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Morphological features of the jejunum and ileum of the middle and heavy goose breeds

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We studied the morphological features of the jejunum and ileum in middle and heavy goose breeds. The geese under six month of age of Gorkovskaya and Legart breeds were used in our research. Geese of a heavy breed had a large intestinal mass, length of the jejunum and ileum, thickness of the mucous membrane of the ileum, and a smaller thickness of the muscular tunic of the jejunum. Legart geese had greater villi density and crypt depth in the jejunum and greater height and density of the villi, the width of the crypts, and the ratio of the height of the villi to the depth of the crypts in the ileum. In all the guts of heavier geese, the density of crypts was lower. The geese of the heavy breed had larger number and area of the ganglia of the mental plexus and smaller number and area in the submucosa in the jejunum, while they had larger area of the ganglia of the submucosal plexus in the ileum. The number of argyrophilic and argentaffin apudocytes in the jejunum of geese of different breeds did not differ, when the Legart breed geese had lesser quantity of apudocytes in the ileum.

Key words: Geese; Intestines; Breed; Morphological marker; Gorkovskaya; Legart

Introduction

One of the main directions of development of industrial poultry farming is to increase the efficiency of animal feed use. This task is solved by purposeful breeding work on the breeding of poultry breeds with the ability to effectively absorb the nutrients of the diet (Sell-Kubiak et al., 2017). It is known that this trait of the organism is hereditary (de Verdal et al., 2013; Mignon-Grasteau et al., 2004). Breeding techniques are one of the most effective ways to reduce the poultry load on the environment. Genetic selection has the potential to reduce feed intake needed for poultry production (Leinonen et al., 2016). Differences in more efficient digestion of nutrients are associated with the anatomical and physiological characteristics of the digestive system. In chickens with a higher rate of nutrient uptake, a large mass of the muscle of the stomach, and a smaller mass of the intestine (Garcia et al., 2007). Correlation analysis showed the relationship between the size of the intestines of chickens and the indicator of the efficiency of feed use (Metzler-Zebeli et al., 2018). Phenotypes of feed use by farm birds, determined by genotypes or gene markers, remain unknown today. There are only limited studies on global gene expression profiling regarding feed efficiency (Lee et al., 2015). Understanding the genetic architecture of the digestive canal and the influence of selection criteria is of particular interest (de Verdal, 2011). Intestinal microstructure morphometric indicators are important markers of poultry productivity that can be used for breeding (Alshamy et al., 2018). In this regard, the search for appropriate morphological markers of high productivity is relevant, which will improve the efficiency of breeding work. The aim of the work was to determine the structural features of the small intestine of the geese of medium and heavy geese breeds.

Research Methodology

We used domestic geese (*Anser anser*) of Gorkovskaya and Legart breeds, which were kept in a poultry house of Kharkiv State Zooveterinary Academy (Ukraine). Gorkovskaya geese are classified as medium breeds with high egg productivity. The live weight of adult females is 5.5-6.0 kg, males – 6.5-7.0 kg (Ernst et al., 1994; Khvostyk, 2008). Legart breed (Danish Legart), which is widely distributed now, belongs to heavy breeds and it was selected in Denmark from domestic fowl. The live weight of adult females is 6.5-7.0 kg, males – 7.5-8.0 kg. Geese of this breed have a high live weight at an early age, excellent meat and feather qualities, are characterized by a high degree of conversion of feed to body weight (Riabokon et al., 2005; Khvostyk, 2008). During the observation, the bird was clinically healthy, received standard complete feed for geese, had free access to water, and used pasture in the summer. The geese were kept and manipulated in accordance with the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes (Strasbourg, 1986). For histological studies, from five heads of geese under six months of age, each breed selected a piece of the middle section of two intestines of the small intestine – jejunum and ileum, which were fixed in 10% neutral formalin solution and embedded in paraffin. For the preparation of preparations, sections were stained with hematoxylin and eosin, azure II and eosin, as well as according to Mallory. To identify the general population of apudocytes, Grimelius staining was used, argentaphine endocrinocytes were used according to Masson in the modification of Hamperl (Singh, 1964; Sarkisov, 1996). Histological preparations were examined under a *Jenamed-2* light microscope. The morphometric parameters of the intestinal microstructures were determined on transverse sections of the intestine using the *Image Tools* 3.6 software as well as the ocular mesh. The density of the villi and crypts was determined on a 1 mm section of the intestine. The number and area of the nerve nodes of the mental and submucous nerve plexuses were determined with subsequent conversion to 1 mm^2 of the area of the muscle and mucous membrane, respectively; the number of endocrinocytes per 1 mm^2 of

compared with those of a lighter breed – Gorkovskaya. Assessment of the statistical significance of measured parameters was performed by one-way ANOVA and Student's t-test. The data in Tables were presented like mean and standard deviation.

