academicJournals

Vol. 5(4), pp. 99-106 April 2013
DOI 10.5897/JVMAH12.036

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Histomorphology of the digestive system of red deer (*Cervus elaphus* L.) in Latvia

Inese Zitare[1]*, Mara Pilmane[2] and Aleksandrs Jemeljanovs[1]

[1]Research Institute of Biotechnology and Veterinary Medicine Sigra, Latvia University of Agriculture, Sigulda, Institute Street 1, LV 2150, Latvia.
[2]Institute of Anatomy and Anthropology, Riga Stradins University, Dzirciema Street 16, Riga, LV 1007, Latvia.

Accepted 25 March, 2013

The present study described the histo-morphological and immuno-histochemical characteristics of the digestive and neuroendocrine systems of captive wild domesticated red deer (*Cervus elaphus*) in Latvia. Results revealed that four (4) out of the five (5) animals studied showed keratinization of the rumen mucosa, with patchy loci of parakeratotic basal cells in the epithelium of the rumen wall. Neutrophilic leukocytes, lymphocytes and rare macrophages had infiltrated the mucosa and muscle layers of the rumen wall. Vacuolization of gangliocytes was seen in the intermuscular nerve plexus. It was observed that red deer's small and large intestines were more innerved than the rumen, despite the variations in some parts of the enveloped mucosa. Small intestines were characterized by abundant expression of serotonin. Focal appearance of neurofilaments (NF)-containing nerve fibers was characteristic of the tissues of the rumen in the direction of the large intestine. Prominent apoptosis was also seen in the rumen. Moderate hepatocyte activity, a small number of apoptotic cells in the *vena centralis* area, with simultaneously distinct expression of interleukins and limited expression of degeneration enzymes was observed in the liver of the animals studied.

Key words: Diffuse neuroendocrine system, digestive tract, red deer, morphology, Latvia.

INTRODUCTION

The digestive tract is an important system in living organisms, and plays a vital role in food processing and absorption (Hill et al., 2008). In food animals the digestive process ensures safety and healthiness of the animal product consumed by humans. Investigation of digestive tract of deer is related to health control issues for the protection of their gene pool in Latvia (Skriba, 2011).

Red deer is a ruminant belonging to herbivorous animals which prefer leguminous food: bushes, hard grass, weeds and tree scions as well as grass and fodder (Fulbright and Ortega, 2006). Fulbright and Ortega (2006) have classified deer as "intertype" animals focusing on leguminous food unlike other herbivorous animals, choosing only herbs or solely grass. Deer eats legumes, as well as hard to digest plants and their parts containing starch, protein and fat rather than grass. It is able to digest cellulose in plant cell walls, although this ability is restricted.

Feed processing starts in the rumen (the largest structure in the digestive tract of deer) with consecutive passage through the small and large intestines. The process in the whole digestive tract is closely connected with the functioning of the diffuse neuro-endocrine system (DNES), described by Ceccarelli et al. (1995) in fallow deer *(Dama dama)* and Franco et al. (2004) as well as Masot et al. (2007) in red deer *(Cervus elaphus)*. Mallard et al. (1998) and Zabielski et al. (1998) suggested impact of neuropeptide-containing

*Corresponding author. E-mail: sigra@lis.lv. Tel +34167976307.

innervations on the intrauterine development of the digestive system, breaking down and absorption of the nutrients, contractions of the walls of hollow organs of the digestive system, as well as local immune responses. Bueno and Fioramonti (1994) found correlations between the changes of neuro-peptide innervations and metabolic disorders of the body. Although, Münnich et al. (2008) have studied the role of DNES in the digestive tract of based on feed selection, there is scarcity of information on the morpho-functional structure of the digestive tract of wild red deer under captivity in Latvia.

Wholesome investigations of the deer digestive tract require concurrent studies of the homeostasis of the liver cells in this animal, as liver is the biochemical laboratory of the body where various processes such as essential metabolic functions, protein synthesis, detoxification of the body, bile secretion, etc occur (Barrett, 2011). Function of the digestive tract in the body is closely related to function of the liver: the absorbed nutrients are carried to the liver where they are processed and stored (Barrett, 2011). According to Junqueira and Carneiro (2003), liver is a coordinator between the digestive tract and blood. Histological studies by Soveri (1993) revealed that in winter, when a deer under natural circumstances is forcefully starved, the parenchyma of its liver changes due to reduction in the size of the liver cells and sinusoidal cavities. According to Knolle and Gerken (2000) and Kmieč (2001), mutual functioning of separate cells of the liver tissues is strictly connected in both healthy and sick deer.

Previous histological studies in red deer (Hartwig and Hartwig, 1975; Hals and Cato Olsen, 1984; Soveri, 1993; Shabadash and Zelikina, 2003), revealed scanty or no data on morpho-functional structures of the digestive system of wild captive species of in Latvia. Studies are needed in order to justify conditions of captivity to comply with the physiological requirements of the bodies of red deer, ensuring their well-being and high quality of the acquired meat. The main goal of our study was to carry out histological and immunohistochemical (IHC) investigations of the morphological structures and neuroendocrine system of the digestive system of captive red deer (*C. elaphus* L.).

MATERIALS AND METHODS

Animals and tissue preparation

The study included five clinically healthy 18 months old red deer (*C. elaphus* L.) farmed in Sigulda municipality of Latvia, in captivity. Each of the animals over the winter period had received daily feed ration of 7 kg of haylage and kg of rolled grain; containing 2.63 kg of dry matter and 121 g (per kg of dry matter) crude protein. The amounts were estimated by means of "RationPro MVP" (USA) software on the bases of the feeding-stuff analysis. Additionally, animals fed themselves on bushes growing within their fence as supplements.

