Currently, the livestock and human nutrition sectors are competing for arable land, which is essential for crop production for animal feed and human food. In addition, in recent years, competition has been intensified by the biofuel sector. In order to limit competition, the efficiency of plant production should be improved, but also the use of feed by animals, which will allow for lower consumption. The sectors of human food production and livestock production are convergent. Food production is largely based on ruminants (dairy cows, beef cattle) as much less human edible feed is needed in the ruminant system than in monogastric animals (pigs, poultry) to produce the same amount of animal protein product. In addition, ruminants use feeds with a high crude fiber content in the production chain, which are not recommended for monogastric or human feeding. However, ruminants have been criticized for the excessive nitrogen emissions into the environment due to low nitrogen utilization from feed. Proper selection of forage and their quality will allow for the improvement of the use and reduction of nitrogen emissions to the environment. One of the widely used high-protein feeds in the nutrition of ruminants, the nitrogen of which is less utilized, is alfalfa silage. The reason is the susceptibility of plant to proteolysis during ensilage, which leads to the degradation of the true feed protein into non-protein nitrogen compounds with higher rumen solubility or in highly degraded forms, which results in a reduction in the efficiency of bacterial protein synthesis in the rumen and reduces its utilization. Proteolysis during alfalfa silage results from the activity of plant enzymes, and in the second stage of bacterial enzymes and its highest intensity is usually observed in the initial stage of ensilage. The primary content of true protein in forage can be reduced by up to 80%.

Attempts to limit or inhibit proteolytic transformations to date have not yielded the expected results. Taking into account the previously undertaken research topics and the knowledge of proteolysis and the activity of proteolytic enzymes, it would be effective to acidify the environment as quickly as possible and reduce the first aerobic phase of fermentation, which has an inhibitory effect on protein degradation. The solution could be carbon dioxide (CO_2), which should displace the air/oxygen present in ensiled herbage, as this gas has a higher density. CO_2 given during ensilage can also compensate for insufficient filling of the free spaces with air between the herbage particles, and thus eliminate its negative impact. The use of gas would have no effect, but dry ice can be used as a source of CO_2, which changes the state of aggregation into gas under the influence of temperature increase. Previous studies on the addition of dry ice to maize ensilage did not take into account proteolysis.

The aim of the project is to investigate the proteolytic and microbiological changes during the ensilage of alfalfa after the use of dry ice as a factor modifying the atmosphere inside the ensiled mass, which will limit the aerobic processes of the first stage of ensilage.

The effectiveness of inhibiting the proteolytic processes of alfalfa protein should be sought in the combination of three factors: the degree of density, the dry matter content and the level of added dry ice as a CO_2 source. These factors converge and can jointly affect the extent of proteolysis. Based on the literature and previous research, it will be assumed that the CO_2 formed as a result of dry ice sublimation will modify the environment of the ensilaged mass, changing the gas composition, will shorten the first oxygen phase of ensilage, reduce the oxygen loss of sugars, create a complex of microorganisms desired for the ensilage process, and shortening the oxygen phase will accelerate the growth of bacteria lactic acids and acidification of the silage mass environment, and as a result, it will lower the activity of plant proteolytic enzymes and change the composition of the silage microbiome. It is assumed that lowering the proteolytic activity will also increase the proportion of protein nitrogen in the silage, which will reduce the ammonia concentration in the rumen, improve the use of nitrogen in the rumen and reduce its emission to the environment.

The project took into account a three-factor system: dry ice dose, dry matter content and the degree of density. The research will be carried out in two stages. Stage I will investigate the proteolytic and microbiological changes following the application of different doses of dry ice. The aim is to determine the optimal proportion of dry ice, degree of wilting and density to obtain high quality silage. Stage I includes determination of the effect of dry ice additive on the basic composition, carbohydrate fraction, crude protein fraction, *in vitro* rumen degradability of crude protein and dry matter, fermentation products and microbiological analysis. Stage II is based on the selection of the best variants from stage I, and then analyzing the dynamics of proteolytic changes and fermentation profile in time periods and the effect of adding dry ice.

By introducing dry ice to the ensiled mass, we introduce CO_2, which may seem undesirable due to the emission to the environment. However, the oxygen that occurs between the plant particles in the first stage of fermentation is metabolized by aerobic microorganisms which produce CO_2 as a result, so introducing dry ice does not harm the environment. In this way, we can obtain anaerobic conditions in the ensiled plant faster, shortening the aerobic processes of the first stage of ensilage.