Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2018, 8(4), 469-475

RESEARCH ARTICLE *UDC 599.735.3 + 591.477*

Influence of environmental factors of on the structural and functional state of its specific skin gland of Red deer (*Cervus elaphus sidiricus* Severtzov, 1872)

N.D. Ovcharenko, E.A Kuchina

Altai State University, Lenina pr. 61, Barnaul, 656049, Russia,
E-mails: ovcharenko-55@mail.ru, kuchina70@mail.ru
Received: 19.10.2018. Accepted: 23.11.2018

The influence of the temperature factor on the functional state of the dermal specific gland of the red deer, which manifests its maximum values in the Altai, both the lowest in the winter period and the highest in the summer period of the year. Initially, a detailed description of the structure of the tail gland of the deer is given due to the fact that the classification of skin glands is still controversial, and data on the structure of such structures in real deer are sporadic. Standard histological, morphometric and statistical research methods were used. In determining the functional state of the tail gland, morphometry of its indicators such as the diameter of the alveoli, the height of the glandular epithelium, the diameter of the excretory ducts, the diameter of the nuclei of the glandular cells and their nuclear-cytoplasmotic ratio was performed. Sexual dimorphism of both macroscopic (length and mass of the gland) and micrometric indices is established, and in both adult and young females they are higher than in analogs of males. In the study of seasonal dynamics of morphological parameters of the gland, their significant change was found only in adult females, which allows us to speak about enhancing its functional state in the summer period. The dynamics of the functional state of the gland in females, depending on their physiological state in the winter season, was also revealed. For comparison, selected groups: non-pregnant animals, pregnant, lactating and at the same time pregnant and lactating. It was established that the functional state of the gland at the beginning of pregnancy (its first trimester) does not significantly change compared with non-pregnant animals. Animals of non-pregnant, but lactating iron are functionally more active than non-lactating. The simultaneous effect of lactation and pregnancy does not affect the functional state of the tail red deer
Keywords: red deer; tail gland; morphological parameters; functional state; environmental factors; pregnancy; lactation; season of the year

Red deer, as a representative of ungulates, is a unique object living in a wild state in the mountains of Altai. Red deer and spotted deer are bred in Altai also in reindeer farms in order to obtain valuable products from them, such as antlers, blood, tails, penises, and embryos. Being in the conditions of park or so-called semi-free keeping, the deer retain their biological features - they are seasonally breeding animals. During the breeding period form temporary groups. Gon have them in the fall. Pregnancy lasts 8.5–9 months. Cubs are born at the beginning of summer (Lunitsin, 2004, 2012; Ovcharenko, 2010).
The leading environmental factors affecting the state of the body, including the skin and its glandular structures in a sharply continental climate, are: solar radiation, temperature, humidity and, as a consequence, the availability of food (Ovcharenko, 2010).
In many deers (zambar, mazama, pudu, fallow deer, elk of northern and spotted deer and others) besides the usual skin glands, so-called specific skin glands are also developed.
The classification of skin glands is still controversial. Their division into sebaceous holocrine and sweat (apocrine and merocrine) is undoubtedly. Some authors single out the so-called hepatoid ones separately (Shabadash, Chernova, 2006). The latter were first described by Shaffer (1924, 1940). The basis of classification according to the researchers can only be the type of secretion. Hepathoid glands are defined by Shaffer as polyptychial merocrine, sebaceous - as polyptychial holocrine, and he considers polyptychial mero-fibrin glandular organs in which the hepatoid glands are combined with the sebaceous

(Kurosumi et al., 1984). Later, the authors, describing the skin glands of mammals, in particular the fox tail gland, the stormy gland of the chamois and the preorbital glands of the bovid, defined earlier as hepatoid, consider them “modified sebaceous” (Richter, 1971, 1973; Kuhn, 1976; Albone, Flood, 1976; Mainoya, 1978; Macdonald, 1985; Atoji et. Al., 1989, 1996; Tosi et. Al., 1990).

