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Research

*Corresponding author
Galyna Blishch, MD
Department of Normal and Pathological Morphology and Forensic Veterinary Medicine, Lviv National University of Veterinary Medicine and Biotechnology
79010 Lviv, Ukraine
Tel. +380679105825
E-mail: galyna.blishch@gmail.com

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Impact of Autonomic Tonus Typological Features on the Duodenum Structure of Chickens

Andrij Tybinka, PhD; Olexander Zaitsev, MD; Galyna Blishch, MD*

Department of Normal and Pathological Morphology and Forensic Veterinary Medicine, Lviv National University of Veterinary Medicine and Biotechnology, 79010 Lviv, Ukraine

ABSTRACT

The goal of this research is to show the relationship between the duodenum morphology of laying hens (Gallus domesticus) of ISA Brown cross and typological peculiarities of autonomic tonus in the poultry organism. To achieve the goal a group of adult chickens (aged one year) was investigated to determine an aggregate tonus of their autonomic centers based on the study of heart rate variability (variation pulsometry). According to the results all the poultry was divided into two groups: sympatheticotonic chickens (hereinafter referred to as ST chickens) and sympathetico-normotonic chickens (hereinafter referred to as ST-NT chickens) The first group of poultry is characterized by a clear predominance of sympathetic centers tonus while within the second group there is a slightly increased tonus of parasympathetic centers. Using a set of various morphological methods a comparative analysis of the duodenum structure in these groups was made. The analysis revealed that ST chickens unlike ST-NT chickens are characterized by a higher value of the duodenum absolute length (by 1.6 cm) and its relative length (by 1.4 %) as well as lower values of its internal perimeter (by 0, 25 cm). Apart from this, an increase of absorption surface of the duodenum mucous membrane is observed in ST-NT chickens. Increase in villus height (by 143.7 micrometers) and their quantity (by 61.5) per 1sm^2 contribute greatly to it. In the duodenum muscle membrane the thickness of both layers has larger values under a distinct sympathetic tonus in ST-NT chickens. In a circular layer difference between poultry groups reaches 21 micrometers while in a longitudinal it approaches 4.9 micrometers. Investigating a connective tissue it was found that the relative area of its fibers near the mucosal crypts of ST chickens is larger by 0.21 % than that of the ST-NT chicken. In the muscle layer area a superiority of ST chickens according to this indicator reduces to 0.15%.

KEYWORDS: Autonomous tone; Chicken; Intestinal wall; Morphometry.

ABBREVIATIONS: ST Chickens: Sympatheticotonic Chickens; ST-NT Chickens: Sympathetico-Normotonic Chickens.

The results of morphological studies indicate that a different combination of autonomic centers tonus causes the development of an entire complex of duodenum structural changes. This is obviously aimed at ensuring the optimal digestive system functioning under various regulatory influences.

INTRODUCTION

Being characterized by a determined cellular and tissue structure the intestine wall is a very dynamic structure of the poultry organism. Adaptation processes that adjust its morphological peculiarities and functional characteristics constantly take place there.[1-6] These processes are influenced by a variety of internal and external environment factors that are mutually intertwined and exert a complex impact on the organism. Genetic factor occupies a dominant position in determining the morphological and functional intestinal wall profile.[7-9] Nervous,

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endocrine and immune systems generate regulatory influence on the intestine in the process of its growth and development.[10-15] They help form the phenotypic expression of genetic potential according to individual living conditions of the poultry. Forage base and climate are of primary importance here too. Living conditions as well as artificial formation of productive qualities through the development of new breeds and crosses become increasingly important for domestic poultry. The use of different feed supplements, growth promoters, antimicrobial and antibacterial drugs, probiotic preparations and many other compounds exerts a significant impact on the intestinal wall.[16-21] Constantly adapting intestinal mucosa that supports necessary digestion parameters and performs the barrier function responds to these factors in the most sensitive way.[22-24] All this eventually impacts the poultry health and productivity.

