## ABSTRACT

Ciliates are the group of microorganisms having an important impact on metabolism of nutrients in the rumen. The ability of protozoa to digest and metabolize the structural and storage polysaccharides were the subject of numerous studies. On the other hand, their contribution to metabolism of the carbohydrates synthesized in the rumen has not been studied to date.

The structural carbohydrates produced in the rumen are chitin and murein. Thy are the components of the fungal and bacterial cell wall. The literature data show that bacteria and fungi are engulfed by ciliates living in the rumen. According to the commonly accepted opinion, bacteria are the major source of protein for protozoa. In opposite to this the role of fungi in the covering of the nutritional requirements of rumen ciliates remains still unknown in spite of their potential role as a source of protein and carbohydrates. This concerns especially to the freely swimming zoospores. The fungal polysaccharide is chitin. Unfortunately, our present knowledge on the possibility of rumen ciliates to utilize the mentioned carbohydrate is restricted to the data concerning of the total chitinolytic activity of natural rumen fauna as well as two species of protozoa. Some results on the chitin degrading enzymes are also available. On the other hand, no ability of the other species of rumen ciliates to digest and metabolize the mentioned carbohydrate were examined to date. The problems listed above were the aim of the studies presented in this dissertation and ciliates *Eudiplodinium maggii* were the subject of the reported researches. These protozoa belong to the common representatives of rumen fauna. The ciliates were isolated from the rumen fluid of sheep and cultured *in vitro* or were inoculated to the rumen of animals after removal of their natural ciliate fauna. The objectives of the studies were: 1) to determine the effect of the supplementation of culture medium with chitin or fungal zoospores on the survival and population density of ciliates *Eudiplodinium maggii* maintained *in vitro;* 2) to examine the ability of this ciliate species to digest and metabolize chitin and the zoospores of rumen fungi; 3) to identify and characterize the enzymes involved in the degradation of chitin in the ciliate cells; 4) to determine the influence of the examined ciliates on the concentration and production of chitin in the rumen; 5) to examme the contribution of ciliates *Eudiplodinium maggii* to metabolism of chitin in the rumen.

The experiments performed *in vitro* showed that the survival of protozoa in the medium composed of the culture salt solution, and chitin or the lyophilized fungal zoospores (0.25 mg/ml/d) was shorter than 4 days. There was also stated that daily ration of chitin (0. 12, 0.25 and 0.37 mg/ml/d) had no effect on the survival of protozoa. The supplementation of the medium with lyophilized rumen fluid (3.75 mg/ml/d) and wheat gluten (0.08 mg/ml/d) prolonged the survival of ciliates up to 4 and 8 days, respectively. The medium composed of the culture salt solution, meadow hay (0.3 mg/ml/d) and wheat gluten (0.08 mg/ml/d) created the appropriate conditions for protozoa to survive for a period of 28 days at least. Chitin supplemented to this medium stimulated the development of the population of *Eudiplodinium maggii* and a positive correlation was found between the density of ciliate population and chitin dose. The supplementation of the lyophilized zoospores of rumen fungi to the same medium was not univocal and a positive effect was observed only during the 8 last days of the cultivation period.

An increase in the concentration of volatile fatty acids (VFA) was found when the protozoa were incubated with chitin or with fungal zoospores. The production rate of VFA was 45 and 46.3 pM/protozoan/h whereas the endogenous production did not exceeded 31 pM VFA/ ciliate cell/h. The molar proportions of acetic acid were 72.0 and 77.7% of total acids and these of butyric and propionic acids - 21.0 and 12.2 and 6.9 and 11.0%, respectively. The obtained results are the evidence that chitin and carbohydrates present in fungal zoospores were utilized by protozoa in energy yielding processes.

The chitinolytic ability was examined using of the crude enzyme preparation of ciliates *Eudiplodinium maggii*. The total chitinolytic activity was 0.035 µM released N-acetylglucosamine /mg protein/min and the optimal condition to degrade colloidal chitin were 4.5 pH and 50°C. There was also stated that crude enzyme preparation enabled the complete degradation of chitin and end products of this reaction were N- acetylglucosamine, chitobiose and chitotriose. The zymographic studies of crude enzyme preparation of ciliates *Eudiplodinium maggii* revealed the presence of enzymes exhibiting the features of endochitinase, exochitinase and chitobiase. Two enzymes from each of the listed groups were identified there.

