
Ph.D. THESIS

Morphological and histochemical study of the digestive system in the domestic rabbit (*Oryctolagus cuniculus*)

SUMMARY OF Ph.D. THESIS

Ph.D. Mircea-Florin Cipou

Scientific coordinator **Prof. Dr. Aurel Damian**



INTRODUCTION

The activity of digestion and absorption of food principles is a particularly complex process and requires the participation of a very large number of substances of great diversity. Moreover, if we take into account the specifics of nutrition in different species of animals, then digestion and absorption presents particular aspects related directly to the food consumed by each species of animals and the biochemical processes necessary for their digestion. The large differences between species from this point of view involve the participation of some substances that may be common, but also some that are specific for a species or a group of species.

The digestive system of the wild rabbit is adapted to a very varied range of plant nourishment, which allows it to live in different or even very different habitats. With the domestication of the rabbit, the situation changed in terms of nutrition, at first a little, but gradually rabbit farms appeared, where the use of a very wide range of vegetables in daily nutrition is difficult and totally unprofitable from a practical point of view.

For increasing the efficiency, including through significantly reduce the number of people needed in maintenance of the farm, rabbit feeding has gradually passed to granulated feed, which is very easy to administer and keep for a certain period of time, if the storage conditions are respected. The use of pelleted feedstuff simplifies many things in the sense of reducing food diversity and even canceling the differences between summer and winter nutrition. From the wild rabbit nutrition with a wide variety of nourishments to restricting the feedstuff to just one, namely pellets, is a very big difference.

The domestic rabbit raised in farms has adapted to this unique feed, relatively easily and without major complications, as long as the production technology is respected. The question arises if the digestive system has adapted to the new conditions of growth and feeding only by operating within large physiological limits, or in the case of some of its organs certain changes have appeared in response to the new demands. The appearance of major adaptive structures can be noticed through anatomical examination. But if it's about changes in some components of the organs and not in the entire organ, these changes cannot be noticed just through an anatomic examination. If in the domestic rabbit such changes have appeared or not can be verified just through a detailed histological and histochemical assessment.

Beside those mentioned, the incidence of rabbits in veterinary practices is constantly increasing. Rabbits are sometimes raised as pets, being loved especially by children. From this point of view, rabbits have an emotional significance that determines their owners to make serious investments of their pets well-being.

THESIS STRUCTURE

The Ph.D. thesis entitled "Morphological and histochemical study of the digestive system in the domestic rabbit" extends on 155 pages and contains a relevant iconography of 125 figures. It is structured in two parts and has been developed according to the methodologies for writing Ph.D. theses of IOSUD Cluj-Napoca.

The first part of the thesis, current state of knowledge, contains 32 pages and is structured in three chapters.

Chapter I, entitled „*Embryonic development of the digestive system*” contains informations about the embryonic development of the digestive system.

Chapter II, entitled „*The digestive system* ” contains informations about the anatomy and topography of the digestive system in the domestic rabbit.

Chapter III, entitled „*Digestion physiology in the domestic* ” comprehends informations about the process of digestion at different levels of the digestive system in relation with the implied anatomical structures.

The second part, personal contribution, extends on 97 pages, structured in 7 chapter and covers notions about the working hypothesis, objectives, materials and methods, the investigations of macroscopic, microscopic and histochemical morphology of the digestive system in domestic rabbits. This part of the manuscript ends with the general conclusions; aspects of originality and the innovative contributions of the thesis.

THE MANUSCRIPT OBJECTIVES

-the anatomic study of the domestic rabbits digestive system for highlighting eventual peculiar anatomical aspects

-the microscopic study of the digestive system organs in the domestic rabbits for highlighting possible histological adaptive structures appeared after nourishment changes;

-the histochemical study mucin secreting cells from the entire digestive system, for assessing the type of mucin secreted by each glandular cell.

MATERIALS AND METHODS

Chapter V entitled „*Materials și methods*” contains information about the biologic material, chemical materials, other types of materials, devices and the research protocol. The research was realised on the digestive system of 10 adult domestic rabbits, clinically healthy and slaughtered for domestic meat production.

RESULTS AND DISCUSSIONS

Chapter VI entitled „*Macroscopic study of the digestive system in the domestic rabbit*“ contains anatomic investigations realized with the aim of surprising possible particular aspects of the digestive system components, with reference to the dimensions, shape, aspect and reports with the proximal structures. In order to achieve this aim, we proposed the following objectives:

-highlighting possible new characteristics of the digestive system in the domestic rabbit

-describing and establishing the topographic relationships of the components of the digestive system

-morphometric investigations of constituent organs and their components.

