Praca oryginalna *Original paper*

Effect of balanced supplementary feeding in winter on qualitative and quantitative changes in the population of microbes colonizing the rumen of red deer[1)]

SEBASTIAN GNAT, ROMAN DZIEDZIC*, ANETA NOWAKIEWICZ, PRZEMYSŁAW ZIĘBA**, ALEKSANDRA TROŚCIAŃCZYK, BARBARA MAJER-DZIEDZIC, GRAŻYNA ZIÓŁKOWSKA, SŁAWOMIR BEEGER*, MARIUSZ WÓJCIK*

Sub-Department of Veterinary Microbiology, Institute of Biological Bases of Animal Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland
*Sub-Department of Animal Ecology and Wildlife, Department of Zoology, Animal Ecology and Wildlife, Faculty of Biology and Animal Breeding, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
**State Veterinary Laboratory, Słowicza 2, 20-336 Lublin, Poland

Received 15.02.2017 *Accepted 20.06.2017*

Gnat S., Dziedzic R., Nowakiewicz A., Zięba P., Trościańczyk A., Majer-Dziedzic B., Ziółkowska G., Beeger S., Wójcik M.

Effect of balanced supplementary feeding in winter on qualitative and quantitative changes in the population of microbes colonizing the rumen of red deer

Summary

Ruminants are a group of animals that process and assimilate their food in a unique manner. The functioning of the digestive tract of these animals is closely related to the abundance and composition of microbes in the forestomach, which is a complex ecosystem of bacteria, protozoa and fungi. Microorganisms present in the rumen, and in particular their effect on physiological processes in the body, influence the animal's physical condition and state of health. Microbiological examination of rumen microbiota ecology is hindered by a lack of selective growth media, as well as by difficulties in isolating bacteria in vitro and accurately identifying them. The aim of the study was to evaluate the effect of food consumed by red deer (Cervus elaphus) on the diversity of their rumen microbiota. Microbes were compared in two study periods. In autumn the animals' diet came exclusively from natural plant sources, while in winter, supplementary feeding was introduced, including specially prepared fodder. The study showed that in deer that did not receive the special fodder in winter, but only natural plant components, the abundance of bacterial flora decreased significantly compared with what it was in autumn, unlike in animals that did receive the fodder, whose composition and caloric value substantially increased the activity of rumen microbes. In winter, changes in proportions of different morphological forms of rumen bacteria were observed, as well as a decline in their total number, particularly in the animals that did not receive the pellets. A similar decline was also observed in the populations of yeasts and protozoa in winter. To sum up the results of the study, the use of the specially prepared high-calorie fodder in winter was shown to influence the rumen ecosystem of red deer. The most significant factor improving the condition of deer receiving supplementary fodder during this period is the stabilization of bacterial flora in the rumen, which directly contributes to the efficiency of digestion.

Keywords: balanced diet, Cervus elaphus, deer, rumen, ruminal bacteria, wild animal microflora

Ruminants are a group of animals characterized by a unique way of processing and assimilating food. Proper functioning of the digestive tract of ruminants is closely linked to the abundance and composition of the microbiota in the forestomach, which is a complex ecosystem comprising a vast diversity of bacteria, protozoa and fungi (6). Microorganisms making up the microflora of the rumen, and in particular the interactions between them and physiological processes in the body, directly affect the animal's physical condition and state of health (3, 9, 17, 28). However, our knowledge of the rich diversity of microbial species in the rumen and our

[1)] Study financed by the General Directorate of the State Forests National Forest Holding in Poland (ZKE-U-6).

understanding of their role in the adaptation of ruminants to different living environments are still incomplete (9).

As research on the ecology of the rumen microbiota has been discredited by a lack of truly selective culture media and difficulties in isolating bacteria in in vitro cultures and identifying them by direct microscopic examination, techniques based on DNA analysis are currently being used (9, 16, 29).

Most ruminal microbes are anaerobes and bacteria requiring the presence of various substances (inorganic salts, sugars or acids), which not only are nutrients, but also create suitable conditions (pH, osmotic pressure, redox potential) in the rumen environment (29, 30).

The cycle of metabolic changes in the rumen has made it possible to identify microbes commonly occurring in it, as well as to determine the ecological interactions taking place between populations (8). The most active ruminal microorganisms are cellulolytic bacteria, mainly of the genera *Ruminococcus* and *Fibrobacter*, as well as fungi and protozoa. The second group consists of microorganisms that acquire energy from the decomposition of sugars, such as *Prevotella*, *Butyrvibrio*, *Selenomonas*, *Megasphaera*, *Veillonella* and *Peptostreptococcus* (3, 9, 29). All of the main bacterial shapes have been found, including short and long bacilli, cocci and coccobacilli, curved cylinders, comma-shaped and helical forms, and spirals. The total number of bacteria in the rumen fluid ranges from 10^9 to 10^{12} cells/ml (10, 15).