Carboxymethylchitin (CM-chitin), p-nitropheny 1-N-acetylglucosamine and p-nitrophenyl-N ,N-diacetylchitobiose were the specific substrates used to determine the activity of particular groups of enzymes. The most effective degradation of the first of them was found at 5.5 pH

and the temperature 50°C. The optima!conditions to degrade the last two substrates were 4.5 pH and 55 and 45°C, respectively . The degradation velocity of CM-chitin and the nitrophenyl derivatives of chitobiose and chitotriose were - 0.028, 0.067 and 0.033 µM of released products/mg protein/min, respectively . The presented results show that the lowest activity revealed the enzymes exhibiting features of endochitinase and the highest - chitobiase.

The separation of protozoa! protein by filtration on Sephadex G-150 column revealed, that chitinolytic enzymes exhibiting the features of endochitinase and chitobiase were present in the fractions No. 16-25 whereas exochitinase - in No 19-25. Only single enzymes belonging to the listed groups were identified in the examined fractions. Further examination showed that molecular mass of endochitinase, chitobiase and exochitinase was 62, 51 and 41 kDa, respectively . Apart of characterization, the result of molecular filtration of crude enzyme preparation were the partially purified enzymes.

The *in vivo* experiments were performed using three sheep. The studies were divided to the control and experimental periods. The sheep were having no protozoa in the rumen during the first of them and only *Eudiplodinium maggii* during the second.

The performed experiments showed that the density of the developed population of *Eudiplodinium maggii* was 14.2-20.3x $10^3$/ml rumen fluid. The numbers of fungal zoospores during the control and experimental period were 9.2-10.3 and 10.0-12.2x$10^3$/ml of rumen fluid, respectively. They were determined by qPCR method. The statistically significant differences between the zoospore numbers in the control and experimental period were found at 4 and 8 h after feeding of sheep. The chitinolytic activities of the rumen fluid of sheep in the control and experimental periods were 2.4-3.4 and 3. 1-3.9 µM N-acetylglucosamine /g D.M. rumen fluid/min, respectively ($P<0.05$). The chitynolytic activity of protozoa contributed only to 7.7-13.9%o of the total chitinolytic activity in the rumen fluid.

lt was stated that the chitin contents in the rumen fluid of sheep during the control and experimental period were 6. 1-6.8 and 6.7-8. 1 mg/g D.M. of rumen fluid, respectively ($P<0.05$). The smallest quantity of chitin was found just before the feeding of sheep and the largest - at 4 h thereafter. It was also stated that chitin derived from zoospores contributed to 81-90% of the total quantity of this polysaccharide in the rumen fluid. On the other hand the dry matter of single zoospore was 2.1 ng. and chitin - 0.23 ng i.e. about 11% of D.M.

It was showed that the total chitin content in the rumen fluid of sheep in the control and experimental periods were 2.I and 3.I g, respectively and the relevant production rate

- 2.3 and 3.I g/I2 h, ($P<0.05$). Thus, the calculated total pool of chitin which could be utilized by ciliates was 6.2 g/I2 h.

It was found, that chitin content in the protozoa isolated from the rumen just before feeding was I.I mg/g their D.M. It increased by about 36% during the first 4 h after meal. The decrease in chitin content by 13% was observed after the next 4 h. These data showed that the polysaccharide content in the ciliate cells diminished by about 3% per hour. This figure seem to characterize the digestion rate of chitin present in the zoospores engulfed by ciliates. Other calculations showed that dry matter of the single cell of ciliate was I96.5 ng and chitin content - 0.27 ng. The obtained results enabled the estimation of the mean content of chitin in all protozoa present in the rumen of sheep. It was about 54 mg i.e. about 1.7% of the total chitin present in the rumen fluid.

**Conclusion:** The performed studies confirmed the ability of protozoa *Eudiplodinium maggii* to digest and utilize chitin synthesized in the rumen. However, this polysaccharide seems to play the role of only supplementary carbon source in energy yielding processes of the examined species of ciliates.