Specific skin glands are most often hypertrophied holocrine sebaceous, apocrine or eccrine sweat glands or their complexes. These glands are usually located on the cranial and caudal parts of the body, as well as on the extremities, accompanying the senses, food production and reproduction (Azbukina, 1970, 1982; Silkin, 2012). The number, type and topography of specific skin glands is determined by the biology of the species. V.Ye. Sokolov and O.F. Chernova (2001) note that the degree of development of many skin glands also depends on the sex of the animal, season, age and the reproductive state of the individuals.

The tail gland of deer is one of the largest iron structures, it is known that it has application in folk medicine and is exported to the countries of Southeast Asia. In noble and spotted deer, it completely surrounds the last eight caudal vertebrae. Morphologically, it is divided into lobes and has an unusually rich vascularization, which is why the metabolism in it is extremely intense, and the structure is very quickly destroyed after the death of animals. This feature makes research difficult and gives rise to contradictions regarding its structure (Hoffmann, Thome, 1986; Ovcharenko, Rzhanitsyna, 1988; Ovcharenko, 1988; Shabadash, Chernova, 2006).

A detailed study of this gland in the red deer on a large amount of factual material from different points of view is carried out by the staff of the Department of Zoology and Physiology of the Altai State University in particular, by professor N. D. Ovcharenko and her students over the past few years.

Material and methods

The red deer tail gland from adults (7–8 years old) and young (1.5–2 years) males and females selected in the winter and summer seasons served as the material for the study. From adult females during the winter period, the material was also collected taking into account their physiological state (non-pregnant, pregnant, lactating, pregnant and lactating at the same time). The age of the deer was determined on the basis of registration in the inventory books. The total number of animals studied is 35.

Glands were obtained during the planned slaughter of animals at a meat processing plant with the aim of obtaining meat products from them.

For histological studies, tissues excised from the middle part of the tail, 1x1 cm in size were used. 10% neutral formalin was used as a fixing medium. In order to compact the tissue, samples were paraffin embedded using the automatic histological wiring system TPC 15. 5–10 μm thick sections were obtained using a rotary microtome for HM 325 Thermo Scientific paraffin sections. Serial sections were made from each animal (at least 10 drugs). The preparations were stained with Ehrlich hematoxylin and eosin (Histologic ..., 2013).

Morphometric analysis was performed using light microscopy at magnification (× 100, × 400 and × 1000) and the Scope Photo 3.0 program installed on a personal computer. Parameters such as the diameter of the alveoli, the height of the cells and the diameter of the nuclei were measured. For each indicator, 50 measurements were taken from at least three animals. Statistical processing of the data array was carried out using Microsoft Excel. When choosing morphological criteria for describing the skin of the studied area, we were guided by the works of V.E. Sokolov et al. (1988, 2001, 2002). The following formula was used to calculate the nuclear cytoplasmic ratio (NCR):

$$NCR = \frac{d^2 max_я}{d^2 max_{кл}},$$

where $d^2\max_я$ – nucleus maximum diameter, $d^2\max_{кл}$ – cell maximum diameter (Bondarenko et al., 2000).

In undifferentiated cells, the NCR index is high. The nuclear cytoplasmic ratio shows the state of the cell. If this ratio is equal to or greater than unity, then these cells are functionally inactive. On the contrary, cells with less than a single NCR are highly differentiated and able to function actively (Didenko et al., 2006).

Micrographs were taken using a LOMO Micmed 6 microscope and a ScopeTek DMC310 camera.

Statistical processing, processing of the data set for individual indicators with the calculation of averages and deviations, was performed using the standard software package STATISTICA 10.0 and Microsoft Excell 2010. The normality of the distribution of quantitative indicators was performed using the Kolmagorov-Smirnov and Lillifors criteria. Assessment of differences between samples using the Kolmagorov-Smirnov non-parametric paired test due to the lack of agreement of data with a normal distribution. The critical level of significance was assumed to be 0.05. Data are presented as arithmetic means (M) and standard deviations of the means (Mastitsky, 2009).

Results

The red deer tail gland, from a morphological point of view, is a large, dark-colored subcutaneous glandular formation that surrounds the caudal vertebrae in the form of two cords (Fig. 1).