Results and Discussion

The live weight of young geese of 6 months of age of the Legart breed was 4870.0 ± 137.0 g, Gorkovskaya – 4285.0 ± 62.4, which was 13.7% more ($P \leq 0.05$). The absolute intestinal mass of the geese of the heavy breed was 24.5% more ($P \leq 0.05$) than the Gorkovskaya ones and amounted to 177.2 ± 17.8 and 142.3 ± 9.62 g, respectively. The difference in the relative mass of the intestine (3.7 ± 0.41 versus 3.3 ± 0.23%) had no significant difference. No reliable interbreed differences between the lengths of the entire intestine and its small part of geese have been established. The absolute length of the jejunum in geese of the Legart breed was 8.2% more ($P \leq 0.05$), and the ileum was 10.8% more ($P \leq 0.05$) (Table 1). The relative length of the thin section and its individual intestines did not have significant breed differences.

Table 1. Indicators of geese small intestine (n=8).

Organ	Breed			
	Gorkovskaya		Legart	
	Absolute Length, Cm	Relative Length,%	Absolute Length, Cm	Relative Length,%
Intestines whole	293.0 ± 7.97	100.00	313.8 ± 10.24	100.00
Small intestine	229.7 ± 6.10	78.4 ± 1.10	244.5 ± 9.71	77.9 ± 0.58
Jejunum	161.7 ± 4.15	55.2 ± 0.13	175.0 ± 3.33*	55.6 ± 0.39
Ileum	26.0 ± 1.15	8.9 ± 0.17	28.8 ± 0.17*	9.3 ± 0.13

*Note: * P ≤ 0.05*

The main processes of digestion and absorption take place in the duodenum and jejunum, the ileum constitutes a reservoir for the absorption of substances (Maev et al., 2005). The diameter of the jejunum was significantly larger in geese of Legart breed, and the ileum did not differ between these breeds (Tables 2 and 3). There was also no significant difference between the wall thickness of both the jejunum and ileum in geese of the same breed. Sklan (2001) suggested that there were no differences in the mass of the duodenum in chickens of the heavy and light breed. At the same time, the weight of the jejunum and ileum of the chickens was characterized by interbreed differences, and these changes fluctuated over time. Microscopically, on the cross section of the intestinal wall of geese, three membranes are distinguished: mucosa, muscle and serous.

Three layers are clearly distinguished in the composition of the mucous membrane: epithelial, own lamina and muscle lamina. The protrusions of the lamina propria form mainly the finger-shaped form of the villi, and the depressions of the epithelium in the lamina propria form the tube-shaped crypts covered with a single-layer single-row prismatic epithelium. Two layers were distinguished in the composition of the muscle plate: the inner and outer layers with the predominantly longitudinal and transverse directions of smooth muscle cells, respectively.

The submucosa in the wall of the small intestine of the geese is absent: only a narrow plate of amorphous substance is revealed between the muscle plate of the mucous membrane and the inner layer of the muscle membrane. Khaleel et al. (2017) and Alshamy et al. (2018) reported the absence or weak development of the submucosa in the intestinal wall of domestic duck and chicken.Mitchell et al. (1991) reported a greater surface area of the mucous membrane of the small intestine of the heavy line of meat chickens compared to light, while Marchini et al. (2011) reported a decrease in the area of the mucous membrane of chickens under the action of heat stress.

The duodenum of the heavy line meat chick embryos compared with the light one had large indicators of the height and area of the villi. At the same time, in other guts of the thin section, such a difference has not been established (Osama et al., 2014). The area of the small intestine mucosa determines the ability of the entire intestine to absorb nutrients (Rougiere et al., 2012; Yamauchi et al., 2000). We found that the thickness of the mucous membrane was 24.9% ($P \leq 0.05$) more in the ileum in young geese of heavy breed. The main structural and functional unit of the mucous membrane of the small intestine is the crypt-villus complex (Maev et al., 2005). In the small intestine of chickens, the height of the villi and their surface area decreases towards the ileum (Marchini et al., 2011).

According to our studies, the Gorkovskaya breed geese had the highest villi height and surface area in the jejunum, and lesser in the duodenum and ileum. In Legart geese, the highest villi height was in the ileum, and the largest surface area was in the jejunum. An increase in the height and width of the villi increases the surface area of absorption of nutrients, increases the growth and productivity of birds (Schaefer et al., 2006).

These indicators are associated with changes in bowel function and can be used to assess villi function (Yamauchi et al., 2000; Maneewan et al., 2004).

In geese of heavy breed, the height and density of villi in the ileum were 49.9% ($P \leq 0.001$) and 23.2% ($P \leq 0.05$), respectively. In the jejunum, the density of the villi was 21.4% less ($P \leq 0.001$). According to Taklimi et al. (2012), the greater height of the villi and the depth of the crypts of the intestines of broiler chickens increased body weight, when using humic acid as a feed additive. Due to Osama et al. (2014), the height and area of the villi of the small intestine of the embryos of meat chickens of the heavy line is greater than that of the light.