Animals were slaughtered at the end of the experiment, and 0.5-1 × 1 cm tissue samples were taken from the back part of *saccus caecus ventralis* of rumen, the medium sections of *duodenum* and *colon* and from the liver. Rumen and intestinal samples were rinsed with warm 0.9% NaCl solution. Subsequently, all the tissue samples were placed in 10% neutral formalin at room temperature for 48 h and then dehydrated in tissue processor (TISSUE-TEKII) and embedded in paraffin blocks. Tissues were processed according to standard procedures (Kiernan, 2008; Carson and Hladik, 2009).

For general histological assessment, the tissue samples were de-paraffinized with xylene and ethyl alcohol, and then stained as described by (Carson and Hladik, 2009).

Immunohistochemistry

Samples for immunohistochemical (IHC) investigation were fixed for twenty-four hours in a mixture consisting of 2% formaldehyde, 0.2% picric acid and 0.1 M phosphate buffer (pH 7.2) (Aughey and Frye, 2001). Then the pieces of tissues were rinsed for 12 h in a thyroid buffer containing 10% sucrose, embedded in paraffin and cut with a microtome in 8 µm thick layers. Prior to immune-staining, sections were de-paraffinized and rehydrated. Sections were processed in a microwave for 20 min in 4% citrate buffer (pH 10), quenched for 10 min with 3% H_2O_2 to block endogenous peroxidase activity, rinsed in a phosphate–buffered saline (pH 7.4), pretreated with a non-immune goat serum for 10 min to block the nonspecific antibody binding and then incubated for 2 h with the primary antibodies (Hsu et al, 1981).

The primary antibodies used in the IHC investigation of gastrointestinal samples were protein gene peptide 9.5 (PGP 9.5) (rabbit polyclonal, working dilution 1:600, code Z5116, DACO, Denmark), serotonin (mouse monoclonal, code M758, working dilution 1:10, DACO, Denmark), neuro-filaments (NF) (mouse monoclonal, code M0762, working dilution 1:160, *Euro-Diagnostika*, Denmark),neuro-peptide Y (NPY) (rabbit polyclonal, code B48-100, working dilution 1:100, Peninsula, Sweden).

Expression of hepatocyte growth factor (HGF) in the liver samples was evaluated by means of HGF antibody (mouse monoclonal, code AF294NA, working dilution 1:300, R&D Systems, Germany). Expression of interleukin 6 (IL-6, mouse monoclonal, code NYRhIL6, working dilution 1: 50, Santa Cruz Biotechnology,, USA) and interleukin 10 (IL-10), rabbit polyclonal, code ab34843, working dilution 1: 400,Abcam, UK) and matrix metalloprotease 13 (MMP13),*Escherichia coli*, code sc 81547, working dilution 1:100, Santa Cruz, USA) were also studied in the liver cells.

Immune reaction was visualized by the avidin-biotin immune-peroxidase method using an LSAB kit (Dako Cytomation, DK) and diaminobenzidine (DAB) solution (Dako Cytomation, DK) was used as chromogen, and hematoxylin was used as the counter-stain.

Apoptosis was detected by the means of TUNEL assay, described by Negoescu et al. (1998) in all the tissue samples from the gastrointestinal tract. *In situ* Cell Death Detection kitPOD (1684817, Roche Diagnostics) and Vector DAB Peroxidase substrate kit were used to perform the TUNEL assay according to the manufacturers' instructions, briefly: deparaffinized sections (xylol 2×4 min, 99% ethanol 2×2 min, 95% ethanol 2×2 min and 70% ethanol 2×2 min) were rinsed with water for 7 to 10 min and transferred to PBS (pH 7.5) for 10 min. Subsequently slides were placed into 50 ml PBS solution with 500 µl 30% hydrogen peroxide for 30 min on shaker to block the endogenous peroxidases. Afterwards, tissue samples were washed with PBS (3×5min), placed into 0.2 M boric acid (pH 7.0), and then placed in microwave (700 W) for 10 min to fix antigen. Content was cooled to room temperature and rinsed with PBS. Subsequently, slides were kept in refrigerator in 0.1% bovine serum albumin (BSA) solution with PBS for 19 min and incubated in TUNEL mix (Tdt – mix of terminal deoxynucleotide transferase and DIG-ladeled deoxynucleotide) for 1 h at 37°C. Then the slides were rinsed with PBS 1:10 and incubated for 30 min at 37°C with POD (anti-fluorescein antibody,

Fab fragment from sheep, conjugated with horse-radish peroxidase). Then the slides were washed with PBS, covered with DAB (diaminobenzidine chromogen) for 7 min, and then rinsed with running water for 5 min. Finally, haematoxylin and eosin staining was performed on each sample. Sections were covered with a polystyrene-based medium and coverslip.

Samples were viewed by Leica DM 5000B microscope (×250 and ×400), and pictures were obtained by Leica DC 300F digital camera using software *Image Pro Plus* version 6.3.0.512 for Windows XP Vista, serial number 41N63000-58567 (Media Cybernetics, USA).

Statistical analysis

The mean values and standard deviations were determined statistically using statistical package SPSS 17.0. Pearson correlation test was applied for determination of the correlation among parameters (Arhipova and Balina, 2006).