Rumen protozoa, which are mainly ciliates, are usually classified on the basis of characteristics of their morphological structure, such as the location of the cilia or the number of skeletal plates (8, 10). The number of protozoa in the rumen can reach 10^6 cells/ml and may account for as much as half of the microbial mass in the rumen (15, 21). Many ciliates store small granules of starch, thereby modulating the speed of fermentation and protecting the animal from metabolic acidosis (37). A substantial number of rumen bacteria may be consumed by protozoa, causing an inverse correlation between the density of protozoa and bacterial cells (15, 37).

Fungi are also encountered in the rumen environment, although for many years they were considered to be strictly aerobic organisms. Some rumen organisms that were previously thought to be ciliate protozoa have been classified as fungal spores (26, 36). Fungi have a relatively long life cycle, from 24 to 32 hours, which substantially exceeds the time that food remains in the rumen. For this reason, only ruminants fed low-quality fodder seem to have large fungal populations, accounting for up to 8% of the biomass of the rumen (16, 21, 38). Another explanation for the low abundance of fungi in the rumen may be the presence of bacteriocins produced by cellulolytic bacteria densely colonizing the niches of the forestomach, which can inhibit their growth (1, 15, 29).

The rumen ecosystem of livestock is generally qualitatively and quantitatively stable, despite the fact that, as an organ, the rumen is not a closed environment (7, 18). In wild animals, microflora is subject to certain changes, particularly in terms of quantity, depending on seasonal changes in food. During the growing season, the dominant food source are herbaceous plants with low fibre content, while outside the growing season the diet is dominated by bushes, shrubs and trees, with high fibre content and fewer calories. Another characteristic of the diet of wild ruminants under moderate climate conditions is the amount of food eaten, which during the growing season is 2-4 times the amount eaten outside of the season (14, 19). During spring and summer, fermentation activity is considerably higher than in autumn and winter (25). Improvement observed in the health of animals after a change to a diet that increases the abundance of rumen microbes is indicative of the beneficial effect of the microorganisms. In the case of livestock, the use of fodder that improves rumen fermentation is beneficial in terms of production (10, 15, 20, 34).

The available literature contains numerous reports on the microbiota of domesticated ruminants and its effect on their well-being (7, 17, 24, 35), but there is very little information on free-living ruminants, particularly with respect to the effect of the type of food consumed on parameters of the forestomach microbiota. Red deer is a species commonly found in Poland (4, 13). The composition of the deer's annual diet depends on many factors, and the main factor is the availability of vegetation in the given habitat and the season. The winter period is special because of the decreasing abundance of food base combined with the increased energy demand due to the costs of thermoregulation (4).

The aim of the study was to evaluate the effect of food consumed by red deer (*Cervus elaphus*) on the diversity of the rumen microbiota. Microorganisms were compared following the change in diet between the autumn and winter periods, taking into account the effect of supplementary feeding with special fodder.

Material and methods

The study was conducted in West Pomerania, Poland, in two forested areas of about 6,000 ha (Forest District Warcino, area A) and about 8,300 ha (Forest District Trzebielino, area B). In terms of physiography, the two areas were very similar (natural topography, water conditions, soil quality and vegetation). In the forests of both areas, coniferous habitats were dominant, and pine accounted for about 75% of woody species, with sparse underbrush and predominance of *Vaccinium myrtillus* in the undergrowth. In terms of food abundance and quality, these areas are assessed as average and poor. Red deer densities are relatively high (about 50 individuals per 1,000 ha), so there is considerable competition for vegetation as a natural food source. In both areas, deer in winter are provided with feed, consisting mainly of hay, beets, carrots and cabbage. The amount of wet bulky fodder provided in winter does not exceed 1 kg per individual per day, and hay does not exceed 0.3 kg. Both the wet and dry bulky fodders have low energy value (Tab. 1). From the beginning of December, the deer in plot A were additionally supplied with special feed in granular form with energy value of 6.7 MJ and fibre content of about 15%. This feed also contained a mineral and vitamin supplement commonly used in feeding domestic cattle, in the recommended concentration (0.3%) (Tab. 1). The feed mixture was placed in hay racks intended for winter feeding of

