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CARACTERÍSTICAS ECOLÓGICAS E BIOLÓGICAS E COMPOSIÇÃO QUANTITATIVA DA FAUNA DE INFUSORIA EM DIFERENTES PARTES DO ESTÔMAGO DE ALCES EUROPEUS (*ALCES ALCES*) QUE VIVEM NAS REGIÕES DE OMSK E CHELYABINSK DA RÚSSIA

THE ECOLOGICAL AND BIOLOGICAL FEATURES AND QUANTITATIVE COMPOSITION OF INFUSORIA FAUNA IN DIFFERENT PARTS OF THE STOMACH OF EUROPEAN ELK (*ALCES ALCES*) LIVING IN THE OMSK AND CHELYABINSK REGIONS OF RUSSIA

ЭКОЛОГО-БИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ И КОЛИЧЕСТВЕННЫЙ СОСТАВ ИНФУЗОРНОЙ ФАУНЫ РАЗЛИЧНЫХ ОТДЕЛОВ ЖЕЛУДКА ЛОСЯ ЕВРОПЕЙСКОГО *(ALCES ALCES)* ОМСКОЙ И ЧЕЛЯБИНСКОЙ ПОПУЛЯЦИЙ РОССИИ

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RESUMO

Os alces (*Alces alces* L.) são os maiores animais da família dos veados e um importante animal comercial e de caça na Rússia. Desde os tempos antigos, os alces oferecem carne nutritiva e peles valiosas. Por esta razão, existem repetidas tentativas de domesticar alces. No entanto, a domesticação dos alces e sua implementação nas fazendas não foi além das fazendas experimentais. Isso se deve em grande parte à fisiologia inexplorada da digestão do alce, incluindo o papel dos ciliados endobiontes. A dieta do alce é muito diversa e inclui brotos, folhas, a casca de várias espécies de árvores e arbustos, várias ervas, rizomas e folhas de plantas aquáticas e cogumelos. Esta pesquisa teve como objetivo realizar um estudo quantitativo e específico da composição do infusório do trato digestivo de alces europeus que vivem no território das regiões de Omsk e Chelyabinsk da Rússia em relação ao habitat, nutrição e clima. São apresentados os resultados de uma análise comparativa das espécies e da composição numérica de protozoários ciliados simbióticos em estômagos de alces europeus. O número médio de espécies individuais de cílios do estômago de alce dependendo do território de habitat do animal, bem como a composição qualitativa e quantitativa de várias partes do estômago é apresentada. O gênero dominante de cílios em alces é *Entodinium*, que inclui 10 espécies. O *Entodinium* representa 63% de todos os protozoários no rúmen dos alces na população de Chelyabinsk e 73% na população de Omsk. A participação da família ophrioscolecidae é responsável por 98% da composição total dos cílios no rúmen dos alces da população de Chelyabinsk e por 96% da população de Omsk. Nenhum cílio foi encontrado no coalho. Os resultados permitem avaliar as perspectivas para a domesticação dos alces europeus.

Palavras-chave: *Alce, Infusoria Fauna, Rúmen, Retículo, Omasum, Ração alimentar de animais comerciais.*

ABSTRACT

Elk (*Alces alces* L.) are the largest animals in the deer family and an important commercial and hunting animal in Russia. Since ancient times, elk have offered nutritious meat and valuable hides. For this reason, there are repeated attempts to domesticate elk. However, the domestication of elk and their implementation on farms has not gone beyond experimental farms. This is mostly due to the elk's digestion's unexplored physiology, including the role of endobiont ciliates. The elk's diet is very diverse and includes shoots, leaves, the bark of various tree species and shrubs, multiple herbs, rhizomes and the leaves of aquatic plants, and mushrooms. This research aimed to perform a quantitative and specific study of the infusory composition of the digestive tract of European elk, which lives on the territory of the Omsk and Chelyabinsk regions of Russia in connection with habitat, nutrition, and climate. A comparative analysis of the species and numerical composition of symbiotic ciliated protozoa in the stomachs of European elk are presented. The average number of individual species of elk stomach cilia depending on the territory of the animal's habitat and the qualitative and quantitative composition of various parts of the stomach are presented. The dominant genus of cilia in elk is *Entodinium*, which includes ten species. *Entodinium* makes up 63% of all protozoa in the rumen of moose in the Chelyabinsk population and 73%

in the Omsk population. The ophrioscolecidae family share accounts for 98% of the total composition of cilia in the moose rumen of the Chelyabinsk population and 96% of the Omsk population. No cilia were found in the rennet. The results allow us to assess the prospects for European elk domestication.

Keywords: *Elk, Infusoria fauna, Rumen, Reticulum, Omasum, Food ration of commercial animals.*

АННОТАЦИЯ

Лось (*Alces alces L.)* - крупнейшее животное семейства оленьих и важное промысловое и охотничье животное в России. С давних времен лоси предлагали питательное мясо и ценные шкуры. По этой причине предпринимаются неоднократные попытки приручить лося. Однако приручение лосей и их внедрение на фермах не вышло за рамки экспериментальных хозяйств. В основном это связано с неизученной физиологией пищеварения лося, в том числе с ролью инфузорий эндобионта. Рацион лося очень разнообразен и включает в себя побеги, листья, кору различных древесных пород и кустарников, множество трав, корневища и листья водных растений, а также грибы. Цель данного исследования заключалась в количественном и качетсвенном изучении инфузионного состава пищеварительного тракта европейского лося, обитающего на территории Омской и Челябинской областей России, в зависимости от среды обитания, питания и климата. Представлены результаты сравнительного анализа видового и численного состава симбиотических мерцательных простейших в желудках европейского лося. Приведено среднее количество отдельных видов инфузорий желудка лося в зависимости от территории ареала животного, а также качественный и количественный состав различных отделов желудка. Доминирующим родом инфузорий лося является *Entodinium*, который включает 10 видов. Энтодиниум составляет 63% всех простейших в рубце лося в челябинской популяции и 73% в омской популяции. На долю семейства *Ophrioscolecidae* приходится 98% от общего состава ресничек рубца лосей челябинской популяции и 96% омской популяции. Реснички у сычужного фермента не обнаружены. Полученные результаты позволяют оценить перспективы одомашнивания европейского лося.

Ключевые слова: *Лось, инфузорийная фауна, рубец, ретикулум, книжка, пищевой рацион промысловых животных.*

1. INTRODUCTION:

Elk (*Alces alces* L., 1758) are the largest animals in the deer family and an important commercial and hunting animal in Russia. Since ancient times, elk have offered nutritious meat and valuable hides. The literature discusses repeated attempts to domesticate elk in various parts of its range (Skalon and Khoroshikh, 1958). It describes how, in Siberia, salmon farming already existed several thousand years ago, but then disappeared, becoming replaced by other, more promising animal husbandry (Kornilova, 2003). Since 1949, there has been an elk farm in the Pechora-Ilych reserve, where it was possible to domesticate European elk and study their biology, ecology, and diseases in detail. Studies on domestication have allowed for a deep analysis of elk ecology, behavior, physiological and morphological features, and diseases (Bełżecki, Miltko, and Michalowski, 2004).