Following the anatomical investigations of the digestive system in the domestic rabbits we achieved the following conclusions:

The arrangement of the upper and lower incisors is peculiar to other domestic mammals and then other experience animals, their occlusal surface enabling the cutting of fibrous forage. The average clinical crown length of upper primary incisors under normal conditions is on average 8.5 mm.

The tongue in the adult rabbit had an average length of 54,16 mm, a weight of 6.29 g, and the dorsal surface of the tongue has a whitish lingual torus. The body of the tongue has the largest share in the total length of the organ.

The esophagus is a musculo-connective duct of which the thoracic segment is the longest, followed by the cervical segment and then the abdominal segment.

The stomach is simple, undivided with a large volume compared to the animal's waist. The mean volume of the stomach is 170 ml. At the level of its lumen in the domestic rabbit, margo plicatus is not present.

The intestine in the rabbit is highly developed reported to the size of the animal, being adapted to retain high quantities of digestive content at this level, it's volume being minimum 58% of the total volume of the digestive system.

Between the loops of the duodenum, the descending loop has the longest length, being in close contact with the descending colon. This is followed by the ascending loop of the duodenum. The length at the level of the caudal flexure is not apparent *in situ* due to the fact that it presents numerous flexuosity's.

The jejunum is the longest segment of the small intestine in the rabbit, with a mean length of 207 cm.

The ileo-ceco-colic complex is a morphofunctional unit, which is not found in other species of domestic animals and is specific to the rabbit.

The papillary process could be considered a distinct lobe of the liver in the rabbit, being attached in the upper part directly at the level of the hepatic hilum. Appearance similar to the presence of the Spiegel lobe in humans. The gallbladder has an average length of 2.66 cm.

Chapter VII entitled „**Microscopic study of the digestive system in the domestic rabbit**” aims to obtain microscopic details on the structure of the digestive organs in domestic rabbits, for completing with the obtained informations the actual existing data in the speciality literature. In order to achieve this goal, we have set ourselves the following objectives:

-verification of the histological structure of all the organs of the digestive system of the domestic rabbit raised with single granulated feed;

-identification of the possible structural changes that occurred at the level of some organs following the effort of adaptations to habitat and forage conditions very different from those in a state of freedom;

-bringing possible new information regarding the microscopic features of the organs of the digestive system in the rabbit and their detailed description.

Following the histological investigations on the digestive system in the domestic rabbit, the following conclusions were drawn:

The filiform papillae on the rabbit tongue are short cylindrical and tall cylindrical on the tip of the tongue, tall conical on the body and short conical on the root, their thickness increases progressively anteroposteriorly, and the highest density is on the tip of the tongue, then on the tongue's body and the smallest on the root.

The oesophagus mucosa presents a stratified squamous type epithelium with a discreet tendency of keratinization and the muscularis of the mucosa consists of longitudinal smooth muscle bundles, which gradually increase in number, being more numerous in the abdominal segment.

The muscularis externa of the oesophagus consists of striated muscle cells arranged in three layers, internal longitudinal, middle circular and external longitudinal, the external layer is thinner in the first two segments, but after they are of a comparable thickness in the abdominal segment.

The total thickness of the oesophageal wall differs from one segment to another, being the largest in the abdominal segment, especially due to the thickest muscle here, an aspect that seems to help the progression of the food bolus on the last portion of the oesophagus.

The cardia region is highly developed in the rabbit, the gastric crypts being less deep in the first portion, gradually growing after reaching a two times bigger depth and even three times further, so that in the last portion to gradually decrease so that in the transition area to the fundic region so become evidently/visibly less deep.

Muscularis mucosae is present but has a peculiar disposition, in the sense that it's disposed at a significant distance from the epithelium, crypts and even glands, made from a single layer of smooth muscle cells disposed rarefied and the majority oriented in the same direction.

Glands are relatively well represented in the cardia region of the rabbit stomach, but are significantly less than in the fundic region and are not disposed to the muscularis mucosae as in other regions, such that the deeper half of the lamina propria doesn't contain glands.

Cardiac glands contain a single cell type just in the immediate proximity of the oesophago-gastric junction, otherwise the cardiac glands are polymorphic and contain more cells types in a different ratio in order with the area.

As a general characteristic, the gastric mucosa from the cardia gastric region of the rabbit, being very thick and loose, which eases the passage of the feedstuff from the oesophagus in the stomach and also adaptation to volume variations.

In the fundic region the gastric crypts are less deep than in the cardia region and the number of gastric glands is very high so that by their number, density and length it occupies the whole space between the bottom of the crypts and the muscularis mucosae.

The gastric mucosa from the pyloric region has a pleated appearance, the crypts here are significantly deeper than in the fundic region and also much wider, resembling in a some measure the crypts from the cardia.

In the duodenal ampulla the Brunner glands are predominant, occupying a large zone that implies a small part from the mucosa and most of the tela submucosa and appear made up by mucous cells which are evidently predominant and in a smaller degree serous cells.