Figure 1. The tail gland of 8 years female red deer.

Its main component is the apocrine glands, which have a complex tubular-alveolar structure.
Functionally, the tail gland belongs to the group of communicative signaling glands. The main purpose of this gland, presumably, is alert about the danger - the selection of the "smell of anxiety" (Sokolov, 2002). The thickness and length of the glandular field in different parts of the tail differ, and there is sexual dimorphism. The mass of the tail gland of females, regardless of the season and age as compared with males, is distinguished by larger size, color and color intensity of gland secretion on histological specimens. Thus, the mass of glandular tissue (without skin), in females in winter averages 130 grams (n = 11), and in males - 100 grams. The color of the gland in females is darker than in males (Kuchina and Ovcharenko, 2011). Bakke and Figenschou (1983), who identified 28 components in it, also point out the gender differences in the qualitative and quantitative composition of the tail gland secretion in other deer.
The glandular parenchyma is divided by connective tissue septa into lobes (Fig. 2). The average size of shares on transverse preparations is 999.36x607.02 microns.

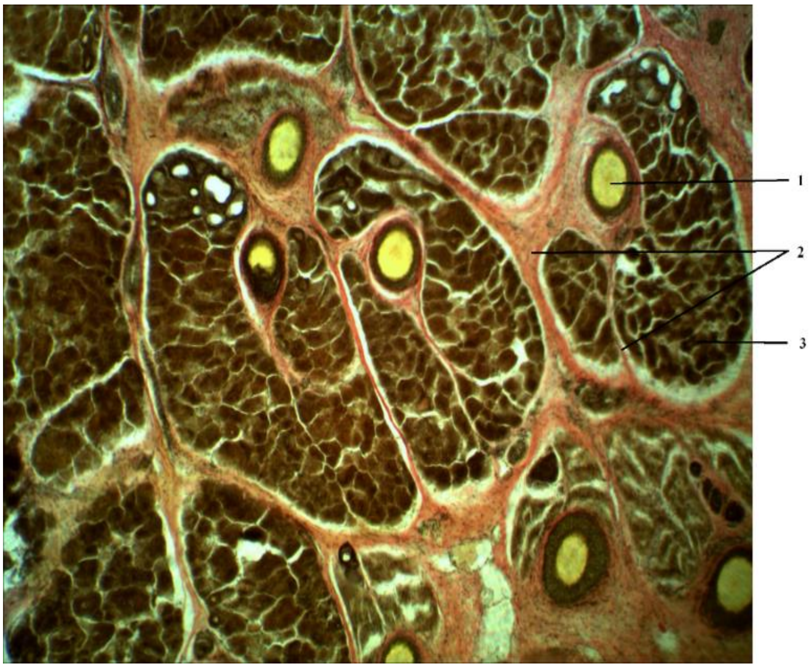

Figure 2. A cross-section of the skin in the tail gland. The red deer female is 7 years old, winter. Coloring according to van Gieson, (SW. X400): 1 - guard hairs; 2 - connective tissue layer; 3 - secretory lobes of the gland.

Inside the lobules, the secretory portions of the apocrine glands are located, most of them having a rounded shape. The volumetric ratios of the stroma and the parenchyma of the gland, per unit area, in animals of both studied groups, are shifted

in favor of the secretory part. However, we found that the proportion of the glandular component in young animals is slightly higher compared with adults (88.97–81.36%).
The glandular tissue itself is represented by a cluster of apocrine glands, their terminal sections ending in alveoli, the walls of which consist of a single layer of secreting cells. The alveoli are surrounded by a layer of myoepithelial cells lying on the basement membrane (Fig. 3).