Wilcoxon's T-criterion test was applied for interdependent groups of samples and statistically significant differences were evaluated at $p<0.05$ (Sheskin, 2011). Quantitative method was applied to count HGF positive structures and apoptotic cells within three freely chosen visual fields around the central vein of the liver and portal tract area at magnification ×400. The expression of cytokines was determined by simplified qualitative counting method, and presence or amount of positive immune structures were evaluated as low (+), medium (++) or high (+++) using a method described by Pilmane et al. (1998).

RESULTS

Examination of the sampled tissue sections under light microscope revealed characteristic keratinization of the *fundus* of rumen mucosa in four of the five animals studied, clear distinctions in two cases. Also some areas of the tissue samples from the rumen wall revealed more or less distinct basal cell hyperplasia of para-keratotic epithelium (Figure 1). Infiltration of patchy inflammatory cells in mucosa and muscular layer was represented by neutrophilic leukocytes, lymphocytes and rare macrophages. Vacuolization of gangliocytes was observed in some areas of the inter-muscular nerve plexus. Changes in the small intestines of four animals were relatively similar: walls, especially mucosa, were infiltrated by high or medium-high number of inflammatory cells. Inflammatory cells were represented by lymphocytes, neutrophilic and eosinophilic leukocytes, macrophages and plasmocytes. In two cases, proliferating connective tissue were detected in the small intestinal villi. Inflammation of the mucosa of the large intestine was prominent in two cases (Figure 2) and of small intestines - in one case. One of the animals had evident atrophy of mucosa in some areas of the large intestine.

The present study showed that the small and large intestines of red deer (*C. elaphus* L.) bred in Latvia was more innervated than the rumen although in some areas mucosal envelope presented variations.

General marker PGP 9.5 for the DNES indicated nerve fibers around arteries; the largest number of peptide-containing nerve fibers and gangliocytes was found in sub-mucosa and inter-muscular nerve plexus of the small and large intestinal walls (Table 1). Wilcoxon's T-criterion test for interdependent groups of samples did not reveal statistically significant differences ($p>0.05$) between the expression of PGP 9.5 in the mucosa of rumen, small and large intestines. Correlation was also insignificant ($p>0.05$) between the expression of PGP 9.5 in the sub-mucosa and the muscle layers of rumen as well as small and large intestines.

A few patchy loci of NPY containing nerve fibers were found in the deer's rumen and the small intestinal wall muscle layer. Some NPY-containing fibers were found in the muscle layer of the wall of large intestines. The insignificant expression of NPY confirmed the minor role of this neuro-peptide on the functions of digestive system of deer. NF-containing nerve fibers were widely found in the basal and muscle layers of the rumen and the small intestines and in all of the wall layers of the large intestine, though they were patchy and fragmentary in some areas. A high amount of serotonin-containing neuroendocrine cells were found in the epithelium of the small intestine while medium-high amount of those cells was found in the epithelium of the large intestine (Figure 3); no cells of this type were found in the epithelium of the rumen. Index of apoptosis was higher in the rumen mucosa (0.17), but was lower in the mucosa of the small intestines and that of the large intestine (Figure 4, Table 2).

Wilcoxon's T-criterion test for interdependent groups of samples revealed significantly higher level of apoptosis in the rumen mucosa comparing to mucosa of the small intestine ($p<0.05$ or $T=0 < T_{0.05;\ 15}=30$). The difference between the levels of apoptosis in the mucosa of the small and large intestine was insignificant ($p>0.05$ or $T=30 > T_{0.05;\ 15}=25$).

Histological investigations of the liver samples of deer revealed well-formed liver structure: a liver lobe with *V. centralis* and portal tracts. Inflammation with lymphocytes was detected in the walls of the blood vessels of portal tracts. Expression of HGF was more distinct in the cytoplasm of the hepatocytes of the portal tracts and less – around *V. centralis.*

As shown in Table 3, 34.4 ± 17.47 HGF positive hepatocytes were found on average in 3 visual fields near the acinus. The average number of apoptotic cells in the portal tract was 5.2 ± 1.09, apoptotic index – from 0.04 to 0.07, in *V. centralis* area - 13.2 ± 2.59, apoptotic index: 0.1 to 0.2. Distinct expression of IL-6 was detected around the blood vessels and bile ducts. IL-10 positive cells were found in the whole liver parenchyma. Weak expression of MMP13 was observed around the blood vessels.

DISCUSSION

Animals included in the study were given 7 kg of haylage and 1 kg of rolled grain during the winter period thus

Table 1. Relative distribution of protein gene peptide 9.5 in separate parts of deer's digestive tract.

Animal	Tissue layer	Rumen	Small intestine	Large intestine
	Mucosa	++	++	+
1.	Submucosa	++	++++	++++
	Muscle layer	++	++	++++
	Mucosa	++	++	+++
2.	Submucosa	++	++++	+++
	Muscle layer	++	+++	+++
	Mucosa	+/-	++++	++++
3.	Submucosa	++	++++	++++
	Muscle layer	+/-	++++	++++
	Mucosa	+/-	++	+
4.	Submucosa	+	++	++
	Muscle layer	+/-	++	+++
	Mucosa	+	+++	++
5.	Submucosa	+	++++	+++
	Muscle layer	+	++++	++++

+/- Rare positive structures in visual field; + few containing structures in visual field; ++ moderate number containing structures in visual field; +++ numerous containing structures in visual field; ++++ very high number of containing structures in visual field.

