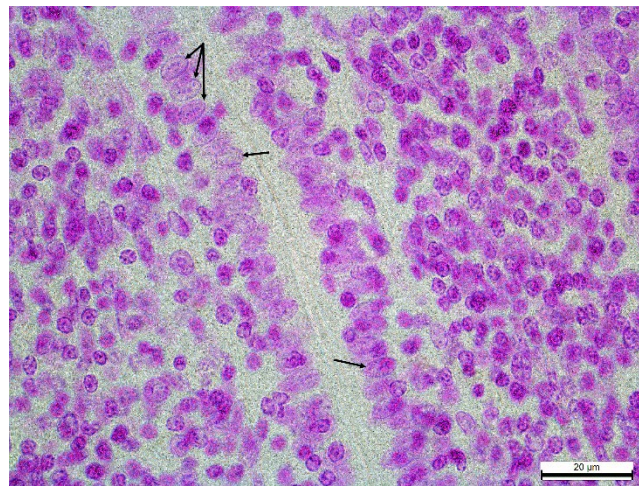


Fig. 13. DNA in the nuclei of mucous membrane's columnar epitheliocytes in ST-NT chicken mucosa- (shown by arrows). Fölgen and Rossenbeck

The intermuscular nerve plexus of the rectum consists of nerve ganglia of various shapes and sizes, which vary significantly in their dimensions (Fig. 14, 15). Approximately 45 % of all ganglia have an area ranging from $10 \times 10^3 \mu\text{m}^2$ to $50 \times 10^3 \mu\text{m}^2$. About 43 % of the ganglia fall within the size range of $51\text{--}100 (\times 10^3) \mu\text{m}^2$. Significantly fewer ganglia (9% in total) have an area of $101\text{--}150 (\times 10^3) \mu\text{m}^2$, and relatively rarely (in 3 % of cases) the ganglia of the maximum size range, i.e., $151\text{--}250 (\times 10^3) \mu\text{m}^2$, are observed. The presented percentage values are average indicators for both groups of chickens since there are specific differences in the sizes of nerve ganglia for each type of autonomous regulation. Considering the average area of nerve ganglia (Table 3), ST chickens have a larger size than ST-NT ones by $5.3 \times 10^3 \mu\text{m}^2$.

Regarding the number of ganglia per 1 mm^2 of the intestinal wall, ST chickens have 0.12 fewer ganglia than ST-NT ones ($P < 0.05$).

Table 3

Morphometric parameters of the rectum's intermuscular nerve plexus in chickens (mean \pm SE)

Indicators	Groups of poultry	
	ST chickens n = 12	ST-NT chickens n = 12
Nerve node area, $\times 10^3 \mu\text{m}^2$	54.2 \pm 4.42	48.9 \pm 5.09
Number of nerve nodes per 1 mm^2	0.70 \pm 0.03	0.82 \pm 0.06*
Number of nerve loops per 1 mm^2	1.57 \pm 0.18	1.68 \pm 0.17
Nerve loop area, mm^2	0.47 \pm 0.046	0.42 \pm 0.041

Note: * – $P < 0.05$

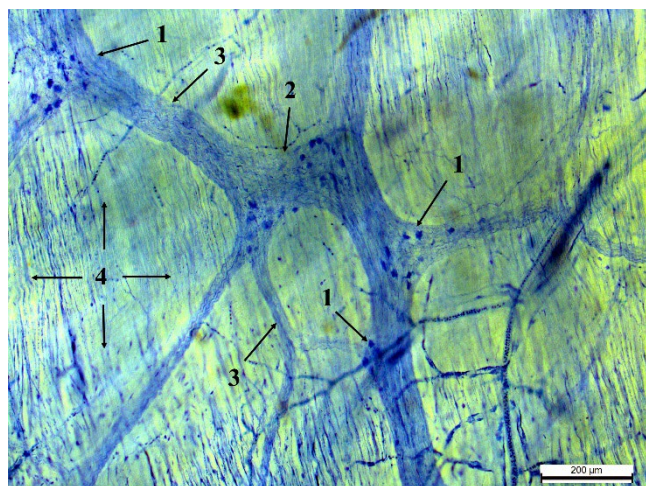


Fig. 14. Nodes of the intermuscular nerve plexus in ST chicken: 1 – node of the first group; 2 – node of the second group; 3 – nerve cord; 4 – nerve loop. Dogel-Shabadash