Brunner's glands from the propper duodenum in rabbits, are totaly disposed in the tela submucosa and appear made up mainly by large cells and aspect of mucin secreing cells and smaller cells with aspect of serous cells.

Lieberkühn's glands from the jejunum are relatively rare, of medium length and from a structural point of view contains two types of glandular cells, enterocytes which occupy the superior 2/3 of the glands and some with aspect of DCS cells, disposed in the deep portion of the glands.

The situation is comparable in the ileum refering the length of the Lieberkühn glands, density and even the presence of DCS cells in the deeper part of the glands, but changes totally in the ileum ampulla where there are present numerous lymphoid structures.

The cecum in the domestic rabbit is highly developed, presents a thick mucosa which in the first two parts presents intestinal villi comparable in a large measure with the ones present in the small intestine, but not in the cecal appendix which has a typical lymphoid structure.

In the colon, the mucosa is relatively thick and presents glands of which in length are over the glands from the jejunum and ileum, and the glands density is the lowest in the ascending colon, visibly increase after in the tranverse colon and mentain high density in the descending colon.

Chapter 8 entitled „**Histochemical study of the digestive tract in the domestic rabbit (*Oryctolagus cuniculus*)**” aimed to identify mucin secreting cells existing in the structure of the organs which made up the digestive system. First of all, it was followed the presence of two types of mucins, respectively neutral and acid. The presence mucins secreting cells from each organ was followed regarding density, distribution and also the type of mucins secreted by each glandular cells. In order to achieve this aim, we had the following objectives:

-quantification of the presence and distribution of the mucin secreting cells from the walls of the digestive's tract cavitory organs;

-quantification of the mucin type/types secreted by each glandular cell from the walls of the digestive's tract cavitory organs.

After the histochemical investigations of the digestive tract in the domestic rabbits, the next conclusions have been drawn:

Cells of the epithelium which covers the gastric mucosa and crypts are intense PAS positive but alcian negative, which proves that these cells secrete important quantities of neutral mucins but no acid mucins.

A part of the gland's cells from the cardia region are discreet PAS and alcian blue positive, the glands from the fundic region are negative on both histochemical reactions and the ones from the pyloric region most are PAS positive and alcian negative.

The overall assessment of the behaviour of the gastric mucosa's cells in the domestic rabbit reported to the histochemical reactions, proves that the gastric mucosa synthesises in the studied species large quantities of mucins, from which the neutral ones are predominant, and the acid ones are in very small quantities.

In the duodenum, the goblet cells are intense PAS positive and intense alcian blue positive, whereas the Brunner's gland cells are positive of moderate intensity on PAS reaction and intense positive on the alcian blue coloration.

As the synthesis of neutral and acid mucins is comparable at the level of goblet cells and the Brunner's glands cells the synthesis of acid mucins is higher than the neutral ones we can state that in the duodenum are synthesised large quantities of mucins from which the acid are predominant.

In the jejunum the only intense positive cells on PAS reaction and also on the alcian blue coloration are the goblet cells, present both in the villi epithelium, intestinal crypts and in the walls of Lieberkühn's glands, more precisely in the superior half.

In the deeper half of Lieberkühn's glands from the rabbit jejunum exists particular cells, morphologically similar to DCS cells, just that these cells are negative on both histochemical reactions, which proves that these cells don't secrete mucins.

In the ileum, the situation resembles the one of the jejunum in the sense that goblet cells from the wily epithelium and from the Lieberkühn's glands are both PAS and alcian blue positive whereas the DCS cells from the deeper half of the glands are negative on the two histochemical reactions.

At the level of the cecum, the single positive cells on the two histochemical reactions are the goblet cells from the villi epithelium and from the walls of Lieberkühn's glands from the three regions of the cecum, respectively base, body and appendix.

Goblet cells are present in all the three segment of the colon, disposed both in the surface epithelium as in the glandular one, being positive on both histochemical reactions, but the intensity is weakly on the PAS reaction as on the alcian blue stain.

Peculiar for the colon is the fact that in the walls of the glands there exists two types of mucin secreting cells, disposed interleaved, some positive on the PAS reaction

and others on the alcian blue stain, but the one with acid secretion outnumbers the ones with neutral secretion.

As the goblet cells secrete both neutral mucins and acid mucins at a comparable level and the other cells involved in the secretion of mucins synthesize higher loads of acid mucins than neutral, we can state that the total secretion of mucopolysaccharides in the rabbit's colon is predominantly acid.

Chapter 9 entitled „**General conclusions**“ synthesizes the aspects highlighted after the anatomical, histological and histochemical investigations, as it follows:

The lingual papillae have a distribution and proportion characteristic for each segment of the tongue to which we refer.