Autonomic nervous system centers are characterized by a different tonic activity in different organisms. That's why their cumulative effect on the organs will have some differences too. This study was aimed at showing the relationship between the chickens duodenum morphology and typological peculiarities of the autonomic tonus.

MATERIALS AND METHODS

The study was conducted on laying hens (Gallus domesticus) of ISA Brown cross, aged one year. All poultry was a subject of electrocardiographic investigation. ECG recording was performed with the aid of R4-02 rheograph and N338-6P recording device at a belt speed of 250 millimeters per second. Received cardiograms allowed to analyze a heart rate variability (variation pulsometry) of poultry organism.[25-27] The research enabled to describe the aggregate tonus of the autonomic centers and divide all the poultry into two groups: sympatheticotonic chickens (ST) and sympathetico-normotonic chickens (ST-NT). The first group is characterized by a distinct predominance of sympathetic centers tonus. The second group has a slightly increased tonus of parasympathetic centers. Sympathetico-normotonic chickens are an intermediate type between two known types of autonomic tonus that are typical for mammals: sympatheticotonics and normotonics.

For the different types of research a certain number of specimens was selected from each group. Euthanasia was performed by an inhalation overdose of chloroform. Intestinal wall tissue samples were taken from the middle third of the duodenum.

To determine the duodenum size 12 ST chickens and 13 ST-NT chickens were selected. With the aid of a caliper an inner intestine perimeter was defined in each investigated group. Absolute duodenum length was determined using a measuring tape. Comparing the latter value with the entire intestine length a relative length of the duodenum was defined (in %). Combining the indicators of absolute duodenum length and its perimeter the area of duodenum mucosa was calculated.

To conduct the study of the duodenum blood supply 4 ST chickens and 5 ST-NT chickens were selected. For the specimens of each group an arterial injection of mesentery with a 10% solution of gelatin colored with red or green pigment (gouache) was performed. After the gelatin hardening a number of intestinal arteries within the duodenum wall was determined.

The research of the fibers number was conducted on 12 ST chickens and 13 ST-NT chickens. The number of fibers per 1 cm^2 of mucosa and the total number of fibers of the entire mucosa was determined.

For morphometric studies the tissue samples were fixed in Carnoy and Bouin fluids, further embedded in paraffin. Paraffin sections were stained according to the methods of Van Gieson and Pacini.[28] Villus height and crypt depth were determined due to the histological slides received from 10 ST chickens and 12 ST-NT chickens. The thickness of the epithelium and mucosa muscle plate as well as thickness of both muscle membrane layers was determined due to the histological slides received from 16 ST chickens and 17 ST-NT chickens. The same quantity of poultry was used to examine a relative area (expressed in %) of the connective tissue fibers in the area of the muscle membrane and mucosa crypts.

Morphometric study was conducted using the Leica DM-2500 microscope equipped with Leica DFC450C camera and software Leica Application Suite Version 4.4 as well as stereoscopic microscope MBS-10 with Konus №5829 camera and software Image-Tool 3 and WCIF ImageJ.

Research was conducted in a full compliance with the general principles of work with experimental animals (Strasbourg, 1986; Kyiv, 2001).

Statistical Analysis

The received data was processed using a standard software StatPlus 2008. Validity difference of each indicator was measured for ST chickens and ST-NT chickens. Differences between two poultry groups were considered valid when $p<0,05$.

RESULTS AND DISCUSSION

The discovered typological peculiarities of autonomic tonus are manifested with both functional and structural signs in the chickens' organism. The investigated duodenum morphological features are a perfect proof of it (see Table 1).