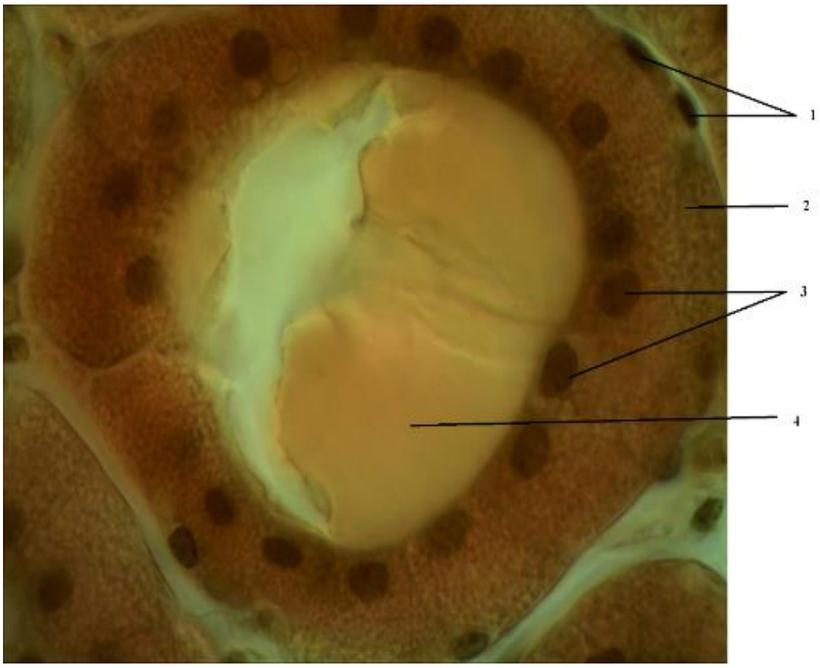

Figure 3. The end of the tail gland. The red deer female is 7 years old, winter. Coloring according to van Gieson (SW. X1000): 1 - myoepithelial cells; 2 - cells of the secretory epithelium; 3 - secretory cell nuclei; 4 - secret.

It should be noted that in young animals alveoli are often found with extended secretory tubes (up to 212 microns), which, as a rule, are located in the upper sections of the glandular parenchyma, their walls are represented by low cubic epithelium. Most of the terminal secretory parts are represented by tall cylindrical cells with somewhat narrowed apical ends. The nuclei in the secretory cells are rounded and displaced into the apical part. Chromatin lumps in the nucleus occupy a peripheral position (Ovcharenko, Kuchina, 2012).
The skin, including the skin glands, is more affected by the temperature factor. We considered the influence of the maximum in the year of both low and high temperatures, which are recorded in the Altai during the winter period (–20 ... –30 ° C) and in the summer (+ 20 ... +30 ° C).
When studying the seasonal dynamics of morphological equivalents of the functional state of the gland, we found that indicators such as the diameter of the alveoli, the height of the glandular epithelial cells and the diameter of their nuclei in females as a whole have higher values in winter and in summer compared to males (Ovcharenko et al., 2016). It should be noted that this pattern is also observed in young animals (Table 1). At the same time, in young people, most of the indicators in the winter period are higher than in adults.

Table 1. Morphometric parameters of the tail gland of male and female red deer depending on age (winter period), M±m

Index	Males		Females	
	Adult	Young	Adult	Youmg
Cell height, μm	18.36 ± 0.62	20.45 ± 0.41*	18.49 ± 0.28	20.78 ± 0.25*
Alveolar diameter, μm	67.00 ± 1.91	72.89 ± 3.91*	89.70 ± 5.13	76.83 ± 2.18*
Nucleus diameter, μm	4.95 ± 0.07	5.15 ± 0.09*	6.11 ± 0.05	6.33 ± 0.05*

*Note: * here and further the differences with previous group significant at* P≤0.05

All the above indicators in summer in adult females have higher rates compared with winter. This indirectly indicates a change in the functional activity of the gland. In our opinion, this may be due to the presence of progeny in females at this time of year, and the signaling function of the gland is quite explicable and connected with taking care of the young. In males, in adults and young, only the volume of the nuclei of glandular cells changes reliably from the studied parameters, on the basis of which it can be assumed that this gland plays an insignificant role in this period of the year, as evidenced by the data in Table 2.