Table 2. Number of apoptotic cells in digestive tract wall of red deer.

Parts of red deer's digestive tract in mucosa	Average number of positive cells from 100 cells in visual field and standard deviation	Index of apoptosis
Rumen	14.7±3.06	0.17
Small intestine	8.7±2.52	0.10
Large intestine	4.7±2.52	0.05

Table 3. Results of immunohistochemical investigationsof red deer (*Cervus elaphus* L.) liver.

Animals	HGF positive cells	Portal tract		*V. centralis* area		Interleukin expression		MMP13 expression
		Number of apoptic cells	Apoptic index	Number of apoptic cells	Apoptic index	IL-6	IL-10	
1.	20	5	0.05	10	0.1	+++	+++	+
2.	29	5	0.05	13	0.15	+++	+++	+
3	35	7	0.07	12	0.14	+++	+++	+
4.	64	5	0.05	17	0.20	++/+++	+++	+
5.	24	4	0.04	14	0.16	++/+++	+++	+
Average	34.4±17.47	5.2±1.09		13.2±2.59				

Positive immune cells are marked by: (+) – few, (++) – moderate, and (+++) – high number of positive cells.

providing 2.63 kg of dry matter and 318.2 g of crude protein as well as sufficient ration of energy (46.7 MJ ME per day). Our findings agree with the study of Josefsen (1997), which reported that changes in the mucosa of

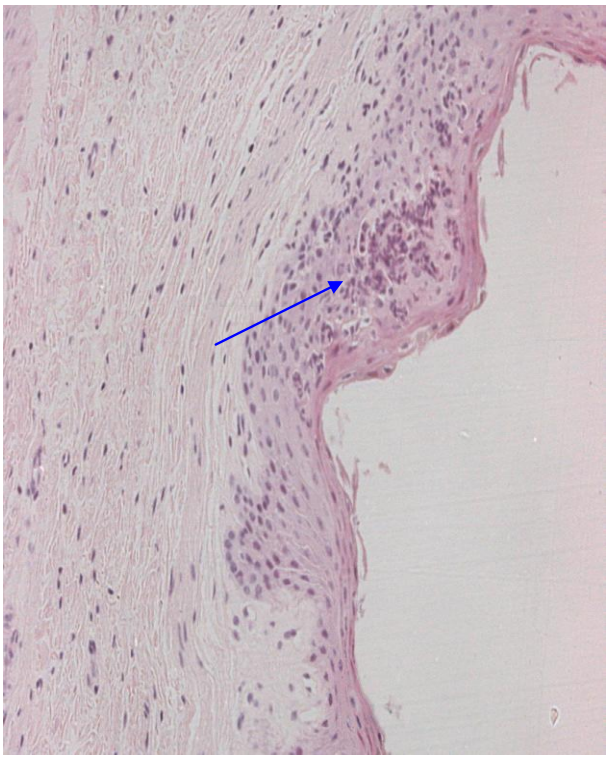

Figure 1. Keratinization of ruminal mucosa (H&E, magnification ×200).

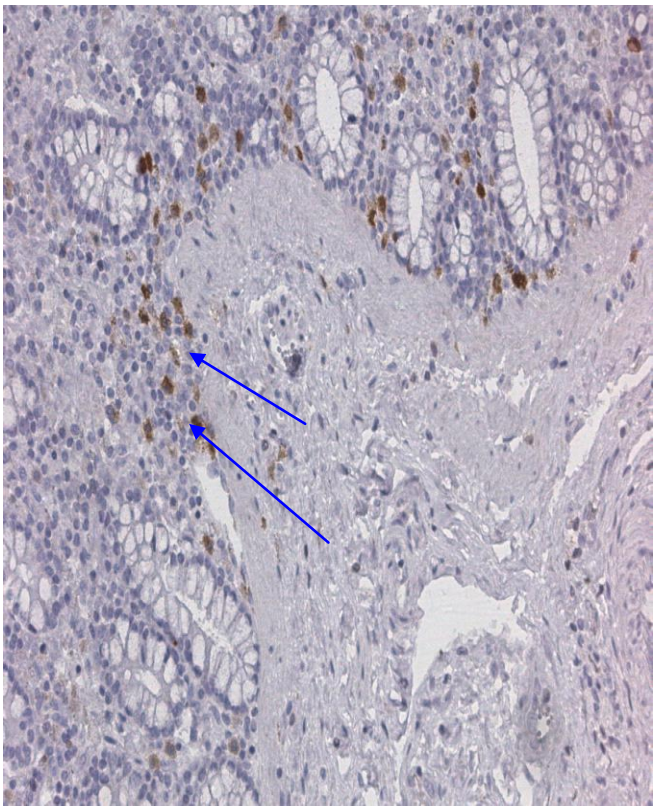

Figure 3. Serotonin expression in colon wall (IHC, magnification ×250).