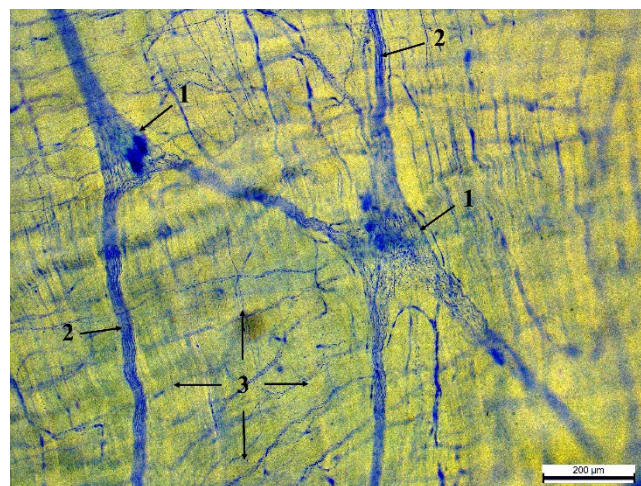


Fig. 15. Nodes of the intermuscular nerve plexus in ST-NT chicken: 1 – node of the first group; 2 – nerve cord; 3 – nerve loop. Dogel-Shabadash

The nerve ganglia are interconnected by nerve bundles, forming closed structures called nerve loops of elongated shape (Fig. 14, 15). The number of these structures per 1 mm^2 of intestinal wall differs by 0.11 units between the two groups, with a dominance of the ST-NT group. The average area of the nerve loops of the rectum, similar to the area of

the nerve ganglia, has larger values in ST chickens, surpassing the other group by 0.05 mm^2 .

3.2 Discussion

The variability of heart rhythm, which is associated with regulatory influences of the autonomous nervous system and forms the basis for our division of poultry into two groups, is

an important physiological and clinical indicator. During the embryonic period, the heart is under the enhanced influence of sympathetic nerves, as indicated by its high frequency and rhythmic contractions. Before or immediately after birth, parasympathetic influences on heart function are enhanced, which is associated with the development of respiratory function (Taylor et al., 2014). This results in the formation of three main types of autonomous tone in mammalian organisms: 1) sympathicotonia – dominance of sympathetic tone over the parasympathetic one; 2) normotonia – balanced tone of both divisions of the autonomous nervous system; 3) parasympathicotonia – dominance of parasympathetic tone over the sympathetic one (Kononenko & Zaitsev, 2009). However, compared to mammals, birds have a higher heart rate, more intensive metabolism, and higher body temperature. These characteristics indicate a high sympathetic tone in the avian body. Therefore, our study could not identify poultry groups with normotonia and parasympathicotonia. In all studied chickens, the sympathetic tone always dominated over the parasympathetic one. Our division of poultry into two groups reflects the level of this dominance.

Heart rhythm is used to characterize metabolism, emotional state, behavioral reactions, growth and development, and overall well-being of animals (Blanchard et al., 2002; Tazawa et al., 2002; Aubert et al., 2004; Borell et al., 2007). The balance between sympathetic and parasympathetic tone is also considered in breeding new lines of domesticated poultry, including chickens (Kjaer & Jorgensen, 2011).