Table 2. Morphometric parameters of the tail gland of male red deer depending on season, M±m

Index	Winter		Summer	
	Adult	Young	Adult	Young
Cell height, µm	18.36 ± 0.62	20.45 ± 0.41	19.26 ± 0.3	20.62 ± 0.6
Alveolar diameter, µm	67.00 ± 1.91	72.89 ± 3.91*	67.23 ± 1.02	75.88 ± 4.72*
Nucleus diameter, µm	4.95 ± 0.07	5.15 ± 0.09*	5.80 ± 0.07	5.70 ± 0.09

It should be noted that in the literature there is information about the enhancement of the functions of the marking glands (the horn glands) in other deer during the rut period (Shabaldash, Chernova, 2006). We have not studied iron during this period of the year (autumn).
Analyzing the data when studying the influence of the physiological state of females on the functional activity of the tail gland, we found that in pregnant and non-pregnant females there are statistically significant differences in the indicators of cell height, diameters of alveoli, ducts and nuclei (Ovcharenko et al., 2018). In this case, the nuclear-cytoplasmic ratio in pregnant and non-pregnant females remains equal, which indirectly indicates a similar functional state of the gland (Table 3).

Table 3. The effect of pregnancy on the morphological parameters of the tail gland, M±m

Index	Pregnant, lactating, n= 3	Non-pregnant, non-lactating, n=3
Cell height, (мкм)	17.63 ± 3.55**	18.72 ± 2.52
Alveolar diameter (мкм)	68.33 ± 13.82**	85.02 ± 19.39
Duct diameter (мкм)	34.39 ± 12.84**	53.83 ± 20.58
Nucleus diameter (мкм)	5.84 ± 0.45**	6.10 ± 0.51
NCR	0.3	0.3

Note: here and further the differences with control group (non-pregnant, non-lactatin) significant at **P≤0.01and *P≤0.05

Considering that the red deer's chase lasts from mid-September to mid-October and this material was selected in the winter period (December), it can be assumed that the females were in the first trimester of pregnancy. Other researchers who studied specific glands in rodents and hamsters also noted a similar pattern (Sokolov et al., 1994; Silkin, 2013).
Analyzing the data obtained, on the effect of lactation on the state of the gland, it was found that in lactating and non-lactating females there are statistically significant differences in all indicators. At the same time, the nuclear-cytoplasmic ratio in lactating females is higher, which may indicate a slight increase in the functional state of the gland in this group (Table 4).

Table 4. Influence of lactation on the tail gland indices, M±m

Index	Lactating, non-pregnant	Non-lactating, non-pregnant
Cell height	17.31 ± 3.42**	18.72 ± 2.52
Alveolar diameter	71.25 ± 11.6**	85.02 ± 19.39
Duct diameter	22.19 ± 7.0**	53.83 ± 20.58
Nucleus diameter	6.23 ± 0.67*	6.10 ± 0.51
NCR	0.4	0.3

The effect of late lactation on the functional state of the tail gland was revealed. In the studied literature, the authors note that the size of specific glands and their functional activity in other animals, in particular rodents and hamsters, changes during lactation (Sokolov et al., 1994). By the end of the lactation period, the activity of the glands decreases.
As a result of the analysis of the obtained data, it was found that with the joint influence of such factors as lactation and pregnancy, there are statistically significant differences between all the studied parameters. At the same time, all indicators (except the height of the cells) are higher in non-lactating non-pregnant females (Table 5).

Table 5. Influence of pregnancy and lactating on tail gland indices, M±M

Index	Lactating, Pregnant	Non-lactating, Non-pregnant
Cell height	19.19 ± 0.46*	18.72 ± 2.52
Alveolar diameter	64.89 ± 2.15**	85.02 ± 19.39
Duct diameter	32.32 ± 1.84**	53.83 ± 20.58
Nucleus diameter	5.83 ± 0.04**	6.10 ± 0.51
NCR	0.3	0.3

Note: differences with the control group (non-lactating, non-pregnant) significant at **P≤0.01; *P≤0.05

However, the NCR valules remains the same, which may presumably indicate the absence of differences in the functional state of animals. We found that pregnancy in its first trimester and simultaneous lactation (its last months) do not have a significant effect on the structural and functional state of the tail gland. We were not able to find in relevant literature the data on the joint effect of lactation and pregnancy on the functional state of specific glands. In conclusion, we suggested that the functional state of the gland is influenced by the season of the year, but is not equivalent for different sexes and different ages of animals. The physiological state of the females affects the functional state of the gland, but it depends on the periods of both pregnancy and lactation.