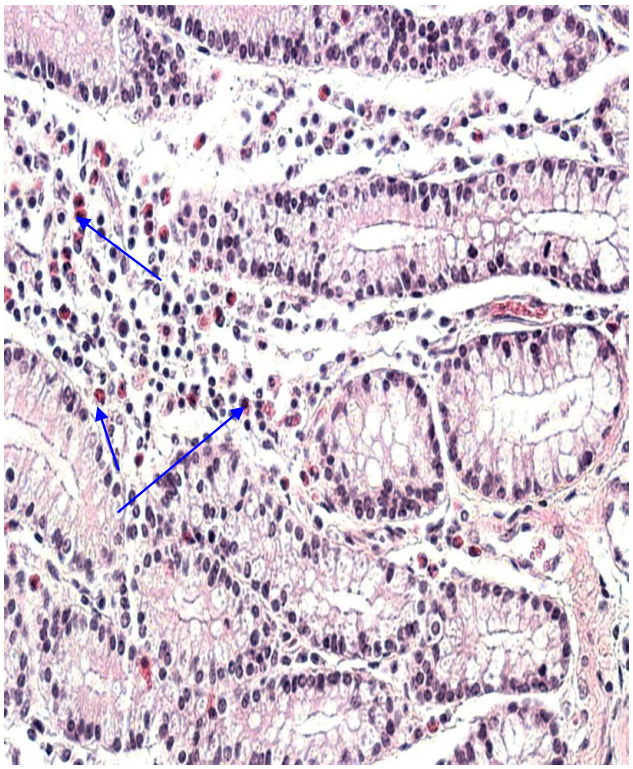

Figure 2. Inflammatory process in large intestines wall (H&E, magnification ×400).

digestive tract of deer (inflammation, para-keratosis and atrophy) may be caused by the influence of local solid forage particles. Atrophy of the mucosal villi was observed in the digestive tract of one of the deer in our study because the deer preferred grazing on bushes. Atrophy of the mucosal villi, found in the digestive tract of deer, according to Mallard et al. (1998), is related to the changes of the local immune system and the DNES. Műnnich et al. (2008) have investigated innervations of the DNES in the rumen wall of fallow deer and found that the rumen of these animals is less controlled in terms of cholin-ergic nerves in comparison with that of cattle, sheep and goats, despite the fact that the majority of neuron sub-populations were found in the inter-muscular nerve plexus of the rumen wall. Development of the DNES in the digestive tract was investigated also for red deer (*C. elaphus* L.) at the stage of prenatal development; no neuro-endocrine cells were found in *rumen, reticulum, omasum* or *abomasum* until the 67th day of fetal development (Franko et al., 2004a, b; Redondo et al., 2005; Masot et al., 2007). In the consecutive days of fetal development they were found in sub-mucosa - *lamina propria* and muscle layer - *tunica muscularis* (Franko et al., 2004 a, b; Redondo et al., 2005; Masot et al., 2007). DNES was not found in the epithelium. Prenatal development of the DNES in deer is

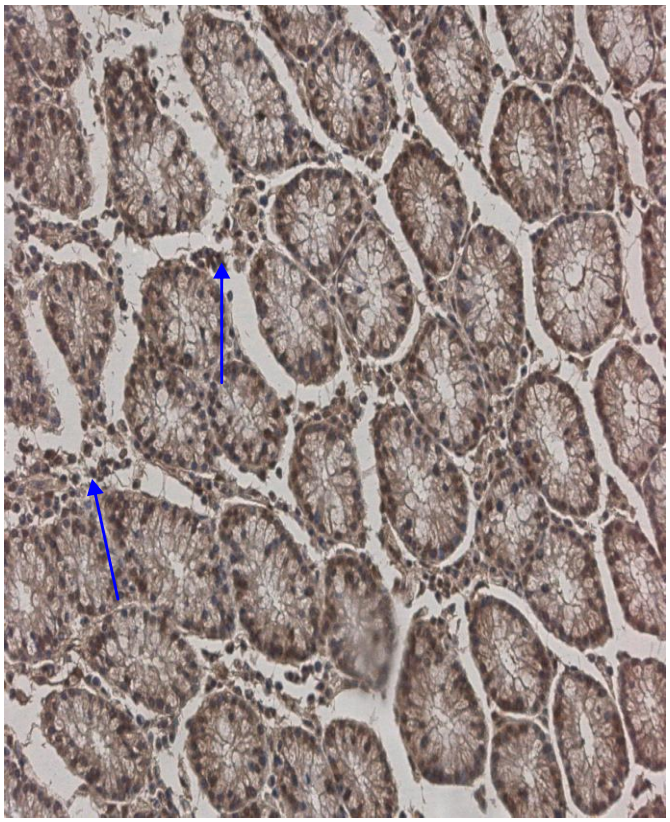

Figure 4. Apoptosis in large intestines wall (TUNEL, magnification ×250).

slower in comparison with that of other ruminants: sheep, goats and cattle (Redondo et al., 2005). Activity of DNES of adult red deer digestive tract (*C. elaphus* L.) is related to its physiological functions by consuming different feed in winter and summer periods (Sibbald and Milne, 1993; Freudenberger et al., 1994). The growth factors and peptide hormones of DNES regulate the splitting up and absorption of the nutrients and contractions of the muscle membrane of whole wall of the digestive tract (Zabielski et al., 1998).

A few patchy loci of NPY-containing nerve fibers found in the muscle layer of the deer rumen and the wall of small intestines as well as some NPY-containing fibers in the muscle layer of the wall of the large intestine could be related to the antibiotic-like ability to disintegrate penetrability of microorganism surface and change the lumen of blood vessel wall as described by Vouldoukis et al. (1996). Although some studies (Franco et al., 2004 a,b; Redondo et al., 2005; Masot et al., 2007) have reported the presence of distinct NPY in submucosa and muscle layer of *rumen, reticulum, omasum* and *abomasum* of red deer embryos before birth, the present study revealed that NPY does not play a significant role in the digestive function of adult deer. The finding in the present study also contradicts those of Allen et al. (1987) which showed that NPY can be found in the nerve fibers of the whole digestive tracts of pigs, rats and guinea-pigs, but are lowly expressed. Low expression of NPY in the digestive tract of the studied animals basically excluded this neuro-peptide from the list of important regulators of digestive system functions of deer.