It is possible to discuss the prospects of domestication of elk connected with the mass introduction of mechanical modes of transport in the everyday life of the Northern taiga regions. Still, there is no denying the versatile significance of domestication experiments of this valuable animal and the need for further development. Also important are considerations regarding the use of wild elk populations that make massive seasonal migrations in the wild. It is very fruitful for the development of science to properly combine its interests with the demands of the national economy (Khaziev, 2011).

However, the domestication of elk and their implementation on farms has not gone beyond experimental farms. This is mostly due to the elk's digestion's unexplored physiology, including the role of endobiont ciliates. The elk's diet is very diverse and includes shoots, leaves, the bark of various tree species and shrubs, multiple herbs, rhizomes and the leaves of aquatic plants, and mushrooms. Simultaneously, unlike most ungulates, elk needs a large amount of salt (Gorobtsova, Khezheva, Uligova, and Tembotov, 2015).

It is useless to undertake any wild animal's domestication without having a clear idea of its specific biological features. Many scientific articles on moose biology and physiology have been published in Russia and other countries. However, the information given in them is very contradictory in many respects. It is possible to clarify them only experimentally and use the data obtained about physiological processes in the future. And especially – the peculiarities of digestion - in the process of domestication of elk. There are data on the study of the composition of protozoa's fauna living in the digestive tract of many wild and commercial animals specific geographical habitats (Korchagina, 2012; Likhachev, Baimakova, and Koplik, 2010; Likhachev, Korchagina, Seryodkin, and Maksimova, 2016). However, individual studies on the elk's gastrointestinal fauna's qualitative and quantitative composition do not create a complete picture of the digestion processes in these animals. This hinders from making scientific recommendations on the composition of feed for elk, which could simplify domesticating these valuable game animals.

The objective of this study was formulated based on the foregoing conditions and encompasses a study of the numerical and species composition of the ciliates that inhabit the digestive tract of European elk to select the diet in different seasons with the prospect of creating elk farms for growing elk and processing its meat and skins.

2. MATERIALS AND METHODS:

The studies were conducted from 2002 to 2015. The objects of study were male and female European elk, commercially caught in the taiga and subtaiga zones of Russia: 10 individuals from the territory of Bolsheukovsky, Tevriz, Znamensky and Tarsky Districts of the Omsk Region and 10 individuals from the territory of the Nizyapetrovsky District of the Chelyabinsk Region (Figure 1).

The studies were carried out in the winter when the elk diet is dominated by branches from deciduous trees, spruce, pine and fir needles, bark, and the shoots of forest raspberries. In the southern parts of the range, elk feed on lichens growing on tree trunks, blueberry and lingonberry shrubs, and sedge where frequent thaws occur. In the winter, elk hardly drink or eat any snow, so as not to lose heat. The qualitative and quantitative composition of protozoa was coordinated with elk diet living in the Omsk and Chelyabinsk regions of Russia. To study the infusoria composition of the digestive tracts of the animals, samples were taken from the contents of the stomach and intestines of each individual immediately after slaughter and were fixed in a 4% formalin solution (Kornilova, 2004).

No more than 20 minutes passed after the elk were slaughtered until the samples were taken. During this time, the temperature of the stomach contents remained unchanged. Infusoria were fixed in the native state, without being affected by external environmental conditions. An incision was made in the wall of each part of the stomach and samples of the food lump were taken directly near the stomach wall. The food lump was squeezed through a filter and divided into two fractions: large particles larger than 0.1 mm and small particles smaller than 0.1 mm. Small particles were placed in a vessel with a 4% formalin solution. Pressing the food lump continued until the jar's bottom formed a solution with a precipitate of 2-3 cm, which contains the infusoria. The obtained material was used to determine the species and study the external morphology of endobiont infusoria.

The research was carried out within the framework of the University's research plan in compliance with international standards on the ethics of animals' treatment. The collection of animal material was carried out by agreement with hunters who shot animals for commercial purposes under the Russian Federation's license. All the conditions for the social welfare of animals were met.

In the laboratory, all material was analyze using cytochemical techniques (Likhachev, 2004). The Cytochemical methods used to identify the features of cell morphology of certain types of endobiont infusoria were performed as follows. Staining was performed only in vitro as the retainers used the liquid Karnua on ethanol (in the laboratory). Vital dyes were used in the study of cell morphology. A 0.001% aqueous solution of neutral red, methylene blue, and methylene green was used to contrast the organelles and cell contours. In the course of the study of General cell morphology, Romanovsky-Giemsa staining and Meyer hematoxylin were used. The cilia were stained with 1% alcohol solution of iodine. Cytostome was detected by adding 2-4% solution of baking soda. The skeletal elements were studied by staining with iodine and Lugol's liquid. To detect glycogen, Lugol's liquid (a solution of iodine in potassium iodide) was used. Identification of acidic mucopolysaccharides was performed using the Stidman method, with the color alcyan blue (pierce, 1968; Krylenko, Levin, Samoilova, 1979). The action of Sudan-3 detected neutral lipids. Lysosomes and digestive vacuoles were detected by the action of a 0.1% aqueous solution of neutral red. To study the processes of intracellular digestion, a 0.1% solution of Congo red was applied. Macronucleus, and in some cases, micronucleus, was detected by the action of 0.1% solution of glacial acetic acid. The core was stained using the Giemsa method with hydrolysis, using the Felgen nucleal reaction (hydrolysis time of 10 min) and Mayer acid hemalaunum staining using the standard method.

The data presented in work were obtained using two brands of BioMed-2 and MBI-6 light microscopes with a phase-contrast device and microphoton attachment. In the field, an MBI-3 microscope was used. The number of ciliates was determined by the calibrated drop method of the Goryaev counting chamber (Baimakova, 2003). Fixed infusoria were counted in the fields of view and in the Goryaev counting chamber. Camera Goryaeva – optical device for counting the number of trophozoites in a given volume of liquid. It consists of a thick slide having a rectangular recess (chamber) with a microscopic grid applied and a thin cover glass. The Goryaev cell grid consists of 225 large squares, 25 divided into 16 small squares. Dimensions of the small square of the Goryaev chamber 0.05×0.05 mm. Dimensions of the large square of the Goryaev camera 0.2×0.2 mm. The camera depth is 0.1 mm. Volume of liquid under 1 small square 0.00025 mm3 (MKL) = 1/4000 mm3 (microliter). Volume of liquid under 1 large square 0.004 mm3 (MKL) = 1/250 mm3 (microliter). The volume of the Goryaev chamber is 0.9 mm3 (MKL). In a square, the infusoria that lies inside it and those that touch the left and upper borders, are considered. Infusoria related to the right and lower borders are not counted.