References

Albone, E.S., Flood, P.F. (1976). The supra caudal scent gland of the red fox Vulpes vulpes. J. Chem. Ecology, 2(2), 167–175.

Atoji, Y., Sugimura, M., Suzuki, Y. (1989). Sebaceous glands of the infraorbital gland of the Japanese serow Capricornis crispus. J. Submicrosc. Cytology Pathology, 21(2), 375–383.

Atoji, Y., Yamamoto, Y., Suzuki, Y. (1996). Infraorbital gland of a male Formosan serow Capricornis crispus swinhoei). Eur. J. Morphology, 34(2), 87–94.

Azbukina, M.D. (1970). Specific skin glands of puppies of the American mink (Mustela vison). Collection of Sc. Papers of All-Russian Research Institute for Hunting Economy and Farming, 30, 60–67 (in Russian).

Azbukina, M.D. (1999). The influence of extirpation of specific preanal glands on the body and the reproductive functions of the American mink. Signals of animals. Moscow. Nauka (in Russian).

Bakke, J., Figenschou, E. (1983). Volatile compounds from the red deer (Cervus elaphus) Secretion from the tail gland. Journal of Chemical Ecology, 9(4), 513–520.

Bondarenko, O.V., Atyasova, O.V., Tokmakova, S.I. (2000). Comparative analysis of methods for calculating the nuclear-cytoplasmic ratio of cells. Questions of theoretical and applied morphology, 5, 61–62 (in Russian).

Didenko, A.A., Dzhandarova, T.I., Sidorov, Yu.S. (2006). Morphofunctional characteristics of the glomerular zone of the adrenal cortex in hyperparathyroidism in the process of adaptation to changes in the light mode. Morphology, 129(2), 35–41 (in Russian).

Histological and histochemical methods of research. (2015). N.D. Ovcharenko, E.A. Kuchina, R.V. Tuzikova (ed.). Barnaul. Altai State University Press (in Russian).

Hofmann, R.R., Thome, H. (1986). Zum funktionellen Morphologie und Mikroarchitectur des Zirkukaudalorgans von Cervus elaphus und C. Nippon. Berl. Munch. Tierarztl. Wochenschrift, 99(12), 418–424.

Kuchina, E.A., Ovcharenko, N.D., Lunitsyn, V.G., Teterina, A.V. (2011). Histostructure of the caudal gland of the red deer (Cervus elaphus sibiricus, Severzov, 1872) and its sexual characteristics. Agrarian Science - Agricultural Production of Sibiria, Mongolia, Kazakhstan and Bulgaria: Proceed. XIV Int. Sc. Conf. Krasnoyarsk. Krasnoyarsk State Agrarian University (in Russian).

Kuhn, H.-J. (1976). Antorbitaldruse und Tranengang von Neotragus pygmaeus. Z. Saűgetierkunde, 41(6), 369–380.

Kurosumi, K., Shibasaki, S., Ito, T. (1984). Cytology of the secretion in mammalian sweat glands. International Review of Cytology, 87, 253–329. DOI: 10.1016/S0074-7696(08)62445-6

Lunitsyn, V.G. (2004). Reindeer husbandry of Russia. Barnaul: Publishing House of All-Russian R&D Institute of Reindeer Herding (in Russian).

Lunitsyn, V.G., Borisov, N.P. (2012). Reindeer Breeding in Russia. Barnaul. ABC (in Russian).

Macdonald, D.W. (1985). The carnivores: order Carnivora (pp. 619-672). In Social Odours in Mammals. R. E. Brown, D. W. Macdonald (Eds.). Oxford.