According to Ulfig et al. (1998) NF are characteristic structural differentiation of the structure of nerves. Pathological changes in NF have a significant impact on the regeneration of motor and sensory axons (Rao et al., 1998). Our results showed that NF-containing nerve fibers are widely seen only in mucosa and muscle layers of the rumen and the small intestines, however some focal points of these nerve fibers were found in all the layers of the large intestine wall. Appearance of patchy loci of NF-containing nerve fibers from the rumen towards the large intestine is a base for patchy loci of cytoskeleton damage and therefore proves the reduced quality of the nerve fibers. Previous study reports that changes in both the cytoskeleton of nerve fibers and the DNES may occur during active metabolic processes (Bueno and Fioramonti, 1994), which was also the assumption in the present study.

Results of the present study also showed the presence of high amount of serotonin-containing cells in the epithelium of the small intestine, and medium-high amount in the epithelium of the large intestine. According to Camilleri (2009), serotonin is involved in many physiological functions of the digestive tract: Its primary role being the prevention of acid secretion in the stomach and may serve as an endogenous enterogastron. Other functions include stimulates the production and secretion of mucus in the stomach and the large intestine, influencing the innervation of intestinal smooth muscle by focusing directly on the mesenteric vascular smooth muscle or through intestinal nerves and exerting impact on the blood flow in the stomach and the intestinal tract. Thus, it be can concluded that abundant expression of serotonin in the epithelium of the small intestine makes these cells the most intense place of metabolic processes in the digestive tract.

Programmed cell death or apoptosis appears when death of the cell is caused by the factors in the cell itself and is also fostered by the expression of *bcl2* gene outside the cell, activating caspase and simultaneously initiating activation mechanism of mitochondrion and lysosomes, as well as, plant oxidative stress (Brunner and Mueller, 2003). In comparison with other parts of the deer's digestive tract, the present study observed the most distinct apoptosis in its largest part– the rumen where the most intense decomposition of feed and absorption processes occurs. These probably increase cell stress and initiate the cascade of programmed cell death.

The walls of the vessels of the portal tract area revealed inflammation processes probably related to the transport of nutrients absorbed in the small intestine.

According to the studies published by Watanabe et al. (2003) and Hironobu et al. (2006), the liver portal tract area shows higher apoptotic index and statistically significantly higher number of HGF positive cells that ensure regeneration ability of the liver. Maher (1993) described that in a healthy liver HGF is found in the cells of sinusoidal endothelium and Kupffer cells. However, during regeneration process of the liver, it is produced by the liver stellate cells or *Ito* cells (Hironobu et al., 2006), as well as fibroblasts, epithelial and endothelial cells, hepatocytes and Kupffer cells (Maher, 1993). Studies by Miyazawa et al. (1996) and Ishikawa et al. (2001) reports that HGF in a healthy liver is found in an inactive-single chain form, but in the cases of pathology it turns into an active-heterodimeric form. By reducing cell apoptosis, HGF participates in the angiogenesis and morphogenesis (Funakoshi and Nakamura, 2003), which corresponds with the findings of the present study: whenever there appears an inflammation in the walls of blood vessels, HGF expression increases and apoptotic index decreases and vice versa in the *V. centralis*.

Matrix metalloproteases (MMP13) are enzymes of cell surface that demolish the majority of peri-cellular substrate (collagen, gelatine, fibronectin, laminin and proteoglycan) participating in many physiological and pathological processes (Nagase and Woessner, 1999). The expression of MMP13 in the parenchyma of the deer's liver was very low. Investigation in to MMP13 revealed that their function in the majority of body development processes and homeostasis, and in a disease process were still uncertain (Birkedal-Hansen et al., 1993; Sternlicht and Werb, 2001). Our study did not reveal any significant expression of MMP13 in the digestive tract of red deer and neither a significant role in their functions.

Our study also revealed distinct expression of interleukins: Expression of IL -6 was mainly found around the blood vessels and bile ducts, and IL-10 markedly expressioned throughout the whole liver parenchyma. Previous studies (Cressman et al., 1996; Heinrich et al., 2003; Coelho et al., 2007) have explained that distinct IL-6 expression around the blood vessels relies on the regeneration processes in the liver. Expression of IL-10 in the liver is related to the immune system and IL-10 is the main regulator of the immune system with its key role in providing anti-inflammatory effect (Howard and O'Garra; 1992; Abbas et al., 1994). Considering the findings of the above studies we concluded that small number of apoptotic cells around *V. centralis* area, high expression of interleukins and simultaneously limited expression of degeneration enzymes on the background of medium distinct hepatocyte activity in the liver of the animals in the present study indicate normal liver activity.