Every biological system, including the digestive system, has a close connection between morphological characteristics and functional features (Scanes & Pierzchala-Koziecb, 2014). It is known that the rectum of birds is involved in water absorption (Son & Karasawa, 2001) and folic acid (Tactacan et al., 2011). It participates in the exchange of sodium (Fan et al., 2013), potassium (Montero & Ilundain, 1989), and nitrogen (Karasawa, 1989). Proteolytic bacteria present in the lumen of the intestine help in protein digestion (King et al., 2009). Therefore, the differences in size and structure of the rectal mucosa in poultry with different types of autonomous regulation, as observed in our study, will undoubtedly reflect these processes' characteristics. For example, ST chickens, with longer intestinal lengths and higher number and height of villi, will have a larger mucosal surface area. ST-NT chickens partially compensate for this difference by having a larger intestinal volume. Therefore, it is evident that the intensity of absorption processes in this section of the intestine will be higher in ST chickens. Goblet cells play an essential role in the process of digestion. Their mucus protects the mucosal lining from proteolytic enzymes and forms an antimicrobial barrier (Kim & Khan, 2013; Tarabova et al., 2016). Mucins are critical components of mucus, which not only participate in the formation of the immune response to infection (Nowarski et al., 2017) but also play an essential role in combating parasitic infections, including nematodes (Hasnain et al., 2013; Sharpe et al., 2018). Based on our results, we cannot reliably determine which type of autonomous regulation leads to higher mucus production, as one group of chickens is characterized by a higher density of goblet cells in the mucosa. In contrast, the other group has a larger cell diameter. In this situation, the chemical composition of the mucus may be more important than the quantity, and further research is needed to elucidate this problem.

In the muscular membrane of the rectum, we discovered two layers of muscles: an outer longitudinal layer and an

inner circular one. This differs significantly from the small intestine's muscular membrane, which, according to certain researchers (Gabella, 1985), contains four layers of muscles: an outer longitudinal layer, an outer circular layer, an inner circular layer, and an inner longitudinal layer. The individual or combined contractions of these muscle layers create the motile activity of the rectum. The longitudinal layer of muscles generates slow, pendulum-like contractions with a large amplitude. In contrast, the circular layer of muscles produces fast peristaltic contractions with a small amplitude (Brummermann & Braun, 1995). Based on the thickness of these muscular layers, it can be assumed that the first type of contractions will be more pronounced in ST-NT chickens, while the second type will be more prominent in ST chickens. In addition to the primary peristaltic contractions of the muscular membrane's circular layer, antiperistaltic contractions can also be observed. This means that the intestine's contents can move both in cranio-caudal and caudo-cranial directions (Lai & Duke, 1978; Duke, 1989). Antiperistaltic contractions are an essential adaptation for water conservation in the body (Dehkordi & Shakaram, 2017).

It is also important to note that despite significant differences in the internal structure of the mucosal and muscular layers in ST and ST-NT chickens, the ratio between these layers remains relatively constant. Similar patterns are observed between indicators such as villi height and crypts' depth. This confirms the previously mentioned compensatory effect and indicates the complex adaptation of the digestive organs' morphology to the influences of the autonomous nervous system. These processes are integral to the regulatory mechanisms to ensure optimal digestive parameters. However, the stable relationship between individual morphological parts of the rectum is characteristic only of biologically mature organisms, as it undergoes substantial changes during ontogenesis (Dehkordi & Ghahremani, 2016).

The limited influence of autonomous regulation on the serous membrane thickness is related to its specific structure (lack of muscle fibers and glandular structures), which accounts for its minimal activity in supporting intestinal wall function.

During the examination of the rectum connective tissue, it was found that the proportion of collagen and elastic fibers in the muscular layer in all investigated poultry groups is significantly more significant than in the mucosal layer. This is due to the specific combination of numerous functions of connective tissue in each layer, such as providing a framework, support, reinforcement, separation, and protection (Verbeke et al., 2001; Langevin, 2006). Additionally, the muscular layer's connective tissue is essential to the gastrointestinal tract's motor function (Bruhin-Feichter et al., 2012). Quantitative and structural differences in connective tissue can even be observed within the same layer but in different parts of the intestine (Tybinka et al., 2018). The reason for this phenomenon lies in the differences in the functional activity of these parts. Based on the quantitative content of connective tissue fibers, we can also assume that each type of autonomous regulation determines specific functional characteristics of the intestinal wall's layers. In ST chickens, a higher amount of these fibers is found in the mucosal membrane, while in ST-NT chickens, it is more prevalent in the muscular layer.

Increased connective tissue fibers are observed around blood vessels and nerve nodes (Pandit et al., 2018). Connec-

tive tissue is essential in the pathogenesis of various intestinal diseases (Verbeke et al., 2001).

