Essential oils are volatile, hydrophobic mixtures of secondary metabolites obtained from plants. Currently, almost all biological properties of this class of compounds have been documented, due to their chemical structure (non-polar low-molecular substances) they are bactericidal and fungicidal. In recent years, however, there has been a growing interest in essential oils in health care, the food industry, cosmetology, and animal production. Currently, applications of natural compounds are sought in animal prophylaxis (antimicrobial and antiparasitic agents), nutrition (e.g. improving the digestibility of feed), and environmental protection (reduction of methane emissions). The observed tendency and planned changes in the legislative use of antibiotics in animal production lead to basic research on the use of biologically active compounds, including essential oils. The so far published studies showing the influence of essential oils or their components did not take into account the activity of the respective enantiomers of the tested compounds.

Some studies have observed the effects of essential oils on the rumen microbiome. However, these studies looked at the limited use of essential oils, and the results were mixed. The project aims to provide new knowledge on the mechanisms of the influence of mixtures, individual enantiomers and their thio-derivatives on the rumen microbiome, biohydrogenation of fatty acids, methanogenesis (*in vitro* and *in vivo*) as well as the metabolism and functional properties of milk, including the fatty acid profile and its flavor. According to the available literature, there is a simple relationship between the profile of rumen microorganisms and the quantitative and qualitative composition of milk fatty acids.

The research work concerns examining for the first time the influence of the relevant essential oils, the optically active ingredients dominant in their composition, and their thio-derivatives on the rumen microbiome of cows, biohydrogenation of fatty acids, and functional parameters of milk (including CLA, OBCFA, phospholipids), and in the environmental aspect on methanogenesis. The project assumes the determination of the optically active compounds - enantiomers - dominating in their composition in the selected essential oils. A range of essential oils will be selected for in vitro tests, where the content of the dominant compound will be no less than 60% with an enantiomeric excess of more than 0.9. The enantiomeric excess will be determined by a classical technique on a GC-MS unit equipped with a surgical column. At the same time the isolation of enantiomeric enriched components, which are not commercially available, will be performed using vacuum distillation. In parallel, research will focus on the isolation of key strains of rumen bacteria, their cultivation under anaerobic conditions using anaerobic chambers and hypoxic incubators. Confirmation of their species affiliation will be carried out using molecular biology methods (sequencing of 16S mRNA gene fragments). Using the established bank of bacterial strains, inhibitory concentrations of essential oils, pure enantiomers, and appropriate thio-derivatives will be determined for them. The aim of the project will be to redirect the microbiome profile in terms of the number of *Butyrivibrio sp.*, *Streptococcus bovis*, and methanogenic bacteria.

Selected compounds (**essential oils, enantiomers, and their thio-derivatives**) will be added to the environment of the rumen microbiome of cows and their influence on the change of fatty acid biohydrogenation and methanogenesis will be determined (*in vitro* model using the DAISY II incubator). Under incubator conditions, the biotransformation of the individual pure components will be assessed. **Then, for the first time for the described compounds, the results obtained in the in vitro model will be correlated with their actual influence on fermentation processes.**

Research on cows will focus on understanding the mechanisms of rumen microbiome control and, consequently, biohydrogenation of fatty acids and the synthesis of OBCFA, CLA, PUFAs, and the antioxidant status through the use of a combination of enantiomers of essential oils and appropriate thio-derivatives. Advantageously modifying the rumen fermentation will reduce methane production. **This will be the first time that the EOs enantiomers and appropriate thio derivatives have been used for this purpose, which may constitute the discovery of the newest method of reducing methane emissions from ruminants.**

The project's findings will contribute to the development of the scientific foundations of animal science, microbiology, and chemistry of natural products. Also, new knowledge on the impact of enantiomers, appropriate thio-derivatives on the rumen microbiome will contribute to the development of applied sciences in the field of the feed industry or the implementation of new reduction methods in environmental protection.