Conclusions

1) The small and large intestines of captive red deer (*C. elaphus* L.) are richly innervated although there are variations of the nerve supply in some places.
2) Abundant expression of serotonin in the epithelium of the small intestines makes this part of the digestive system to be the most intense place of metabolic processes in the digestive tract, while the low NPY expression basically exclude this neuro-peptide from the list of important regulators of the deer's digestive system functions.
3) Focal appearance of NF-containing nerve fibers from the rumen towards the large intestine is a base for patchy loci of cytoskeleton damage and therefore reduces the quality of nerve fibers.
4) Intense processes of apoptosis in the rumen probably rely on the intense food cleavage and absorption processes and could be evaluated as a compensatory reaction on the side of tissues.
5) The small number of apoptotic cells around *V. centralis* area, the distinct expression of interleukins and at the same time limited expression of degeneration enzymes on the background of medium hepatocyte activity in the livers of the animals studied most probably indicate normal liver tissue activity.

ACKNOWLEDGEMENTS

The present work is funded by State Research Programme "Sustainable use of local resources (earth entrails, forests, food and transport) – new products and technologies (NatRes)" and publication has been supported by project "Raising Awareness and Fostering International Cooperation of the Research Institute of Biotechnology and Veterinary Medicine "Sigra"", agreement No 2010/0197/2DP/2.1.1.2.0/10/APIA/VIAA/016.

REFERENCES

Abbas A, Lichtman A, Pober J (1994). Cellular and Molecular Immunology. 2nd Ed., WB Saunders Company, Philadelphia. pp. 1-572.

Allen JM, Hughes J, Bloom SR (1987). Presence, distribution, and pharmacological effects of neuropeptide Y in mammalian gastrointestinal tract. Dig. Dis. Sci. 32(5):506-512.

Arhipova I, Balina S (2006).Statistics in economy. Solutions with SPSS and Microsoft Excel (in Latvian). 2nd Ed., Computer Science center, Riga, Latvia 352 p.

Aughey E, Frye FL (2001). Comparative veterinary histology with clinical correlates. Manson Pub.Ltd, London pp 14-127.

Barrett KE (2011). Functional anatomy of the liver and biliary system. In: Raff H and Levitzky MG Eds. Medical physiology: A systems approach. McGraw-Hill Professional Publishing New York, USA pp 559-565.

Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A, Engler JA (1993). Matrix metalloproteinasis: review. Crit. Rev. Oral Biol. Med. 4(2):197-250.

Brunner T, Mueller C (2003). Programmed cell death: Chapter 9 Apoptosis in disease: About shortage and excess. Essays Biochem. 39:119-130.

Bueno L, Fioramonti J (1994). Neurohormonal control of intestinal transit [neuropeptides, motilin, CCK (cholecystokinin)]. J. Reprod. Nutr. Dev. 34(16):513-525.

