

For several years, there has been an increase in interest in diet supplementation with vitamin D as a way to improve immunity and functioning of the skeletal system. Pharmaceutical companies are offering new products with better effectiveness in rising plasma vitamin D content. The internet contains a lot of information about the wonderful properties of vitamin D for many civilisation diseases such as cardiovascular diseases, neurodegenerative diseases and cancer. Part of this information is confirmed in the scientific literature. However, there are many studies whose results contradict any effect of vitamin D supplementation on diseases not related to the skeletal system. In recent years, the *Lancet Diabetes and Endocrinology* journal published an article describing the analysis of research carried out during 2013-2017 on the impact of vitamin D supplementation on various aspects of health. According to the authors of this article, the only well-documented effect of vitamin D dietary supplementation is its effect on the skeletal system and the occurrence of upper respiratory tract infections and asthma. Regarding other diseases (cardiovascular diseases, cancer, mood disorders, muscle function), there is no convincing evidence for the positive effect of vitamin D dietary supplementation. Scientists are still not sure about the impact of vitamin D supplementation and the recommended ranges determining the normative content vitamin D in the body. It is suggested that the dose of vitamin D should be selected individually for the patient according to the individually calculated index. Many of these doubts could be explained if the effects of vitamin D supplementation at the molecular level were better identified. Previous studies conducted in rodents and in vitro cultures have not given satisfactory answers to questions bothering scientists. The goal of our research is to determine whether vitamin D supplementation changes and expression of genes and proteins in crucial organs (liver, muscle, adipose). Such studies cannot be performed in humans. On the other hand, the pig is currently successfully used as a model animal in biomedical studies due to similar physiology and body size.

In our experiment, 30 pigs will be divided into three groups (no supplementation, standard supplementation – 5000 U/Kg of feed, supplementation 10000U/Kg of feed). After three months, pigs will be slaughtered, organ fragments will be taken for analysis of gene and protein expression, and blood for biochemical (lipid profile, vitamin D in blood content, vitamin D in meat and fat) and haematological analysis. Ribonucleic acid (RNA) will be isolated from the removed organs, which after special processing (creation of cDNA libraries) will be sequenced on a device for NGS - Next Generation Sequencing. The 3' quant mRNA method will be used here, which allows quantitative evaluation of the expression of all genes at a relatively low cost. After this analysis, we will obtain information whether there have been any changes in the level of gene expression under the influence of vitamin D supplementation and possibly a list of genes that are activated or silenced under the influence of a standard or high dose of vitamin D. The list of genes will be subjected to functional analysis using special bioinformatics tools. Thanks to this we will get the answer whether these genes are characteristic of specific biological processes or pathways related to the pathogenesis of some diseases. Besides, we will check whether changes at the RNA level are reflected at the protein level. The final stage of research will be to link all results together, i.e. to check if changes at the level of mRNA and proteins reflect changes in the content of vitamin D and calcium in the blood, meat and fat, blood count, cholesterol, and triglyceride levels. We hope that the research will help us find the cause of so many conflicting reports about the effectiveness of vitamin D supplementation. Insight into the central metabolic pathways associated with vitamin D may help identify various adaptive mechanisms that are triggered in a vitamin D deficiency situation that endure its harmful effects, but they cause irreversible cell changes in the long term.