Tab. 1. Qualitative composition of red deer diet in the two study seasons

Autumn	Winter	
	Not including supplementation with pellets	Including supplementation with pellets*
▪ natural forest vegetation (herbaceous vegetation, trees and bushes)	▪ forest vegetation dominated by trees and bushes ▪ supplementary feeding with a mixture of hay, fodder beet, carrot and cabbage	▪ forest vegetation dominated by trees and bushes ▪ supplementary feeding with a mixture of hay, fodder beet, carrot and cabbage ▪ supplementary feeding with pellets containing ➢ oat grain ➢ maize grain ➢ oat bran ➢ flax seed cake ➢ meadow hay ➢ vitamin and mineral supplement for cattle The pellets contained no preservatives, growth inhibitors or hormone additives
The average energy values of diet [MJ/kg]		
3.2	4.6	11.3

Explanation: *The pellets used in winter as supplementary feed for red deer have a patent pending in the Patent Office of the Republic of Poland (UPRP)

deer in an amount of 0.3 kg of pellets per individual per day. The animals consumed the mixture placed in the hay racks within 2-3 days.

During hunting, rumen contents were collected in sterile containers in an amount of about 500 ml from each individual. The material was kept under refrigeration and shipped within 12 hours to the laboratory in a heat-stable box. All animals from which samples were taken were over 3 years of age. In October, experimental material was collected from 12 red deer (stags) in each of the two study areas. In January, samples were taken from 11 stags in plot A, where the pellets were provided, and from 15 stags in plot B.

The rumen contents were drained, and the solid phase was discarded. Only the rumen fluid was used for further analysis. Due to the very strict growth requirements of rumen microbes (strictly anaerobic conditions, substrates supplemented with specific growth substances), the procedure was limited to direct examinations consisting of determination of the total number of bacteria, yeasts and protozoa. The rumen fluid was diluted 1 : 100. The number of protozoa was determined in a Fuchs-Rosenthal counting chamber using trypan blue staining (0.2%), and the number of yeasts in a Burker chamber without staining. The abundance of bacteria, their morphological types and their response to Gram-staining were evaluated on microscope slides with identical dilutions. The abundance of microbial populations of various morphological types was determined in three replications, with 20 separate, randomly chosen fields of view analysed each time. The microorganisms were counted using an Olympus BX51 (Japan) microscope, immersion lens with a magnification of 100 ×, and a High Resolution Camera Olympus DP72 (Japan). Bacteria and protozoa were counted both manually and by an automatic system built into the software CellF* (Olympus, Japan). There were no differences in the results obtained between the systems applied to count the microorganisms. Examples of the field of view used in microscopy are shown in Figure 1. Preparations from cultures of known titers, made in the same manner as controls.

Results and discussion

In autumn, the total number of bacteria and yeasts and yeast-like fungi in the rumen was 25.73×10^9. In January, in the area without supplementary feeding with special pellets (area B), the number decreased to 10.69×10^9, while in the forest habitats where the diet of the deer was supplemented with the pellets (area A) it was 20.49×10^9. Differences were also observed in the percentages of different morphological forms of bacteria and of yeasts and yeast-like fungi (Fig. 1). In October, the percentage of cocci was 28.45%, whereas in January, the percentage of this form increased to 41.81% in the

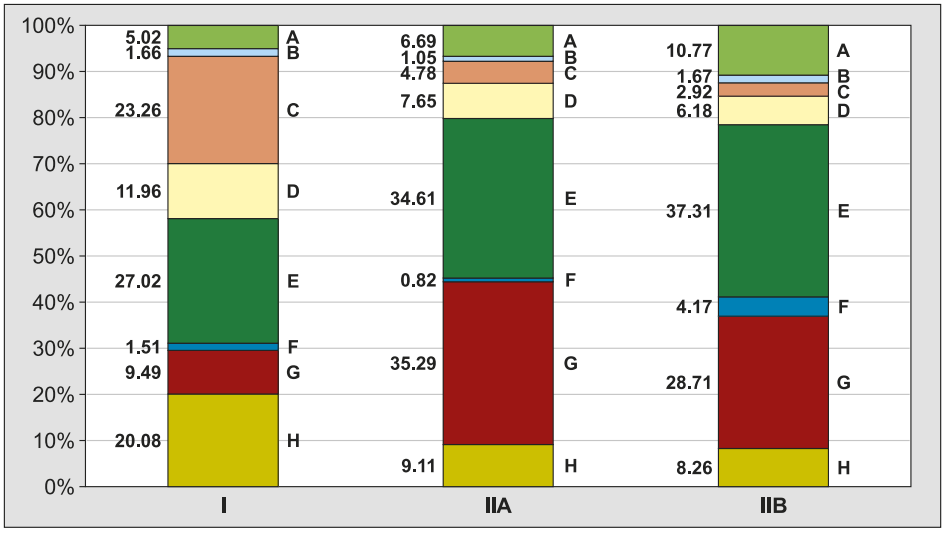

Fig. 1. Percentage shares of different morphological forms of microbes in the two study seasons