Characterizing the duodenum size and its blood supply it was found out that the absolute duodenum length in ST chickens is 1.6 cm ($p<0,05$) higher comparing to the ST-NT chickens. A similar situation is observed with the duodenum relative length. In this case the first group of poultry dominates over the second one by 1.4%. The inverse relationship on autonomic tonus is typical for the internal perimeter of the duodenum. On the

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Indexes	ST chickens	ST-NT chickens
Absolute length of intestine, cm	25,0±0,57*	23,4±0,50
The relative length of the intestine, %	13,8±1,84	12,4±1,22
The inner perimeter intestine, cm	2,92±0,150	3,17±0,156***
Area mucosa, cm²	73,0±3,12	74,2±3,46
Number intestinal arteries, pieces	29,5±0,96*	25,2±0,66
number of fibers, piece /cm²	415,4±22,05	476,9±29,92*
The total number of fibers, piece	30324,2±568,97	35375,5±649,19
villus height, mkm	1252,4±23,19	1396,1±26,02*
The depth of the crypts, mkm	289,6±11,68*	261,3±8,94
The ratio, villus height / crypt depth	4,3±0,34	5,3±0,47*
The thickness of the epithelium villi, mkm	33,7±0,30	33,6±0,43
The thickness of the muscle plate mucosa, mkm	36,8±0,35	37,2±0,45
The thickness of the layer of circular muscle layer, mkm	276,5±3,62	255,5±1,81
The length of the longitudinal muscle layer shell, mkm	58,5±0,87*	53,6±0,94
The relative area of connective tissue fibers in the field of mucosal crypts, %	5,80±0,110**	5,59±0,128
The relative area of the fibers of the connective tissue in the area of the muscle layer, %	9,62±0,135*	9,47±0,165

Values expressed as mean±SD.
* – p<0,05;** – p<0,01;*** – p<0,001.

Table 1: Morphological indicators of chickens duodenum.

contrary, its value is 0.25 cm (p<0,001) higher in ST-NT chickens. Thus, we see the formation of compensation processes between the length and perimeter of the duodenum in different poultry groups. This is also indicated by literally the same mucosa area which is determined considering two previous indicators and is only 1.4 cm² higher in ST-NT chickens. Practical significance of these indicators is that the decrease in the relative length of the duodenum may indicate an increase in the body mass of the poultry.[29,30]

Small arterial vessels (see Figure 1) entering the duodenum are an offshoot of the right branch of the abdominal artery. The number of such arteries was 4.3 higher (p<0,05) in ST chickens. Still, we can't claim that the increase in autonomic centers tonus enhances the duodenum blood supply. To receive a full picture it is necessary to explore the diameter of these blood vessels and intensity of blood flow in a specified area.

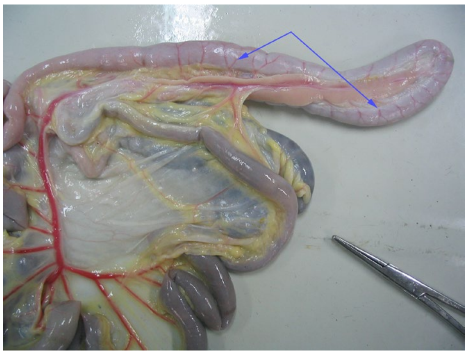

Figure 1: Intestinal vessels of chicken ST-NT, pour gelatin. The arrows show intestinal artery duodenum.

The next stage of our study reveals the peculiarities of relationship between the separate membranes of the duodenum wall and aggregate autonomic tonus.

It is known that the functional characteristics of the intestinal mucosa are closely related to the number of fibers, their size and structure.[31-33] Our study established that the increase in parasympathetic centers tonus in ST-NT chickens also increases the number of fibers per 1 sm² of duodenum mucosa. In ST chickens the number turned out to be 61.5 villi (p<0,01) lower (see Figure 2). Combining this figure with the total mucosa area it was determined that the total number of villi was 5051.3 higher for ST-NT chickens.

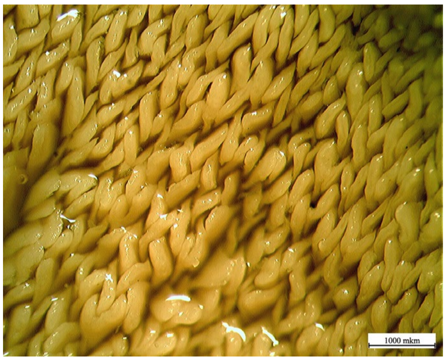

Figure 2: Villi mucosa of ST chicken.