Infusoria were measured using an ocular micrometer on random samples. Moreover, no less than 100 specimens were measured, and for single species no less than 10-20 specimens were found. To determine the percentage of species, all infusoria in several total preparations from each sample were calculated. The number of infusoria was calculated using the "calibrated drop" method, i.e. all trophozoites of infusoria that fell into a drop of 0.1 ml when diluting the sample in a fixator in a strict ratio of 1:2 were counted . A drop from the sample was taken with a precisely measured pipette, placed on a slide under a cover glass, and organisms in several fields of view of the microscope were taken into account by visual examination. The number of organisms in a drop is determined by counting, and then by converting to 1 ml. After viewing 3-5 preparations (each preparation in 10 fields of view), i.e. in 30-50 fields of view, the arithmetic mean for 1 field of view is found and the formula determined the number of organisms in 1 ml:

$D = Sd/ r2\ p$, where

D - the number of organisms studied in ml of liquid;

d - the number of organisms in one field of view (the arithmetic mean of the number of viewed fields of view);

r2 - the area of the lens field of view in mm (the ruler of the object-micrometer determines the radius r of the lens field of view);

S - cover glass area in mm (18x18 or 20x20).

p - volume of the drop.

Ofrioscolecid identification tables were used to assess ciliates' species composition (Bełżecki *et al*., 2004; Dogel, 1929). Variance analysis was used for statistical evaluation of the obtained indicators. These studies were carried out in triplicate; for statistical processing, the computer programs “Statistica for Windows V6.0” and “STATAN - 2006” were used.

3. RESULTS AND DISCUSSION:

It was found that all the species diversity of ciliates is concentrated only in the stomach of the animal, and protozoa were not found in the intestine (cecum, rectum). The distribution of endobionts in the stomach is heterogeneous. Their number and species diversity decrease from rumen to abomasum, which is associated with the medium's acidity in these sections of the stomach (Figure 2a, 2b and 2c). Ciliates in a slightly alkaline or neutral environment are in optimal conditions to thrive.

Representatives of the *Ophryoscolecidae* family with the genera *Entodinium*, *Diplodinium*, Epidinium, and the *Isotrichidae* family with one species *Dasytricha ruminantium*, form the basis of the infusoria fauna of the elk’s stomach. Among the rumen's ciliate population, the leading genus of ofrioscolecid is the genus *Entodinium,* represented in the rumen by 10 species for elk in both populations (Figure 3).

In the rumens of elk in the Chelyabinsk population, the dominant abundant *Entodinium* ciliate is *E. simulans-dubardi* with a frequency of occurrence of 54.1 ind./ml, which is 17% of the total number of *Entodinium*s in the rumen. *E. nanellum* (51.9 ind./ml) and *E. exiguum* (49.2 ind./ml) make up 16% of the total number of representatives of this genus in the rumen, and *E. ovinum* (44.6 ind./ml) makes up 15% of all *Entodinium*s in the rumen. It was found the species *E. furca nanellum* only in the rumen with a total number of 38.1 ind./ml, which accounted for 11% of the total number of rumen *Entodinium*s. Representatives of the species *E. simplex* account for 10% (35.2 ind./ml) of the total representatives from this genus. The least represented species of the genus *Entodinium* in the rumen are *E. bursa,* *E. alces, E. caudatum,* and *E. longinucleatum*, which together make up about 15%.

In the rumens of elk in the Omsk population, the dominant abundant *Entodinium* ciliate is *Entodinium furca nanellum* at 65.2 ind./ml, which is 26% of the total number of rumen *Entodinium*s. The subdominant species of the genus *Entodinium* include *E. exiguum* (56.3 ind./ml), *E. simulans-dubardi* (40.8 ind./ml) and *E. ovinum* (36.8 ind./ml), each of which make up 23%, 17% and 15%, respectively, of the total representatives of this genus in the rumen. The species *E. simplex* accounts for 11% (26.4 ind./ml) of the total, which is similar to this species' population among rumen *Entodinium* in the elk from the Chelyabinsk population. Low abundance in the rumen is noted for the species *E. bursa, E. alces, E. caudatum, E. nanellum, E. longinucleatum*, ranging from 1.1 to 7.4 ind./ml, which amounts to 7% of the total number of all *Entodinium*s in the rumen. Upon study of the infusoria population in the reticulum, there was a decrease in the number of *Entodinium*s to 139.8 ind./ml (Chelyabinsk population) and 109.5 ind./ml (Omsk population), as well as a decrease in the number of species of *Entodinium*s to seven in elk of the Chelyabinsk population and six in the Omsk population (Figure 4).

It should be noted that for the elk in the Chelyabinsk population, the dominant genus of ciliates, as in the rumen, is from the genus *Entodinium,* and the most numerous species of *Entodinium*s is *E. simulans-dubardi* with a frequency of occurrence of 43.2 ind./ml, which is 31% of the total number of reticulum *Entodinium*s. Subdominant species include *E. ovinum* (27.6 ind./ml), *E. nanellum* (22.5 ind./ml), and *E. simplex* (23.4 ind./ml).The abundance of *E. ovinum*is 20% of the total number of reticulum *Entodinium*s, and *E. nanellum* and *E. simplex* make up 16% each. The abundance of the species *E. longinucleatum* in the elk reticulum of the Chelyabinsk population only slightly decreased and amounted to 15.2 ind./ml or 11% of the total number of species of this genus in the reticulum. The least frequent species of the genus *Entodinium* in the reticulum are *E. bursa* and *E. alces* with a population of 5.7 ind./ml and 2.2 ind./ml, respectively, which makes up 6% of the total number of representatives of this genus in the reticulum.

In the reticulum of elk in the Omsk population, the dominant abundant *Entodinium* ciliate is *E. simulans-dubardi* with a frequency of 33.6 ind./ml is 31% of the total number of *Entodinium*s in the reticulum. *Entodinium furca nanellum* with a total number of 29.4 ind./ml is the subdominant species, which makes up 27% of the total number of reticulum *Entodinium*s. The species *E. exiguum, E. ovinum,* and *E. simplex* have approximately the same numbers in the reticulum – from 12.6 to 18.5 ind./ml, which is 12%, 13%, and 16% of the total number of reticulum *Entodinium*s for each species, respectively. Meanwhile, the *Entodinium* species *E. furca nanellum* and *E. exiguum* were not observed in elk reticulum from the Chelyabinsk population.

In the omasum of elk in both populations, the number of *Entodinium*s is extremely small and the species diversity consists of four (for the Chelyabinsk elk population) and two species (for the Omsk elk population) of ciliates (Figure 5). *E. nanellum* is a subdominant species in this section of the stomach, numbering 9.3 ind./ml or 27%of the total number of omasum *Entodinium*s. The species *E. exiguum* (5.7 ind./ml) and *E. bursa* (1.4 ind./ml) are extremely infrequent in this section and together account for 11% of the total number of omasum *Entodinium*s. The omasum of elk in the Omsk population contain two species, out of which *E. simulans-dubardi* (11.8 ind./ml), as in the reticulum, is the dominant species and accounts for 86% of the total population of this genus. The species *E. furca nanellum* with 2.6 ind./ml makes up 14% of the total number of omasum *Entodinium*s. The genus *Diplodinium* also makes up infusoria fauna in the stomach of elk in both populations; it is only necessary to note that the number of species and the number of diplodiniums are significantly inferior to the leading genus of *Entodinium* (Figure 6).