Explanations: I – rumen microbes in autumn without supplementary feeding; IIA – rumen microbes in winter with supplementary feeding; IIB – rumen microbes in winter without supplementary feeding; A – cells of yeasts and yeast-like fungi; B – spiral-shaped bacteria; C – long thin rod-shaped bacteria; D – coccobacilli; E – cocci; F – oval bacteria; G – straight rod-shaped bacteria; H – curved rod-shaped bacteria

area without supplementation with the pellets, and to 37.09% in the area where the pellets were provided. In October, the second most dominant morphological type of microorganism in the rumen in terms of percentage share were long thin rods (24.49%). In January, the percentage of this form decreased to 3.27% in area B (without pellets) and to 5.12% in area A. The percentage of bacteria in the shape of curved rods was 21.14% in October, and decreased in January to 9.76% in area A and to 9.26% in area B. Straight rods accounted for 9.99% of the total number in autumn, while in winter their percentage increased to 37.82% in area A and to 32.18% in area B. These four morphological forms were dominant, as in each period of the study and in each area they constituted about 80% of the total number of bacteria (Tab. 2). The abundance of yeasts and yeast-like fungi in the rumen ecosystem did not differ significantly in the study periods, irrespective of the type of diet (Tab. 2).

Tab. 2. Population sizes of different morphological forms of bacteria, yeasts and yeast-like fungi (n/ml – n × 10^9) in the rumen of red deer in the two study seasons ($\bar{x}$ ± SD)

Morphological type	Autumn – October		Winter – January			
	No supplementation with pellets (areas A + B) n = 12		Supplementation with pellets (area A) n = 11		No supplementation with pellets (area B) n = 15	
	n/ml	%	n/ml	%	n/ml	%
Curved rods	$5.44^{a} \pm 1.63$	21.14	$2.00^{b} \pm 0.54$	9.76	$0.99^{c} \pm 0.26$	9.26
Straight rods	$2.57^{c} \pm 0.80$	9.99	$7.75^{a} \pm 2.24$	37.82	$3.44^{b} \pm 0.96$	32.18
Ovals	$0.41^{a} \pm 0.08$	1.59	$0.18^{b} \pm 0.04$	0.88	$0.50^{a} \pm 0.10$	4.67
Cocci	$7.32^{a} \pm 1.31$	28.45	$7.60^{a} \pm 1.51$	37.09	$4.47^{b} \pm 0.78$	41.81
Coccobacilli	$3.24^{a} \pm 0.74$	12.59	$1.68^{b} \pm 0.40$	8.2	$0.74^{c} \pm 0.22$	6.92
Long thin rods	$6.30^{a} \pm 2.26$	24.49	$1.05^{b} \pm 0.27$	5.12	$0.35^{c} \pm 0.09$	3.27
Spiral	$0.45^{a} \pm 0.12$	1.75	$0.23^{b} \pm 0.07$	1.12	$0.20^{b} \pm 0.80$	1.87
Total bacteria	$25.73^{a} \pm 8.39$	100.00	$20.49^{a} \pm 8.58$	100.00	$10.69^{b} \pm 3.73$	100.00
Yeasts and yeast-like fungi	$1.36^{a} \pm 0.37$	100.00	$1.47^{a} \pm 0.47$	100.00	$1.29^{a} \pm 0.28$	100.00

Explanation: a, b, c – means with different superscript letters differ significantly in the row at $p \leq 0.05$

The number of G+ bacteria in October was 14.23 × 10^9; in January it was 10.10 × 10^9 in the area where the pellets were supplied and 7.94 × 10^9 in the area where no pellets were provided (Tab. 3). In area A the percentage share of G+ bacteria in the total number of microorganisms was similar in autumn and winter – 55.31% and 49.29%, respectively, while in the area where no pellets were supplied it increased to 74.27%. In terms of the morphological forms of bacteria, the percentage shares of long thin rods in the experimental groups were similar, ranging from 2.28% to 3.03%, but substantial differences were noted for the other morphological forms. The percentage of cocci in the rumen was the highest in all of the groups analysed: in autumn it was 44.05%, and in January it was 48.17% in the area where no pellets were provided (area B) and 62.9% in area A. The percentage of curved rods was initially 27.16%, but in January it decreased to 3.17% in area A and to 6.05% in area B. The corresponding percentage of straight rods increased from 11.61% to 20.34% and 35.06% (Tab. 3).