The same regularity is typical for the villus height. For ST chickens this figure was 143.7 micrometers (p<0,05) higher comparing to ST-NT chickens (see Figure 3). However, increase in sympathetic tonus exerts a stimulating influence on the depth of crypts which causes a 28.3 micrometers (p<0,05) higher value of this indicator in ST chickens.

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Figure 3: The structure of the wall of the duodenum ST-NT chicken (stain Van Gieson).

Since the absolute values of villus height and crypt depth belong to different types of autonomic tonus, the relationship between these parameters is also different. For ST chickens it is 1.0 micrometers (or 19.2%) lower compared to ST-NT chickens.

Thus, the increase in parasympathetic centers tonus positively affects the increase in absorption surface of the duodenum mucous membrane rising the number and size of the villi. The indicated regularity should promote a better digestion and absorption of food nutrients, since it is widely known that the growth of these indicators is combined with a greater mass of the poultry.[34-36]

The thickness of the epithelium and mucosa muscle plate appeared to be the least dependent upon the tonic features of autonomic centers. Meanwhile, in different groups of poultry the epithelial layer thickness differed by only 0.1 micrometers with advantage in ST chickens. However, ST chickens had a somehow greater advantage (0.4 micrometers) in the thickness of the mucosa muscle plate.

Describing the duodenum muscle membrane, first of all it should be noted that the thickness of its two layers becomes larger under a distinct sympathetic tonus in ST chickens. In the circular layer of the membrane differences between the poultry groups reach a value of 21 micrometers. Longitudinal muscle layer of the membrane is much thinner than the previous one (see Figure 4). That's why its advantage in thickness is 4.9 micrometers (p<0,05) for ST-NT chickens.

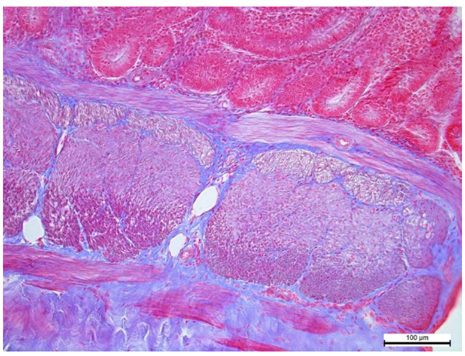

Figure 4: The structure of the muscle layer of the duodenum ST-NT chicken (stain Pacini).

The represented morphological characteristics of the muscle membrane within the explored groups of poultry, of course, must be characterized by some functional manifestations. The main purpose of both layers is to provide a duodenum motor activity in the form of peristaltic, anti-peristaltic and segment contractions.[37,38] Obviously, each type of autonomic tonus adjusts the very character and intensity of the duodenum motor activity, therefore affecting the intensity of feed mixing and its passage speed.

The amount of connective tissue within the duodenum wall structure is a direct evidence of its functional, adaptive and protective properties. Our research established that in the area of duodenum mucous membrane crypts of the ST chickens the relative area occupied by the connective tissue fibers is 0,21% (p<0,01) higher than the same indicator for ST-NT chickens. In the area of the duodenum muscle membrane the percentage of the area occupied by connective tissue fibers increases in both poultry groups. Herewith, the advantage of ST chickens over ST-NT chickens still preserves but is less expressed and accounts for about 0,15% (p<0,05).

It should also be mentioned that for each type of autonomic tonus the amount of connective tissue fibers increases significantly around blood vessels and nerve nodes. It looks like some "connective tissue membranes" are formed around these structures.

CONCLUSION

Summarizing all the research material we can conclude that the increase in the sympathetic or parasympathetic tonus which is a characteristic feature of the selected groups of chickens causes the formation of structural changes complex in the duodenum wall. These morphological adjustments are inextricably linked with functional processes both in the intestine and the whole organism and are aimed at ensuring the optimal dissimilation and assimilation parameters in the organism of the poultry.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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