Five species of diplodiniums were noted for the elk rumen of the Chelyabinsk population, the dominant of D. monocanthum with 56 ind./ml, which accounts for 48% of genus *Diplodinium* species in the rumen. *Diplodinium rangiferi* (29.2 ind./ml) in the rumen is a subdominant species and makes up 27% of the total number of rumen diplodiniums. The species *D. anisacanthum* (19.2 ind./ml), *D. bubalidis consors* (3.3 ind./ml) and *D. bubalidis bubalidis* (4.9 ind./ml) account for 17%, 5% and 3% of rumen diplodiniums, respectively.

In the reticulum of elk in the Chelyabinsk population, the species diversity of diplodiniums went down to three species with a total number of 19.8 ind./ml, however, the dominant and subdominant species are the same as in the rumen (Figure 7). The most numerous species of diplodiniums in the reticulum are D. monocanthum (10.1 ind./ml) or 53% of the total number. The subdominant species is *Diplodinium rangiferi* (7.1 ind./ml), which is 37% of the total number of diplodiniums in the reticulum. Representatives of the species *D. bubalidis bubalidis* (2.6 ind./ml) make up 10% of the reticulum's total number. In the omasum of elk in the Chelyabinsk population, an extremely scarce amount of diplodiniums is noted of only two species with a low number of ciliate species *D. monocanthum* (2.4 ind./ml) and *D. bubalidis bubalidis* (1.2 ind./ml).

Four species of diplodiniums were found in the rumen of the Omsk population. The dominant species was *D. monocanthum* (33.2 ind./ml), accounting for 66% of the total number of rumen diplodiniums (Figure 6). The species *Diplodinium rangiferi* in the rumen became subdominant with many 14.1 ind./ml, which amounts to 28% of the total number of diplodiniums in the rumen. It should be noted that both species of diplodiniums are identical to those of the elk in the Chelyabinsk population, but are significantly inferior in number.

The most infrequent rumen species consists of *D. bubalidis consors* (1.2 ind./ml) and *D. bubalidis bubalidis* (1.6 ind./ml), which together make up only 6% of the total number of rumen diplodiniums. Diplodiniums were not found in the reticulum and omasum of elk in the Omsk population. The genus *Epidinium* is represented in the stomach of elk in both populations by only one species – *Ep. ecaudatum ecaudatum*, and the abundance of this species in the rumen is much higher than in the reticulum, while it was not found at all in the omasum (Figure 8).

There are very few representatives of the genus *Dasytricha*: in the rumen, with the highest numbers, they make up from 9.6 to 14.2 ind./ml (2% and 5%, respectively), in the reticulum - from 5.2 to 9.1 ind./ml (4% and 3% of the total number of ciliates in the reticulum) , in the omasum - from 1.2 to 1.4 ind./ml, which is about 1% of the total number of ciliates in the omasum for elk in both populations (Figure 9). Representatives of this species were found in all sections of the elk stomach of both populations.

Elk feed in the snowless times of year is unlimited, and the animals, as a rule, have it available to them almost any place. However, even in the summer, with an abundance of leaves, grassy and wetland vegetation, the basis of elk nutrition is branch feed. In severe frosts, the percentage of branches and needles from fir, spruce, and juniper in the total food mass of elk is 85%, 15%, and 8%, while in warming periods, it is 22%, 0%, and 0%. This diet sets the pace for a normal digestion cycle (Baimakova, 2003; Kornilova, 2003).

Animals that feed on plant foods face obtaining essential amino acids contained only in animal proteins. In ruminants, this process is carried out in a complex stomach (which is why they are called “protist-eaters”). They use ciliates and other protozoa that breed in the rumen, reticulum and omasum to digest feed. Many researchers suggest that ciliates can serve as one source of nitrogen for the host, since the amount of nitrogen in a ciliate’s body is very significant and, between the rumen to the abomasum, they are easily digested (Chornaya, 2015).

When studying the infusoria population of each European digestive tract of elk, it is found and described 17 species of ciliates in the Chelyabinsk population and 16 species in the animals of the Omsk population. The anatomical structure of and conditions in the rumen almost entirely meet the life requirements of microorganisms. The first three sections – the rumen, the reticulum, and the omasum –form the so-called stomach and are lined with a multilayer epithelium. The stomach is devoid of digestive glands and only bacterial fermentation occurs there, involving the symbionts inhabiting it, which can exist only in a slightly alkaline or neutral environment. The ciliates use the food fed into the rumen for their nutrition, building their own bodies, and digesting the fiber using bacteria living in symbiosis with them.

The feeding regimen affects the digestibility of feed, rumen function and ciliate populations. Food that decomposes relatively quickly in the rumen (fruits and herbaceous food) goes into the omasum more quickly. After processing in the rumen, solid foods (branches, shoots) get into the reticulum and require further decomposition to be digested. Any change in the composition of the feed disturbs the metabolic processes in the animal’s body, leading to further changes in the normal functioning of other systems (Korchagina and Likhaschev, 2005). Based on the analysis of the data obtained, it was found that the rumen is the most densely populated part of the stomach in elk from both populations, and is where the most species were found.

Utilization in the rumen of ruminant monosaccharides (glucose, fructose, xylose), coming from the feed, and mainly formed during the hydrolysis of polysaccharides, is carried out mainly by rumen microorganisms (Dehority, 1996). Ciliates inhabit the rumens of animals in huge quantities (Agatha, 2004). The population of endobiont ciliates consists of representatives of the *Ophrioscolecidae* family, and they dominate both in terms of species diversity and in numbers (Aescht, 2001).

The conditions existing in the rumen contribute to the development of abundant microflora there. Among them are a favorable and constant temperature, a rumen environment with near-neutral acidity, and an abundance and constant influx of nutrients. In addition to processes for the microbial decomposition of feed, very active synthesis processes occur in the rumen. Considering the qualitative and quantitative composition of stomach ciliates in the animals studied, it can be said that the rumen is a very important specialized chamber in a complex stomach, where protozoa develop. In this capacity, they use fiber as feed. The dominant species of the omasum's *Entodinium*s, as in the rumen and reticulum, of elk in the Chelyabinsk population is *E. simulans-dubardi* frequency of occurrence of 16.9 ind./ml, which is 52% of the total number of omasum *Entodinium*s. Rumen ciliates are actively involved in the metabolism of easily fermentable carbohydrates, proteins, and lipids (Bergen, 2004). Therefore, cooperation, or symbiosis, takes place. In the rumen, the feed is delayed long enough so that the components of plant fibers accessible to microorganisms can be broken down.