Tab. 3. The population sizes of different morphological forms of G+ bacteria (n/ml – n × 10^9) in the two study seasons ($\bar{x}$ ± SD)

Morphological type	Autumn – October		Winter – January			
	No supplementation with pellets (areas A + B) n = 12		Supplementation with pellets (area A) n = 8		No supplementation with pellets (area B) n = 5	
	n/ml	%	n/ml	%	n/ml	%
Curved rods	$3.86^{a} \pm 0.85$	27.16	$0.32^{b} \pm 0.12$	3.17	$0.48^{b} \pm 0.17$	6.05
Straight rods	$1.65^{b} \pm 0.54$	11.61	$2.05^{ab} \pm 0.61$	20.34	$2.78^{a} \pm 0.86$	35.06
Cocci	$6.27^{a} \pm 1.69$	44.05	$6.35^{a} \pm 1.62$	62.9	$3.82^{b} \pm 1.11$	48.17
Coccobacilli	$2.06^{a} \pm 0.57$	14.43	$1.14^{b} \pm 0.32$	11.31	$0.62^{b} \pm 0.24$	7.82
Long thin rods	$0.39^{a} \pm 0.13$	2.74	$0.24^{a} \pm 0.06$	2.28	$0.24^{a} \pm 0.12$	3.03
Total	$14.23^{a} \pm 4.06$	100.00	$10.10^{ab} \pm 3.66$	100.00	$7.94^{b} \pm 2.87$	100.00

Explanation: as in Tab. 2

Tab. 4. The population sizes of different morphological forms of G+ bacteria (n/ml – n × 10^9) in the two study seasons ($\bar{x}$ ± SD)

Morphological type	Autumn – October		Winter – January			
	No supplementation with pellets (areas A + B) n = 12		Supplementation with pellets (area A) n = 8		No supplementation with pellets (area B) n = 12	
	n/ml	%	n/ml	%	n/ml	%
Curved rods	$1.57^{a} \pm 0.47$	13.6	$1.68^{a} \pm 0.32$	16.17	$0.51^{b} \pm 0.21$	18.54
Straight rods	$0.92^{b} \pm 0.28$	8.00	$5.70^{a} \pm 1.55$	54.86	$0.65^{b} \pm 0.24$	23.64
Ovals	$0.41^{a} \pm 0.21$	3.56	$0.18^{a} \pm 0.14$	1.73	$0.50^{a} \pm 0.16$	18.18
Cocci	$1.06^{a} \pm 0.24$	9.22	$1.26^{a} \pm 0.33$	12.13	$0.65^{b} \pm 0.21$	23.64
Coccobacilli	$1.18^{a} \pm 0.30$	10.26	$0.53^{b} \pm 0.17$	5.10	$0.13^{c} \pm 0.05$	4.73
Long thin rods	$5.91^{a} \pm 2.04$	51.39	$0.81^{b} \pm 0.22$	7.79	$0.11^{c} \pm 0.06$	4.00
Spirals	$0.45^{a} \pm 0.18$	3.91	$0.23^{b} \pm 0.11$	2.21	$0.20^{b} \pm 0.09$	7.27
Total	$11.50^{a} \pm 3.32$	100.00	$10.39^{a} \pm 3.08$	100.00	$2.75^{b} \pm 0.96$	100.00

Explanation: as in Tab. 2

The number of G− and questionably stained bacteria was 11.50 × 10^9 in October; in January, it was 10.39 × 10^9 in area A and 2.75 × 10^9 in the area where no pellets were provided (B) (Tab. 4). All morphological forms were

present within this group of bacteria, but their percentages were varied. The percentage share of spiral forms was the lowest and the most stable, as in January it was 2.21% (the lowest) in area A and 7.27% (the highest) in area B. In autumn, long thin rods accounted for 51.39% of the total number of G− bacteria; in January the percentage decreased to 7.79% in area A and to 4.00% in area B. The percentage of straight rods was 8.00% in autumn, and increased in January to 23.64% in area B and to as much as 54.86% in area A. A similar upward trend was observed in morphological cocci, but the increase was smaller: in October the percentage was 9.22%, and in January 12.13% in area A and 23.64% in area B. In autumn, long thin rods were dominant (51.39%) and, together with curved rods and coccobacilli, accounted for 75.25% of the total number of G− microbes. In January, in the area with the supplementary pellet feeding (area A), straight rods were dominant (54.86%) and, together with curved rods and oval forms, accounted for 83.16% of all G− bacteria. In area B, no single morphological form dominated, as the percentage of curved rods, straight rods, cocci and oval forms ranged from 18% to 24% and together accounted for 84.00% of all bacteria (Tab. 4).

The number of protozoa in October was 304 × 10^3 per ml of rumen contents. In January, it increased to 424 × 10^3 in the area where the deer were not supplied with the pellets (area B) and to 506 × 10^3 in area A (Tab. 5). The numbers of protozoa classified as large and medium-sized were stable: 11-13 × 10^3 and 87-89 × 10^3 per ml, respectively. The changes in their percentage shares of the total number of protozoa were due to changes in the number of small protozoa. The number of small protozoa in autumn was 204 × 10^3 per ml of rumen contents, while in January it was 324 × 10^3 in area A and 406 × 10^3 in area B (Tab. 5).