A decrease in the live weight of broiler chickens and a corresponding decrease in the depth of crypts and the height of the intestinal villi under the influence of thermal stress was reported by Marchini et al. (2011). At the same time, these indicators of the jejunum and ileum did not differ. According to our data, in comparison with the Gorkovskaya breed bird, the depth of crypts of the jejunum was greater by 12.3% ($P \leq 0.05$) in Legart geese, and in the ileum it did not have a significant difference.

Table 2. Morphometric indicators of geese jejunum (n=5).

Indicators	Breed		
	Gorkovskaya (G)	Legart (L)	L to G, %
Intestine diameter, mm	5.3 ± 0.16	6.0 ± 0.16*	114.2
Villi height, μm	796.7 ± 62.86	786.7 ± 15.63	98.7
Villi density	43.8 ± 1.73	34.5 ± 1.59**	78.6
Area of villi surface, μm^2, × 10^3	106.6 ± 7.02	107.0 ± 6.01	100.4
Crypts depth, μm	308.1 ± 20.75	346.1 ± 11.41*	112.3
Crypts density	333.1 ± 9.83	268.6 ± 6.18***	80.7
Crypts width, μm	28.8 ± 0.88	31.2 ± 2.10	108.5
Villi height to crypts depth	2.6 ± 0.11	2.3 ± 0.09	87.6
Lamina muscularis mucosae thickness, μm	43.7 ± 4.79	56.1 ± 1.48	128.3
Mucous membrane thickness, μm	1161.3 ± 81.58	1207.4 ± 6.90	103.9
Muscle tunic thickness, μm	320.1 ± 25.17	257.3 ± 7.70*	80.4
Intestinal wall thickness, μm	1491.2 ± 128.92	1474.7 ± 114.35	98.9
Number of myenteric ganglia	0.9 ± 0.08	1.5 ± 0.09**	158.7
Number of submucous ganglia	1.6 ± 0.12	1.2 ± 0.08*	77.6
Area of myenteric ganglia, μm^2	2.5 ± 0.21	4.2 ± 0.33**	165.0
Area of submucous ganglia, μm^2	3.2 ± 0.29	2.3 ± 0.19*	70.3
The number of argyrophil cells	35.5 ± 1.15	36.0 ± 5.97	101.2
The number of argentaffin cells	13.2 ± 0.75	12.4 ± 0.81	93.9

*Here and further: * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001.*

Table 3. Morphometric indicators of geese ileum (n=5).

Indicators	Breed		
	Gorkovskaya (G)	Legart (L)	L to G, %
Gut diameter, mm	5.6 ± 0.12	5.9 ± 0.12	105.8
Villi height, μm	536.4 ± 73.07	804.1 ± 21.08**	149.9
Villi density	39.6 ± 2.72	48.7 ± 2.25*	123.2
Area of villi surface, μm^2, × 10^3	72.46 ± 4.70	79.2 ± 4.421	109.3
Crypts depth, μm	331.4 ± 6.22	316.5 ± 13.14	95.5
Crypts density	343.4 ± 6.63	267.0 ± 7.08***	77.8
Crypts width, μm	31.9 ± 1.07	36.2 ± 1.01*	113.5
Villi height to crypts depth	1.6 ± 0.08	2.5 ± 0.17**	157.0
Lamina muscularis mucosae thickness, μm	43.7 ± 4.79	56.1 ± 1.48*	157.8
Mucous membrane thickness, μm	962.0 ± 71.84	1201.4 ± 32.63*	124.9
Muscle tunic thickness, μm	630.3 ± 37.97	537.4 ± 29.60	85.2
Intestinal wall thickness, μm	1601.1 ± 63.04	1748.6 ± 70.37	109.2
Number of myenteric ganglia	0.6 ± 0.04	0.6 ± 0.02	95.1
Number of submucous ganglia	1.4 ± 0.11	1.9 ± 0.18	130.3
Area of myenteric ganglia, μm^2	1.9 ± 0.07	2.2 ± 0.11	116.2
Area of submucous ganglia, μm^2	4.7 ± 0.21	6.4 ± 0.34**	136.9
The number of argyrophil cells	51.1 ± 5.14	34.3 ± 4.38*	63.6
The number of argentaffin cells	28.8 ± 1.92	20.6 ± 3.97	71.7

Thus, an increase in the indices of the studied microstructures in one gut and the absence of a difference in another was observed in heavy breed birds. Higher values of the microscopic parameters of the duodenum: The area of the mucous membrane, the height of the villi, the depth of the crypts and smaller – in other guts of the small intestine in birds with a larger body weight under the influence of a pedigree or some other factor, a number of researchers report (Maneewan et al., 2004; Marchini et all., 2011; Rougiere et al., 2012; Osama et al., 2014). Both the absolute and relative thickness of the muscular tunic in all the studied two intestines of the thin section of the geese of a heavy breed was less, which is consistent with the information of Garcia et al., 2007; de Verdal, 2010; Rougiere et al., 2012, according to which, in the wall of the duodenum, jejunum and ileum of chickens of the light line of meat chickens compared with the heavy, the muscular tunic was significantly thicker, which, in the authors' opinion, compensated for the low functional activity of their stomach. The intestine is the largest endocrine organ in the body (Rehfeld et al., 2012).