Ofrioscolecides absorb carbohydrates entering the rumen, mainly in the form of starch. All ciliates of this family can use starch grains for food. Endobiont ciliates can extract starch grains from the liquid fraction of the stomach contents, where the ciliates make the starch inaccessible to bacteria (Gocmen, Dehority, Talu, and Rastgeldy, 2001). As with most representatives of the deer family (*Cervidae*), in elk, the dominant genus of ciliates is *Entodinium*, which is made up of 10 species. The proportion of all *Entodinium*s in the rumen, in the most densely populated section of the stomach, is 63% among the elk in the Chelyabinsk population, and 73% of the total number of protozoa in the Omsk population. They turn food proteins and broken down fiber components into glycogen, which serves as a source of nutrition for both the ciliates and the ruminants. Offrioscolecid accounts for 98% of the total infusoria composition in the stomachs of elk in the Chelyabinsk population and 96% in the Omsk population. Thus, ciliates' positive role is that they, by consuming an often inferior vegetable protein, turn it into a complete one for the animal's nutrition.

During the seasonal change of feed, microorganisms adapt to new feeds, and digestion is not impaired. The food processed by the ciliates rolls into balls in another part of the stomach - the reticulum - and from there returns to the mouth as cud. Then the food goes to the third section - the omasum, then to the next section - the abomasum. Here, food is processed by digestive juices.

The diet of ofrioscolecid is quite diverse, and there are known specializations in different species. The smallest species of the genus *entodynia* feed on bacteria, starch grains, fungi and other small particles. Many medium and large ofrioscolecides absorb plant tissue particles, which make up the bulk of the contents in the rumen. The endoplasm of some species is clogged with plant particles.

4. CONCLUSION:

Thus, the species composition of the symbiotic fauna in European elk depends on their habitat, but namely on a diet. Whether this gives any advantages to the host remains unclear. All these questions are of great practical interest since it has been discussed about the digestion of ruminants – important objects of animal husbandry. The faunistic and ecological studies conducted make it possible to assess the infusoria fauna in the stomachs of elk to identify the distribution patterns of endobiont ciliates in different parts of the elk's stomach, and the nature of the spatial dynamics of the number and species diversity of ciliates in the host organism. This significantly expands the understanding of fauna, morphology, biology, and ecology of endobiont infusoria in European elk and commercial ungulates that live in other habitats that differ in the climate and composition of animals diet.

With the results obtained, it can be hypothesized that the features of infusoria fauna are determined by the species-related ties of the hosts and the natural and territorial conditions of their habitat. Further research prospects must assess the possible ways endobiont ciliates are exchanged between different hosts. A further study of the fauna, biology, and ecology of wild ruminant ciliates in different world regions should be carried out to expand the ideas about these unique and well-adapted protozoa.

5. CONFLICT OF INTEREST STATEMENT:

The authors declare that they have no conflict of interest.

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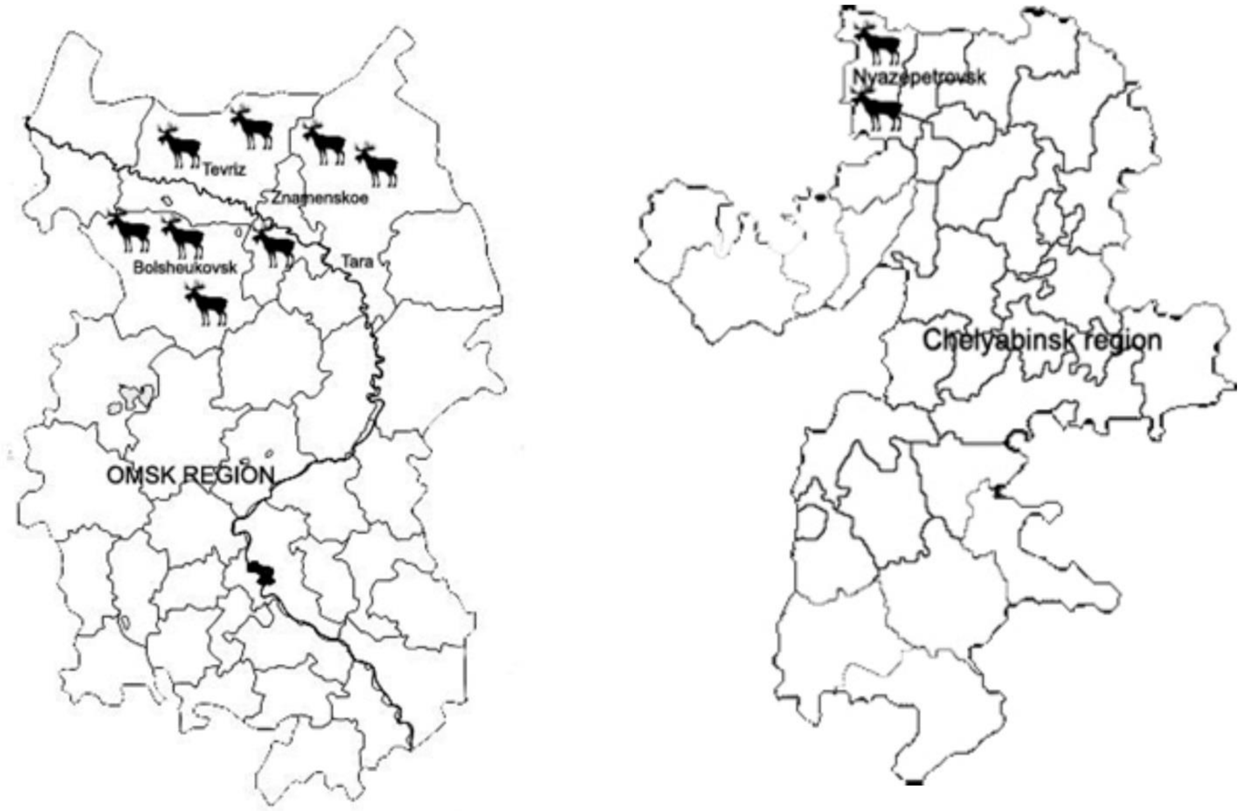