The rumen ecosystem is a special environment with a vast diversity of microbes whose qualitative and quantitative composition determines the assimilation of consumed plants in ruminants. Digestion takes place mainly owing to enzymes produced by the flora of the rumen. Fermentation processes lead to the production of SCFA (short-chain fatty acids), which are used to meet the energy requirements of ruminants, as well as for synthesis of amino acids and proteins by the bacteria themselves (2, 3, 10, 12, 16, 32). Diet affects the composition of the rumen microbiota and fermentation pathways. When cellulose content is high, acetic acid becomes dominant, whereas concentrates contained in fodder increase the production of propionic acid (7, 31, 33). For wild animals, winter is a particularly difficult period to survive. The diet of red deer is then based on browse trees and shrubs, with large amounts of fibre and low nutritional value. Our study compared the composition of the rumen microbes of red deer *Cervus elaphus* in autumn (October) and winter (January). In winter, the animals' natural diet was supplemented with wet bulky feed (fodder beets, carrot and cabbage) and dry bulky feed (meadow hay), as well as specially developed pellets (their composition and the energy value of the components will be available after completion of the patent procedure in the Patent Office). The bacterial system of the rumen of the deer, whose autumn diet was based mainly on herbaceous food sources, was found to contain a large number of G+ curved rods, G+ cocci and G− long thin rods (Tab. 2, 3). In winter, the population size of different morphological forms of bacteria changed, and the total number of bacteria decreased substantially, particularly in animals whose diet was not supplemented with the pellets (Tab. 2).

The seasonal change in the availability of food substantially decreased the number of G+ and G− curved rods, G+ and G− coccobacilli, G+ cocci, G− long thin rods and G− spiral forms (Tab. 3 and 4). The number of bacteria increased considerably in deer whose diet was supplemented with the pellets. This increase was due to the large number of G− straight rods, whereas the abundance of other morphological forms of bacteria decreased significantly (G+ curved rods, G+ coccobacilli and G− long thin rods) or remained at a similar level as in the autumn period (G+ straight rods, G+ cocci, G+ long thin rods, G− curved rods, and G− cocci) (Tab. 2, 3, 4).

The study showed that the activity of bacterial flora in winter decreased significantly in red deer whose diet was not supplemented with the pellets. Supplementary feeding substantially increased this activity, and the total number of bacteria did not differ significantly ($p \leq 0.05$) from that noted in autumn. Foroozandeh et al. (11), comparing the ability of different rumen microorganisms to decompose fibres from alfalfa hay and wheat straw, found that bacteria were more active in this degradation than protozoa or fungi. Our study found that the number of yeasts and yeast-like fungi remained at a similar level irrespective of the type of diet (Tab. 2), but in winter there was a significant increase ($p \leq 0.05$) in the number of protozoa of the family Ophryoscolecidae, irrespec-

Tab. 5. Abundance of protozoa of the family *Ophryoscolecidae* (n × 10^3/ml) in the rumen contents of red deer in the two study seasons ($\bar{x}$ ± SD)

Protozoa	Autumn – October		Winter – January			
	No supplementation with pellets (areas A + B) n = 12		Supplementation with pellets (area A) n = 8		No supplementation with pellets (areas A + B) n = 12	
	n/ml	%	n/ml	%	n/ml	%
Large (≥ 60 µm)	13.2^a ± 4.42	4.28	11.1^a ± 4.23	2.17	11.6^a ± 3.11	2.83
Medium-sized (40-60 µm)	86.8^a ± 24.1	28.61	88.9^a ± 20.6	17.59	88.4^a ± 28.4	20.75
Small (≤ 40 µm)	204.0^c ± 42.6	67.10	406.0^a ± 101.1	80.24	324.0^b ± 110.8	76.41
Total	304.0^c ± 120.3	100.00	506.0^a ± 135.5	100.00	424.0^b ± 144.6	100.00

Explanation: a, b, c – $p \leq 0.05$ in the row between values n/ml; Genus*: Entodinium* – classified in the results as small (32.7 to 49 µm), Genus: *Epidinium* – classified in the results as medium-sized and large (96-111 µm)

tive of the type of food consumed. A study by Rezaeian et al. (27) shows that fungi, which can account for up to 20% of the total microbiological biomass, are better able to colonize ligneous components of plants and degrade them more efficiently than bacteria. Protozoa, on the other hand, are associated with damaged regions of fresh plant material and decompose starch from plant fibres and protein from bacteria. As they pass from the rumen to the further parts of the stomach, they are digested and supply the ruminants with high-value animal protein. Lee (22), however, found that protozoa were not capable of fermenting the cell walls of extracts from orchard hay. In the author's opinion, physiological interactions may occur between rumen microbes that lead to initial degradation of fodder and to continuation of the later stages of this process by protozoa. This was confirmed by Foroozandeh et al. (11), who demonstrated that degradation of fodder components was more efficient when protozoa were not monocultures and were added to bacteria in a later period.