Camilleri M (2009).Serotonin in the gastrointestinal tract. Curr. Opin. Endocrinol. Diabetes Obes. 16(1):53-59.
Carson FL, Hladik Ch (2009). Histotechnology. A Self-Instructional Text. Third Ed., Chicago ASCP Press, 400 p.
Ceccarelli P, Pedini V, Gargiulo AM (1995). The Endocrine Cells in the Gastro-enteric Tract of Adult Fallow Deer (*Dama dama* L.). Anatomia Histologia Embryologia 24(3):171-174.
Coelho MC, Tannuri U, Yannuri AC, Mello ES, dos Santos NA (2007). Expression of interleucin 6 and apoptosis-related genes in suckling and weaning rat models of hepatectomy and liver regeneration. J. Pediatr. Surg. 42(4):623- 619.
Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R (1996). Liver Failure and Defective Hepatocyte Regeneration in Interleukin-6- Deficient Mice. Science 274(5291):1379-1383.
Franco A, Masot AJ, Gómez L, Redondo E (2004a). Morphometric and immunohistochemical study of the rumen of red deer during prenatal development. J. Anat. 204(6):501–513.
Franco A, Redondo E, Masot AJ (2004b). Morphometric and immunohistochemical study of the reticulum of red deer during prenatal development. J. Anat. 205(4):277–289.
Freudenberger DO, Touakawa K, Barry TN, Ball AJ, Suttie JM (1994). Seasonality in digestion and rumen metabolism in red deer (*Cervus elaphus*) fed on a forage diet. Brit. J. Nutr. 71(4):489-499.
Fulbright TE, Ortega-Santos JA (2006). White-tailed deer habitat: ecology and management on rangelands. First Ed., Texas A&M University Press, College Station, Texas, USA 241 p.
Funakoshi H, Nakamura T (2003). Hepatocyte growth factor: from diagnosis to clinical applications. Clinica Chimica Acta 327:1-23.
Hals E, Cato Olsen, H (1984). Scanning electron and incident light microscopy of giant tubules in red deer dentin. Scand. J. Dental Res. 92(4):269-274.
Hartwig H, Hartwig HG (1975). The spleen of the cervidae (Gray, 1821). Quantitative-morphological studies on the spleens of stags (*Cervus elaphus* L. 1785) and of deers (*Capreolus capreolus* L. 1758). Gegenbaurs morphologisches Jahrbuch 121(6):669-697.
Heinrich PC, BehrmannI HS, Hermanns HM, Műller- Newen G, Schaper F (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem. J. 374(1):1-20.
Hill RW, Wyse GA, Anderson M (2008). Animal Physiology. 2nd Ed., Sinauer Associates, Inc., 762 p.
Hironobu M, Hiroaki S, Hiroshi I, Fumio K, Satoshi A, Akira T, Masayuki O, Hiroyuki Y, Atsushi K, Hideyuki Y, Shigeaki S, Masaru M (2006). Changes in growth factor and cytokine expression in biliary obstructed rat liver and their relationship with delayed liver regeneration after partial hepatectomy. World J. Gastroenterol. 12(13):2053-2059.
Howard M, O'Garra A (1992). Biological properties of interleukin 10. Immunol. Today 13(6):198-200.
Hsu SM, Raine L, Fanger H (1981). The use of antiavidin antibody and avidin-biotin peroxidase complex in immunoperoxidase technics. Am. J. Clin. Pathol. 75(12):816 -821.
Ishikawa KS, Masui T, Ishikawa K, Shiojiri N (2001). Immunolocalization of hepatocyte growth factor and its receptor (c-Met) during mouse liver development. Histochem.Cell Biol. 116(5):453-462.
Josefsen TD (1997). Influence of diet on the occurrence of intraepithelial microabscesses and foreign bodies in the ruminal of reindeer calves (*Rangifer tarandus tarandus*). Zentralblatt für Veterinarmedizin 44(5):249-257.
Junqueira LC, Carneiro J (2003).Organs associated with the digestive tract. In: Basic histology. Text & Atlas10th Ed., McGraw-Hill Publishing Co, Starnford. pp 332-344.
Kiernan J.A. (2008). Histological and histochemical methods. 4th Ed., Scion Publishing Ltd. 606 p.
Kmieč Z (2001). Cooperation of liver cells in health and disease. Adv. Anat. Embriol. Cell Biol. 161(III-XIII):1-151.
Knolle PA, Gerken G (2000). Local control of the immune response in the liver. Immunol. Rev. 174(1):21-34.
Maher JJ (1993). Cell specific Expression of Hepatocyte growth factor in liver. J. Clin. Invest. 91:2244-2252.
Mallard BA, Dekkers JC, Ireland MJ, Leslie KE, Sharif S, Vankampen CL, Wagter L, Wilkie BN (1998). Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. J. Dairy Sci. 81(2):585-595.
Masot AJ, Franco AJ, Redondo E (2007). Morphometric and immunohistochemical study of the abomasum of red deer during prenatal parental development. J. Anat. 211(3):376-386.
Miyazawa K, Shimomura T, Kitamura N (1996). Activation of hepatocyte growth factor in injured tissues is mediated by hepatocyte growth factor activator. J. Biochem. Chem. 271(7):3615-3618.
Münnich J, Gotthold G, Pfannkuche H (2008). Intrinsic ruminal innervation in ruminants of different feeding types. J. Anat. 213(4):361–498.
Nagase H, Woessner JF (1999). Matrix matalloproteinases. J. Biol. Chem. 274(31):21491-21494.
Negoescu A, Guillermet Ch, Lorimer Ph, Robert C, Lantuejoul S, Brambilla E, Labat-Moleur F (1998). TUNEL apoptotic cell detection in archived paraffin-embedded tissues. Biochemica 3:36-41.
Pilmane M, Rumba I, Sundler F, Luts A (1998).Patterns of occurrence and distribution of neuroendocrine elements in lungs of humans with chronic lung diseases. Proc. Latvian Acad. Sci. 52(596/597):144-152.
Rao MV, Houseweart MK, Williamson TL, Crawford TO, Folmer J, Cleveland DW (1998). Neurofilament-dependent radial growth of motor axons and axonal organization of neurofilaments does not require the neuofilament heavy submit (NF-H) of its phosphorylation. J. Cell Biol. 143(1):171-181.
Redondo E, Franco AJ, Masot AJ (2005). Morphometric and immunohistochemical study of the omasum of red deer during prenatal development. J. Anat. 206(6):543-555.
Shabadash SA, Zelikina TI (2003). Unknown hepatoid glands of some species of cats and deer. Izvestiia Akademii nauk. Seriia biologicheskaia 4:462-472.
Sheskin DJ (2011). Handbook of Parametric and Nonparametric Statistical Procedures Fifth Ed., Chapman and Hall/CRC. 1026p.
Sibbald AM, Milne JA (1993). Physical characteristics of the alimentary tract in relation to seasonal changes in voluntary food intake by red deer. J. Agri. Sci., Cambridge 120(1):99-102.
Skriba G (2011). Red Deer Origin, Distribution and Breeding in Latvia (in Latvian). Publisher Staltbriezi, Latvia 617 p.
Soveri T (1993). Liver Histology of Reindeer Calves during the Winter Season. Anat. Hist. Embriol. 22(4):313-318.
Sternlicht MD, Werb Z (2001). How matrix metalloproteinases regulate cell behavior. Am. Rev. Cell Dev. Biol. 17:463-516.
Ulfig N, Nickel J, Bohl J (1998). Monoclonal antibodies SMI 311 and SMI 312 as tools to investigate the maturation of nerve cells and axonal patterns in human fetal brain. Cells Tiss. Res. 291(3):433-443.
Vouldoukis I, Shai Y, Nicolas P, Mor A (1996). Broad spectrum antibiotic activity of the skin- PYY. FEBS Lett. 380(3):237-240.
Watanabe H, Sumi S, Kitamura Y, Nio Y, Nigami T (2003). Immunohistochemical Analysis of Vascular Endothelial Growth Factor and Hepatocyte Growth Factor, and Their Receptors, Transplanted Islets in Rats. Surgery Today 33:854-860.
Zabielski R, Dardillat C, Le Huërou-Luron I, Bernard C, Chayvialle JA, Guilloteau P (1998). Periodic fluctuations of gut regulatory peptides in phase with the duodenal migrating myoelectric complex in preruminant calves: Effect of different sources of dietary protein. Brit. J. Nutr. 79(3):287-296.