Omsk Region **Chelyabinsk Region**

Figure 1. *European elk hunting areas in Russia. Source: The authors.*

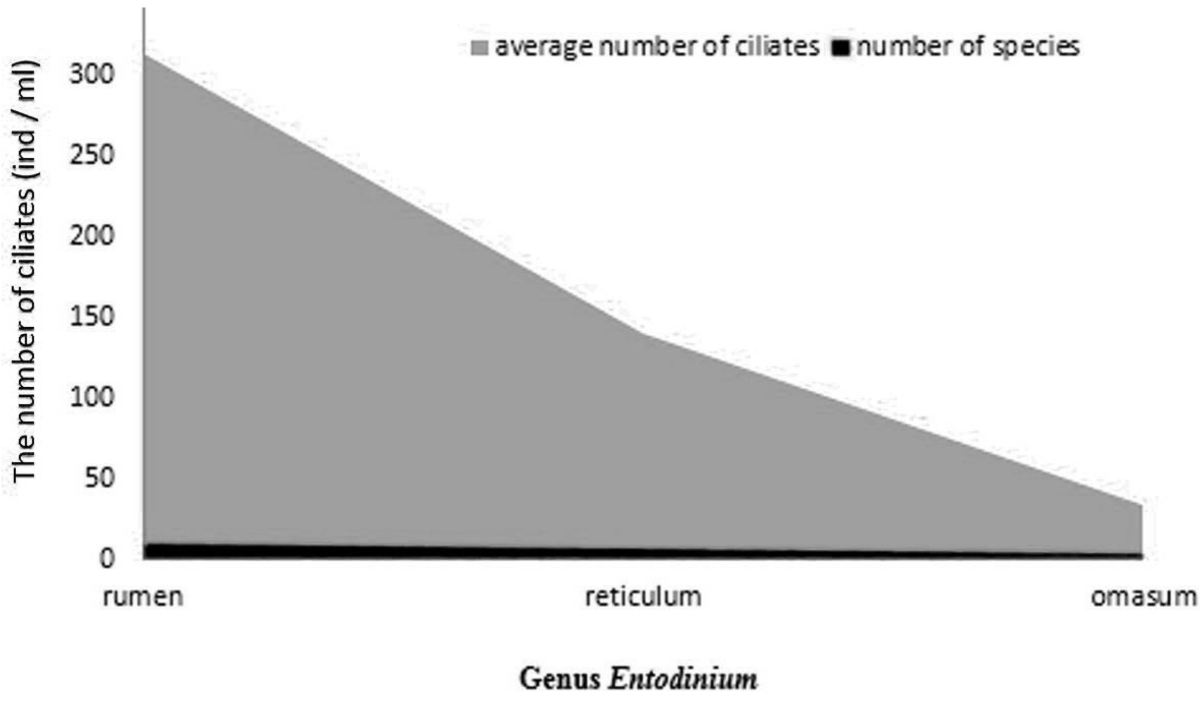

Figure 2a. *The average number of ciliates, ind./ml (number of individuals per milliliter), and the number of species by stomach section (Genius Entodinium).*

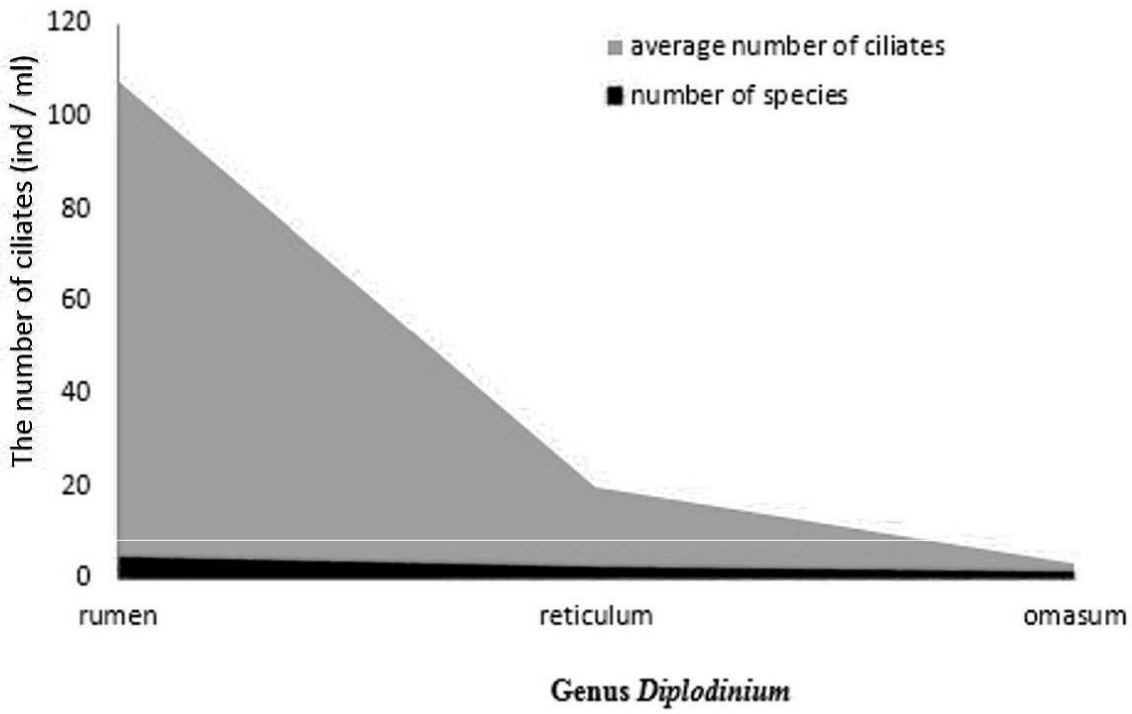

Figure 2b. *The average number of ciliates, ind./ml (number of individuals per milliliter), and the number of species by stomach section (Genius Diplodinium).*

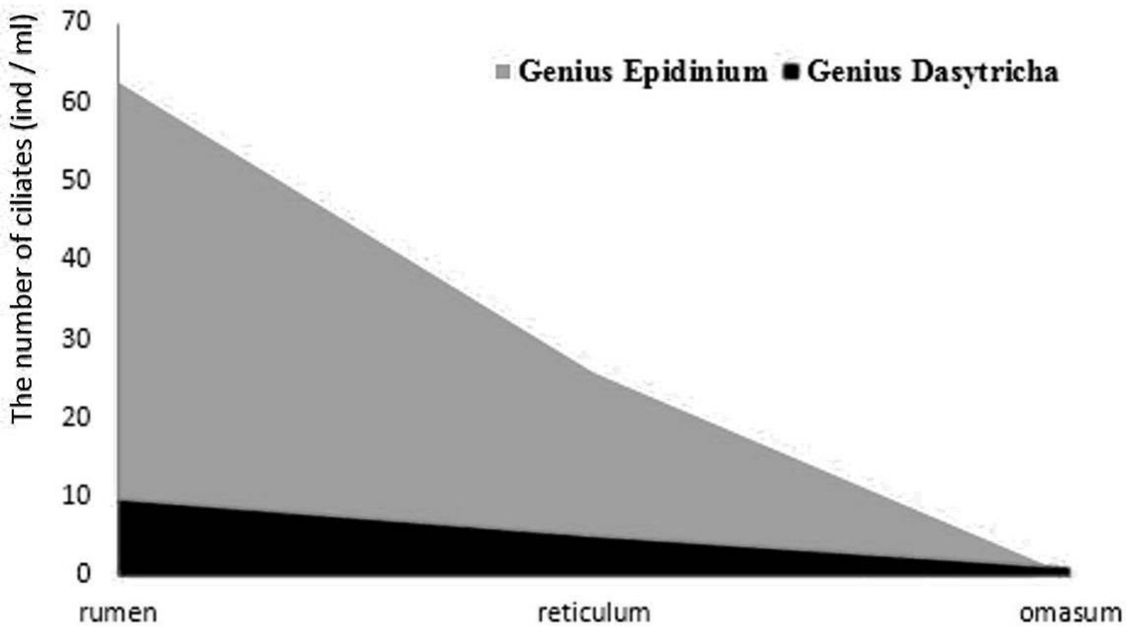

Figure 2c. *The average number of ciliates, ind./ml (number of individuals per milliliter), and the number of species by stomach section (Genius Epidinium, Genius Dasytricha).*