The results obtained in the present study regarding the activity of protozoa in winter confirm this line of reasoning.

To sum up the results of the study, the composition of fodder had both qualitative and quantitative influence on the rumen ecosystem of red deer during the winter period. The high degree of stabilization of bacterial flora, both G+ and G−, in the rumen of deer whose diet was supplemented with the pellets may have improved the digestive activity of enzymes produced by these microorganisms, which indirectly contributed to the improvement observed in the condition of the deer (2, 7, 23).

Although rumen bacteria, due to their dominance and metabolic diversity, are believed to play the primary role in digestion of food, interactions between different groups of microbes are involved in the degradation and fermentation of plant material. Better understanding of these processes requires further research (5, 6).

References

1. *Akin D. E., Borneman W. S.*: Role of rumen fungi in fiber degradation. J. Dairy Sci. 1990, 73, 3023-3031.
2. *Annison E. F., Bryden W.*: Perspectives on ruminant nutrition and metabolism. Nutrition Res. Rev. 1998;11, 173-198.
3. *Bach Knudsen K. E.*: Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed. Sci. Technol. 1997, 67, 319-338.
4. *Bobek B., Morow K., Perzanowski K., Kosobudzka M.*: Jeleń – monografia przyrodniczo-łowiecka. Wyd. Świat, Warszawa 1992.
5. *Cheng K. J., Kudo H., Duncan S. H.*: Prevention of fungal colonization and digestion of cellulose by the addition of methyl cellulose. Can. J. Microbiol. 1991, 37, 484-487.
6. *Church D. C.*: Ruminant animal: digestive physiology and nutrition. Prentice-Hall, England Cliffs, 1993.
7. *Claesson M. J., Jeffery I. B., Conde S., Power S. E., O'Connor E. M., Cusack S., Harris H. M. B., Coakley M., Lakshminarayanan B., O'Sullivan O., Fitzgerald G. F., Deane J., O'Connor M., Harnedy N., O'Connor K., O'Mahony D., van Sinderen D., Wallace M., Brennan L., Stanton C., Marchesi J. R., Fitzgerald A. P., Shanahan F., Hill C., Ross R. P., O'Toole P. W.*: Gut microbiota composition correlates with diet and health in the elderly. Nature 2012, 488, 178-184.
8. *Dehority B. A., Tirabasso P. A.*: Antibiosis between Ruminal Bacteria and Ruminal Fungi Appl. Environ. Microbiol. 2000, 66, 2921.
9. *Fernando S. C., Purris H. T., Najer F. Z., Sukharikov L. O., Krehbiel C. R., Nagaraja T. G., Roe B. A., DeSilva H.*: Rumen microbial population dynamics during adaptation to a high-grain diet. Appl. Environ. Microbial. 2010, 7482--7490.
10. *Flint H. J.*: The rumen microbial ecosystem – some recent developments. Trends Microbiol. 1997, 5, 483-488.
11. *Foroozandeh A. D., Rezaeian M., Balaly G. R., Alikhani M.*: Relative contributions of ruminal bacteria, protozoa and fungi to degradation of forage fiber fractions. J. Animal and Vet. Adv. 2009, 8, 603-607.
12. *Frey J. C., Pell A. N., Berthiaume R., Lapierre H., Lee S., Ha J. K., Mendell J. E., Angert E. R.*: Comparative studies of microbial populations in the rumen, duodenum, ileum and faeces of lactating dairy cows. J. Appl. Microbiol. 2010, 108, 1982-1993.
13. *Gnat S., Trościańczyk A., Nowakiewicz A., Majer-Dziedzic B., Ziółkowska G., Dziedzic R., Zięba P., Teodorowski O.*: Experimental studies of microbial populations and incidence of zoonotic pathogens in the faeces in red deer (Cervus elaphus). Lett. Appl. Microbiol. 2015, 61, 446-452.
14. *Hancock D. T., Besser J., Lejeune M., Davis M., Rice D.*: The control of VTEC in the animal reservoir. Int. J. Food Microbiol. 2001, 66, 71-78.
15. *Hobson P. N.*: The rumen microbial ecosystem. Elsevier Appl. Sci., London and New York 1988.
16. *Hungate R. E.*: The Rumen and Its Microbes. Academic Press, New York 1966.