Mainoya, J.R. (1978). Histological aspects of the preorbital and interdigital glands of the red duiker (Cephalopus natalensis). East Afr. Wildlife J., 16(4), 265–272.

Mastitsky, S.E. (2009). Methodical manual on the use of the Statistica program in the processing of biological research data. Minsk: RUE “Institute of Fishery” (in Russian).

Ovcharenko, N.D., Bondyreva, L.A., Gribanova, O.G., Vlasova, O.E., Kudryashova, I.V. (2010). Endocrine regulation of the growth and development of the deer organism. Barnaul. Altai State Agrarian University Press (in Russian).

Ovcharenko, N.D., Kuchina, E.A. (2012). Age-related features of the macro- and micromorphology of the tail gland of red deer females (Cervus elaphus sibiricus, Severtzov, 1872). Izvesiya of Altai State University, 3 75), 49–52 (in Russian).

Ovcharenko, N.D., Kuchina, E.A., Ebel, L.B. (2016). Morphometric indicators of the tail red deer gland (Cervus elaphus sibiricus Severzov, 1872), depending on the season of the year and the age of the animal. Altai Zoological Journal, 10, 76–79 (in Russian).
Ovcharenko, N.D., Kuchina, E.A., Semenikhina, N.M., Chertovskikh, E.E. (2018) Morphometric indicators of the tail gland of the red deer females (Cervus elaphus sibiricus Severrtzov, 1872) depending on the physiological state. Actual problems of biology and Ecology. Proceed. Int. Sci. Conf. Karaganda. Karaganda State University Publishing House (in Russian).
Ovcharenko, N.D., Rzhanitsyna, I.G.(1998). Some histological indicators and nerve elements of the caudal gland of spotted deer. In Ecology and functional morphology of the skin of mammals. Moscow. Nauka (in Russian).
Richter, J. (1971). Untersuchugen an Antorbitaldrusen von Madoqua (Bovidae, Mammalia). Z. Saűgetierkunde, 36(6), 334–342.
Richter, J. (1973). Zur Kenntnis der an Antorbitaldrűsen der Cephalophinae (Bovidae, Mammalia). Z. Saugetierkunde, 38(5), 303–313.
Schaffer, J. (1924). Zur Eiteilung der Hautdrusen. Anat. Anzeiger., 57(18), 353–372.
Schaffer, J. (1940). Die Hautdrűsenorgane der Saugetiere. Berlin, Urban, Schwarzenberg.
Shabaldash, S.A., Chernova, O.F. (2006). Hepatoid skin glands of mammals. Moscow. KMK (in Russian).
Silkin, I.I., Popov, A.P. (2012). Histological changes and the content of protein components in musk prepucial glands of males of muskrats at the stages of postnatal ontogenesis. Scientific journal of Kuban State Agrarian University, 78(04), 1–12 (in Russian).
Sokolov, V.E., Angelova, P.A., Evgenieva, T.P., Baleva, K.P. (1994). Hormonal regulation of the development of specific skin glands of the golden hamster in vivo and in vitro. Structure, growth and some aspects of the hormonal regulation of the development of specific glands. Moscow. Nauka (in Russian).
Sokolov, V.E., Chernova, O.F. (2001). Skin glands of mammals. Moscow. GEOS (in Russian).
Sokolov, V.Ye. (2002). Selected Works. Vol. 1: Morphology, systematics, faunistics, evolution of mammals. Moscow. Nauka (in Russian).
Sokolov, V.Ye., Genevskaya, R.N. (1988). Guide to the study of the skin of mammals. Moscow. Nauka (in Russian).
Tosi, G., Vailati, G., Arduino, S., Paccetti, G. (1990). Morphology and functionality of the supraoccipital glands in the alpine chamois Rucicapra rucicapra rucicapra. Bolletino Zool., 57(2), 131–138.

Citation:
Ovcharenko, N.D., Kuchina, E.A. (2018). Influence of environmental factors of on the structural and functional state of its specific skin gland of Red deer (Cervus elaphus sidiricus Severtzov, 1872). Ukrainian Journal of Ecology, 8(4), 469-475.