The active role of the intestinal wall in antibacterial defense is indicated by its abundance of specialized cells, including macrophages, eosinophils, and plasma cells. These cells are predominantly located in the mucosal region between the fibers and fibroblasts of the connective tissue (Deane, 1964). Alongside plasma cells, their precursors, such as plasmablasts, can also be found as they undergo differentiation (Roth et al., 2014), with a more excellent localization in the lymphoid nodules of the intestinal wall. The increased number of plasma cells we identified inside the villi compared to the crypts suggests a more prominent involvement of the villi in forming the mucosal membrane's protective barrier. The results also demonstrate that not all parts of the mucosal membrane are equally engaged in the immune defense process, as evidenced by the uneven distribution of plasma cells across the mucosal surface. Additionally, we did not establish a significant influence of autonomous regulation typology on the quantity and distribution of plasma cells. However, this influence may manifest in other unexplored indicators, particularly in producing numerous antibodies by plasma cells, making them critical structures in the formation of humoral immunity (Hoh & Boyd, 2018; Wang et al., 2019).

The high density of nerve plexuses in the rectal wall is also confirmed by the findings of Yang et al. (2013). However, our study's number of plexuses per 1 mm² was almost three times lower. We attribute this difference primarily to the different age groups of the poultry. In our study, the chickens were 1.5 years old, whereas the mentioned researchers studied 7 to 40 days old chickens. Throughout their 33-day study, the number of plexuses in the nerve plexus decreased by 1.7 times. Therefore, it can be assumed that when the chickens reach a similar age, the number of plexuses per 1 mm² would be proportionate to our findings. This assumption is also supported by the results of other researchers (Bagyanszki et al., 2000), who found an even higher number of nerve plexuses per 1 mm² in the thick intestine of chicken embryos.

Additionally, from 12 to 19 days of incubation, the number of plexuses decreased from 98.47 to 10.8. The age-related decrease in the number of nerve plexuses occurs concurrently with an increase in their size and the quantity of the neurotransmitter acetylcholine (O'Donnell et al., 2006; O'Donnell & Puri, 2009). The number and morpho-functional characteristics of the nerve cells within the plexuses depend on their size and localization.

Therefore, the structural characteristics of the intestinal wall identified in our study, determined by the respective types of autonomous regulation, along with a complex array of other regulatory mechanisms (Chelakkot et al., 2018), contribute to the morphological integrity of the intestinal barrier and define its functional characteristics.

4. Conclusions

The overall balance of sympathetic and parasympathetic centers in the chicken's organism gives rise to two types of autonomous regulation, which allow us to classify poultry into two groups: sympathetic-tonic chickens and sympathetic-normotonic chickens. Each group's size, structure, and blood supply of the rectum differs. The autonomous regulation type significantly impacts the thickness of the intestinal wall's mucosal and muscular layers and has almost no effect

on the serous layer thickness. The thickness of all mentioned layers and the entire intestinal wall is more significant in sympathetic-tonic chickens. Sympathetic-normotonic chickens exhibit increased thickness only in the muscular plate of the mucosal layer and the longitudinal layer of the muscular layer. In both groups, the density of collagen and elastic fibers in the muscular layer is significantly higher than in the mucosal layer. However, sympathetic-tonic chickens are characterized by a greater quantity of these fibers in the mucosal membrane, while sympathetic-normotonic chickens have more fibers in the muscular membrane. No significant influence of the autonomous regulation type on the quantity and localization of plasma cells has been established. Functional indicators often demonstrate the effect of morphometric compensation, where higher values of one parameter are combined with lower values of the other. For example, sympathetic-tonic chickens have a longer but narrower intestine, a smaller number of goblet cells per villus, but a larger area of the secretory compartment of the cell, a larger area of the nerve node in the intramuscular nerve plexus, but a lower number of these nodes per 1 mm² of the intestinal wall. These patterns allow for maintaining a balanced morpho-functional structure at the level of individual layers and the entire intestinal wall, enabling effective adaptation to the corresponding regulatory influences from the autonomous nervous system.

Conflict of interest

The author report no conflict of interest in the presented work.

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