17. *Jarvis G. N., Strompl C., Burgess D. M., Skillman L. C., Moore E. R., Joblin K. N.*: Ruminal methanogens from grazing cattle. Curr. Microbiol. 2000, 40, 327-332.
18. *Kent A. D., Smith D. J., Benson B. J., Triplett E. W.*: Establishing populations of Megasphera elsdenii YE34 and Butyrvibrio fibisolvens YE44 in the rumen of cattle fed high grain diets. J. Appl. Microbiol. 2003, 95, 621-630.
19. *Kocherginskaya S. A., Aminov R. I., White B. A.*: Analysis of rumen bacterial diversity under two different diet conditions using denaturing gradient gel electrophoresis, random sequencing, and statistical ecology approaches. Anaerobe 2001, 7, 119-134.
20. *Kolida S., Gibson G. R.*: Prebiotic capacity of inulin-type fructans. J. Nutrition 2007, 137, 2503S-2506S.
21. *Krause D. O., Denman S. E., Mackie R. J., Morrison M., Rae A. L., Attwood G. T., McSweeney C. S.*: Opportunities to improve fiber degradation in the rumen: microbiology, ecology and genomics. FEMS Microbiol. Rev. 2003, 27, 663-693.
22. *Lee S. S., Ha J. K., Cheng K. J.*: Relative contributions of bacteria, protozoa and fungi to in vitro degradation of orchard grass cell walls and their interactions. Applied Environ. Microbiol. 2000, 66, 3807-3813.
23. *Li Z., Wright A. G., Liu H., Bao K., Zhang T., Wang K., Cui X., Yang F., Zhang Z., Li G.*: Bacterial community composition and fermentation patterns in the rumen of sika deer (Cervus nippon) fed three different diets. Microb. Ecol. 2015, 69, 307-318.
24. *Lopez S., McIntosh F. N., Wallace R. J., Newbold C. J.*: Effect of adding acetogenic bacteria on methane production by mixed rumen microorganisms. Anim. Feed Sci. Technol. 1999, 78, 1-9.
25. *Nagaraja T. G., Chengappa M. M.*: Liver abscesses in feedlot cattle: a review. J. Anim. Sci. 1998, 76, 287.
26. *Orpin C. G.*: The role of ciliate protozoa and fungi in the rumen digestion of plant cell walls. Anim. Feed Sci. Technol. 1984, 10, 121-143.
27. *Rezaeian M., Beakes G. W., Parker D. S.*: Distribution and estimation of anaerobic zoosporic fungi along the digestive tracts of sheep. Mycol. Res. 2004, 108, 1227-1233.
28. *Robinson C. J., Bohannan J. M., Young V. B.*: From structure to function: the ecology of host-associated microbial communities. Microbiol. Mol. Biology Rev. 2010, 74, 453-476.
29. *Russell J. B., Onodera R., Hino T.*: Physiological Aspects of Digestion and Metabolism in Ruminants, chap. 24. Academic Press, San Diego, CA 1991.
30. *Russell J. B., Rychlik J.*: Factors that alter rumen microbial ecology. Science 2001, 292, 1119-1122.
31. *Sharp R., Ziemer C. J., Stern M. D., Stahl D. A.*: Taxon-specific associations between protozoal and methanogen populations in the rumen and a model rumen system. FEMS Microbiol. Lett. 1998, 26, 71-78.
32. *Smith R. A.*: Impact of Disease on Feedlot Performance: A Review. J. Anim. Sci. 1998, 76, 272.
33. *Tajima K., Arai S., Ogata K., Nagamine T., Matsui H., Nakamura M., Aminov R. I., Benno Y.*: Rumen bacterial community transition during adaptation to high-grain diet. Anaerobe 2000, 6, 273-284.
34. *Vanloo J.*: How chicory fructans contribute to zootechnical performance and well-being in livestock and companion animals. J. Nutrition 2007, 137, 2594S--2597S.
35. *Vasoest P. J., Robertson J. B., Lewis B. A.*: Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 1991, 74, 3483-3597.
36. *Weimer P. J., Waghorn G. C., Odt C. L., Mertens D. R.*: Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. J. Dairy. Sci 1999, 82, 122-134.
37. *Williams A. G., Coleman G. S.*: The Rumen Protozoa. Springer-Verlag, New York 1992.
38. *Windham W. R., Akin D. E.*: Rumen fungi and forage fiber degradation. Appl. Environ. Microbiol. 1984, 48, 473-476.

Corresponding author: Sebastian Gnat PhD, Akademicka 12, 20-033 Lublin, Poland; e-mail: sebastian.gnat@up.lublin.pl