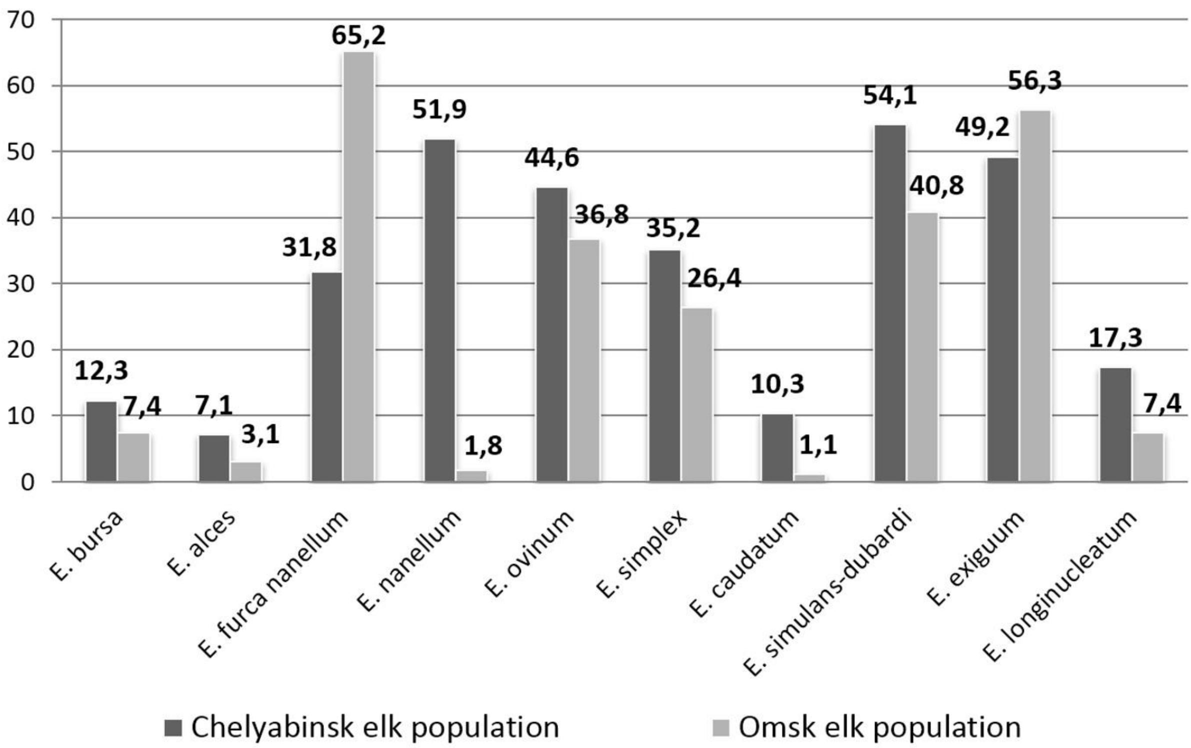

Figure 3. *The number of ciliates of the genus Entodinium in the rumen of elk in the Chelyabinsk and Omsk populations, ind./ml (number of individuals per milliliter)*

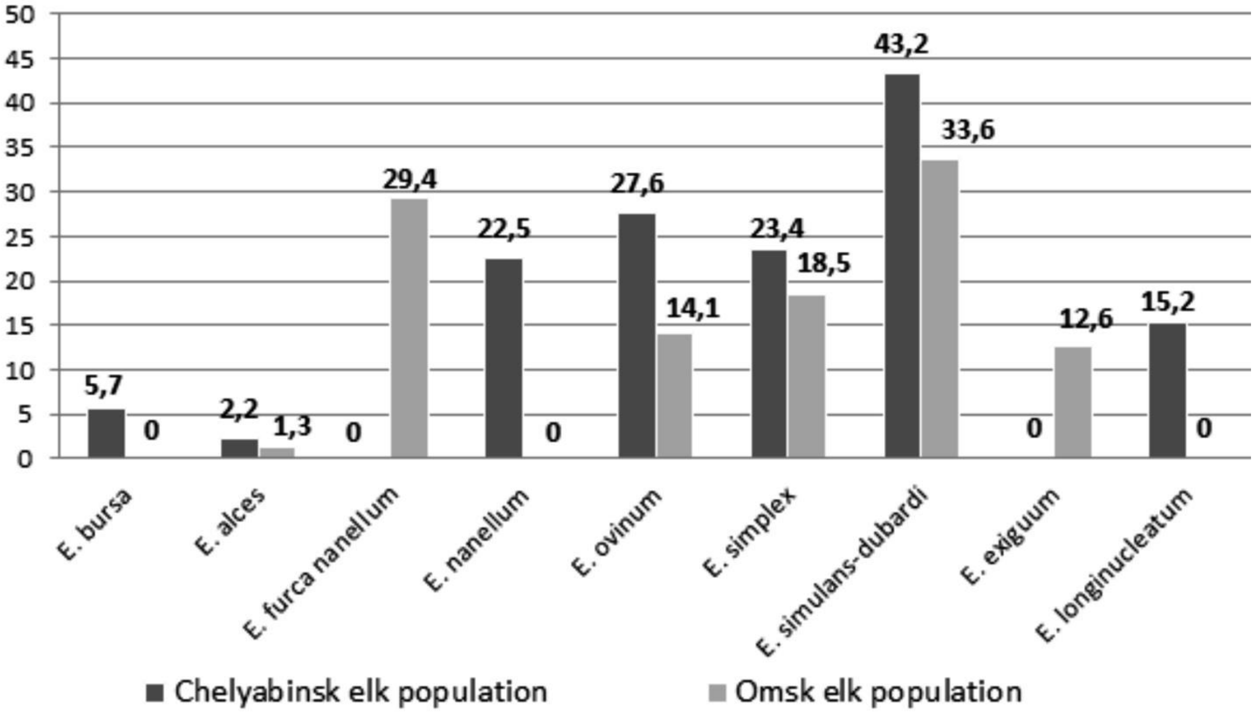

Figure 4. *The number of ciliates of the genus Entodinium in the reticulum of elk in the Chelyabinsk and Omsk populations, ind./ml (number of individuals per milliliter)*