The esophagus has the longest portion in the thoracic segment, then the cervical segment and after in the abdominal segment.

The stomach is simple, non-compartmentalized with a relative large volume reported to the animal's size, in the domestic rabbit it does not exist at the level of the lumen, *margo plicatus*.

The liver in rabbits, present a papillary process which can be considered a distinct lobe, fixed directly at the level of the hilus, in the dorsal part-a comparable aspect with the Spiegelian lobe in humans, and the gallbladder has a mean length of 2,66 cm.

The descending segment of the duodenum is the longest, followed by the ascending segment and the caudal flexure which presents numerous flexuosities, but the jejunum is the longest segment of the small intestine with a mean length of 207 cm.

The ileo-ceco-colic complex is a morphofunctional unit which is not present in other domestic animals, and the liver presents 5 lobes and contains a papillary process which can be considered a distinct lobe and a gallbladder with a mean length of 2,66 cm.

The cardia region of the stomach is very particular, presents a very thick mucosa and a thick and loose chorion, a muscularis mucosae that is at distance from the bottom of the glands and a ill-defined disposition, these overall assuring the adaptation to large volume variations.

In the fundic region of the stomach the crypts are less deep, the number of gastric glands very high whereas in the pyloric region the mucosa has a pleated appearance and the crypts are significantly deeper and larger than in the fundic region.

In the duodenum the Brunner glands are very well represented, disposed in the mucosa and submucosa in the duodenal ampulla, but just in the submucosa in the proper duodenum, and as for their structure it contains mucous cells which are visibly predominant and a small number of serous cells.

The Lieberkühn glands from the jejunum and ileum are relatively rare, of medium length, being made up of enterocytes, goblet cells and cells morphologically similar to DCS cells, arranged in the deep portion of the glands, and in the ileal ampulla there are also numerous lymphoid structures.

The cecum is highly developed, with a thick mucosa provided in the first two segments with villi comparable to those in the small intestine, but not at the level of the cecal appendix which has a typical lymphoid structure.

The mucosa of the colon is relatively thick and has glands whose length exceeds the one of the glands in the jejunum and ileum, and their density is the lowest in the ascending colon, then increases visibly in the transverse colon and remains at a high density in the descending colon.

The two histochemical reactions have proved that the cells from the gastric mucosa synthesizes very large quantities of mucins, and as quantitative representation the neutral ones are visibly predominant and the acid ones are in very small quantities, thus the total secretion is predominantly neutral.

At the level of the duodenum, the goblet cells are intense PAS positive and intense alcian positive, whereas the cells of Brunner's glands are faintly positive and intensely positive on the alcian blue stain, thus that in the duodenum the secretion is predominantly acid.

In the jejunum and ileum, the only positive cells both on the PAS reaction as on the alcian blue stain are the goblet cells, and the intensity of the secretion is comparable, thus we can say that the secretion of neutral mucins is comparable with that of the acid mucins.

The goblet cells from the three segments of the colon, are positive on both histochemical reaction but the intensity is weak on the PAS reaction and on the alcian blue stain, and the other cells the majority secrete acid mucins.

Taking into consideration that the goblet cells secrete both neutral and acid mucins at a approximate level and the other cells involved in the secretion of mucins synthesize higher quantities of acid mucins than of neutral, it results that the overall mucin secretion in the colon is predominantly acid.

Chapter 10 entitled „**Originality and innovative contribution of the thesis**“ points the most important aspects seen in the research made in this thesis, as it follows:

The macroscopical studies made, complete the existing informations from the speciality literature.

From the suggestive aspect we remember:

The body of the tongue has the largest proportion in the total length of the organ;

The stomach of the domestic rabbit doesn't present *margo plicatus*;

The rabbit's intestine is complex and very developed reported to the animals size

The ileo-ceco-colic complex is a morphofunctional unit specific for the rabbit

The papillary process can be considered a distinct lobe of the liver

The histological studies have revealed the following particular aspects:

The tongue filiform papillae differ in density, dimensions and keratinization level in order with the parts of the tongue;

The cardia region of the stomach is well developed and with a particular structure which allows the zone to volume variations;

The duodenal Brunner glands are well developed, numerous and also present in the first part of the jejunum.

In the cecum there are villi comparable to those in the small intestine;

The histochemical studies have highlighted the following peculiar aspects:

In the stomach the neutral mucins are predominant and the acid ones are in very small quantities;

In the duodenum are synthesised large quantities of mucins and the acid are predominant;

Goblet cells secrete from the intestine secrete comparable quantities of neutral and respective acid mucins.

Goblet cells from the colon have a reduced secretory activity than those in the small intestine;

In the colon beside the goblet cells there are other types of mucin secreting cells, represented by glandular mucous cells with different staining properties. The overall secretion of mucins at the level of the colon is predominant acid.