The epithelial layer of the mucous membrane of the small intestine contains a large number of different types of endocrine cells of the gastroenteropancreatic (GEP) endocrine system, which is part of the APUD system (Liu et al., 2010). The APUD system is the largest and most complex endocrine organ in the body of vertebrate animals (Sjolund et al., 1983). In addition to the leading role in the regulation of digestion, endocrinocytes of the GEP system – apudocytes are actively involved in maintaining homeostasis of the body, synthesizing more than 100 different hormonally active peptides (Rehfeld, 2012). The composition of argyrophilic apudocytes includes almost the entire population of endocrinocytes of the GEP system, the argentaffin – the largest, which is represented by EC cells. EC cells make up almost half of all apudocytes of the GEP system and are the main producers of endogenous serotonin and extrapineal melatonin (Wang et al., 2017). These cells play a special role in the morphofunctional status of the GEP system, serotonin has a very wide range of biological effects (Mawe et al., 2013).

Compared with the geese of the Gorkovskaya breed, the number of argyrophilic and argentaffin cells in the jejunum, and argentaffin cells in the jejunum did not differ significantly in the Legart bird. At the same time, the number of argyrophilic apudocytes in the ileum was 32.9% less ($P \leq 0.05$). The leading role in the nervous regulation of intestinal functions is played by the enterosympathetic nervous system, which is represented by the mental (muscle sheath) *(plexus myentericus)* and submucosal *(plexus submucosus)* nerve plexuses, which include nerve nodes (ganglia) – accumulations of nerve cell bodies and nerve cords, who connect them (Ali et al., 1978; Yang et al., 2013). The nerve nodes of the plexus of the muscle membrane of the small intestine of the geese are clearly distinguishable between the smooth muscle cells of muscle tissue. They have an oval or rounded shape and are located mainly in the outer layer of the muscle membrane. According to Kuder et al. (2003), the pigeon nervous network is located between the layers of the muscle membrane, and in hens it was in the inner layer of the muscle membrane. The thickness of the duodenal plexus of the duodenum was significantly greater in the pigeon and chicken than in the jejunum and ileum. However, according to Yang et al. (2013), no significant differences between the various segments of the intestine in the number of neurons in the myenteric plexus were found.

In our research, geese of both breeds had the elements of the submucosal plexus on the transverse sections of the intestine in form of narrow, often long strips, which were surrounded by cellular and non-cellular structures of loose fibrous connective tissue; they were mainly between the inner and outer layers of the muscular plate of the mucous membrane.

Along the longitudinal axis of the jejunum and ileum of the geese of both breeds, the number of submucosal ganglia decreased, while the number of myenteric ganglia increased. The general pattern of changes in the average area of the ganglion of the mental nerve plexus along the longitudinal axis of the intestines of geese of both breeds was their decrease, and submucosal increase. At the same time, the number and area of the ganglia of the mental nerve plexus was 58.7 ($P \leq 0.01$) and 65.0% ($P \leq 0.01$) more in in the jejunum of heavy geese, while the submucosa was less by 22.4 ($P \leq 0.05$) and 29.7% ($P \leq 0.05$). In the ileum of geese of different breeds, the number of ganglia of the mental and submucosal plexuses did not significantly differ, however, the area of submucous ganglia in the Legart bird was 36.9% ($P \leq 0.01$) greater.

Conclusion

In geese of a heavier breed, there was a greater absolute intestinal mass, length of the jejunum and ileum, the absolute thickness of the mucous membrane of the duodenum and ileum, and less muscle thickness of the jejunum compared to middle breed. The villi density and crypt depth were greater in the jejnum of Lagert geese, while the height and density of the villi, the width of the crypts and the ratio of the height of the villi to the depth of the crypts were greater in the ileum. In all examined guts of heavier geese, the density of crypts was lower. In the jejunum of the geese of the heavy breed, the number and area of the ganglia of the myenteric plexus were larger and smaller in the submucosa, whereas the area of the ganglia of the submucosal plexus was larger in the ileum. The number of argyrophilic and argentaffin apudocytes in the jejunum of geese of different breeds did not differ, when the Legart breed geese had lesser quantity of apudocytes in the ileum.

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