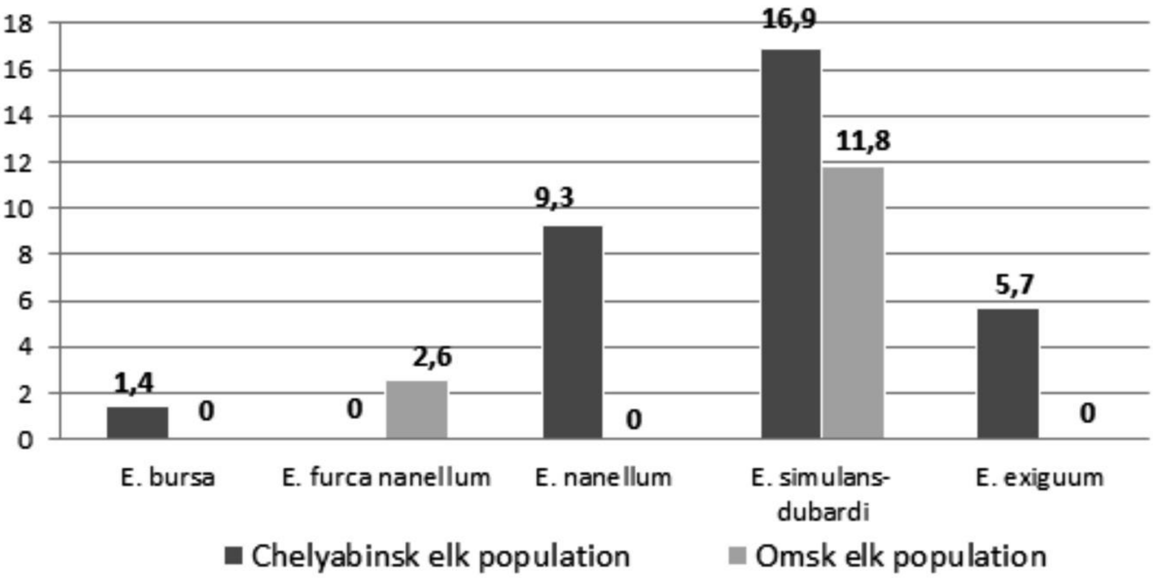

Figure 5. *The number of ciliates of the genus Entodinium in the omasum of elk of the Chelyabinsk and Omsk populations, ind./ml (number of individuals per milliliter)*

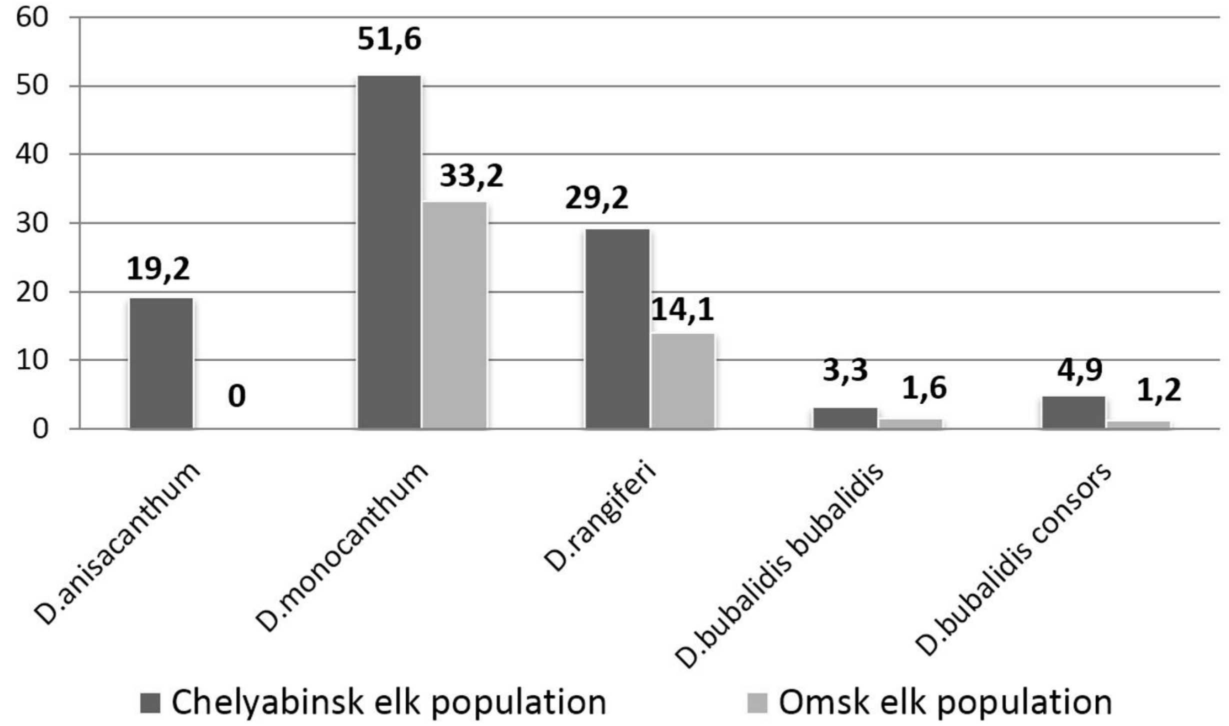

Figure 6. *The number of ciliates of the genus Diplodinium in the rumen of elk in the Chelyabinsk and Omsk populations, ind./ml (number of individuals per milliliter)*

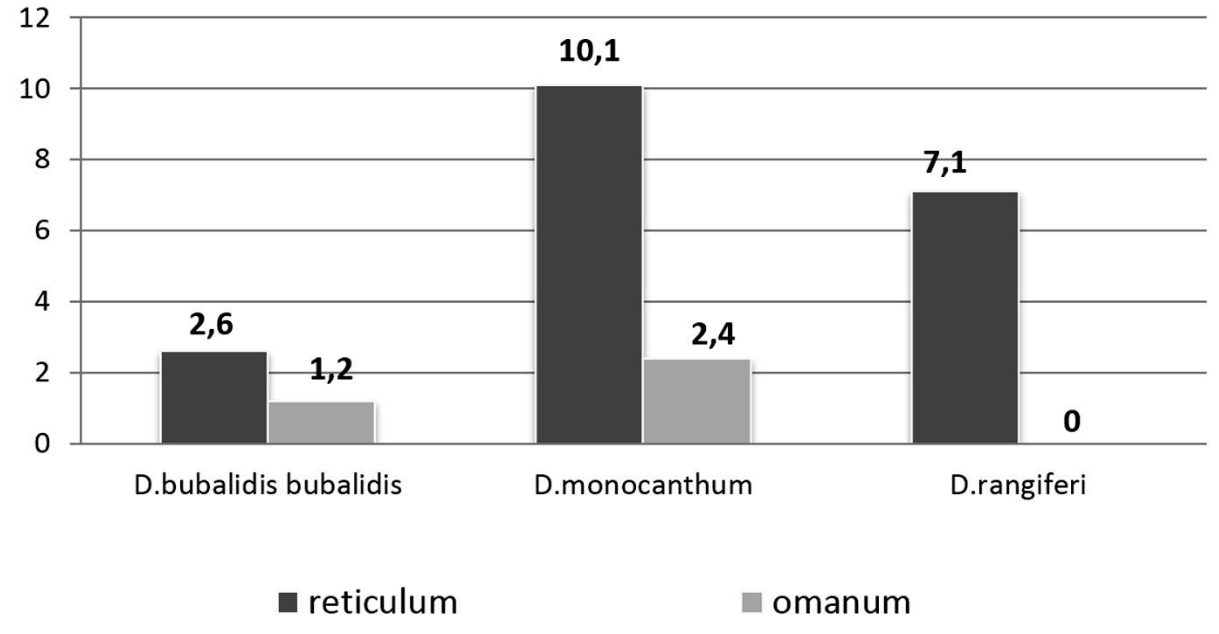

Figure 7. *The number of ciliates of the genus Diplodinium in the reticulum and omasum of elk in the Chelyabinsk populations, ind./ml (number of individuals per milliliter)*