

1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: Transcriptome analysis of liver to determine the influence of supplementary diet *Silybum Marianum* herb extract in the prevention against liver dysfunction using pig as an animal model.

The hepatoprotective medicinal herb *Silybum marianum* (Silymarin), which has a beneficial effect on liver cells and the gastrointestinal tract, can also attenuate the undesirable effects of an unhealthy diet. Bioactive components may significantly modulate gene expression involved in biological processes and metabolic pathways related to detoxification and cellular stress protection. *Silybum marianum*, commonly known as milk thistle and a natural compound derived from the herbs is silymarin, the active compounds of which include silybin, isosilybin, silychristin, dihydrosilybin, silydianin. Studies on rats showed the hepatoprotective effects of *silybum marianum* against oxidative stress, lipid peroxidation, and transaminase reactions connected with liver dysfunction (Rasool, Mahmood et al 2014). In fattening pigs' studies showed that milk thistle seeds (*Silybum marianum*) could be a helpful feed admixture to improve meat quality and oxidative stability. However, a higher dose of milk thistle seeds (6%) appeared to be more effective in improving weight gains, feed utilization, PUFA content in tissues, and water-holding and antioxidant capacities (Grela, et al. 2020), and modulating cardiac lipotoxicity in the ischemic myocardium and to improve the ventricular remodelling (Aicha et al 2021). Therefore, detailed studies of putative changes in the activities of potential genes are desirable, along with a combination of large-scale multi-Omics analyses. Transcriptomic analyses are the key to a better understanding and elucidation of the hepatoprotective molecular mechanism of action of the herb mentioned above in preventing liver dysfunction. Young pigs with a highly reactive organism are the best model to evaluate hepatoprotective action. Moreover, the similarity between humans and pigs causes the pig model to be the best for evaluation to allow for gene expression changes, which would be comparable to humans and allow further indication for specific treatment of liver disease in animals and humans.

Project goals: Specific and operational goals (WP-1 to WP3) of the proposed PhD project are:

WP-1: Collection of research materials, feeding experiments, histopathological, morphometrical, hematological, and blood biochemical examinations, and the phenotypic data management of commercial crossbred piglets

WP-2. miRNA-seq sequencing and determination the effect of supplementation with milk thistle extract (*Silybum Marianum*) on the prevention of liver dysfunction based on transcriptomic analysis in a pig animal model.

WP-3: Validation experiments of porcine liver for miRNA-seq, experiment using RT-PCR

WP1: The feeding experiments (*Silybum Marianum* herbs) on pigs and laboratory examinations on blood morphometric, haematological, and biochemical parameters, and histopathological evaluation of the liver, and to verify the hepatoprotective effect of medicinal plant extracts (*Silybum Marianum* herbs) on the liver function will be carried out.

WP2: Determination of effect of *Silybum Marianum* medicinal herbs in counteracting the hepatoprotective effect on porcine liver transcriptome, and miRNA-seq NGS sequencing of liver tissues will be performed.

WP3: identification and validation of DEGs miRNA transcripts in context to hepatoprotective action of liver, as well as identification and validation of the role of key genes in biological processes and

metabolic pathways altered by SM herbal extracts will be done. Finally, to explain the metabolic changes in porcine liver and to consolidate coordinated multi-level Omics analysis efforts, by integrating experimental data by comprehensive bioinformatics assessment of WP1-WP3 will be done.

1.1. Outline:

During the COVID-19 pandemic period, "diet-oriented problems" became particularly noticeable as a significant issue to human health. The proposed PhD project refers to a healthy diet-oriented experiment based on the action of hepatoprotective medicinal herbs: Silybum Marianum (SM) in the liver using pig model. The hepatoprotective medicinal herb SM, having a beneficial effect on liver cells. The research hypothesis assumes that hepatoprotective herbs like SM may restore disturbed liver metabolism via the induction of phase I and/or II liver enzymes and cellular stress preventing mechanisms such as chaperones, heat shock proteins and oxidative stress preventing enzymes. This type of bioactive components may significantly modulate genes expression involved in biological processes and metabolic pathways related to detoxification and cellular stress protection. Therefore, detailed studies of putative potential genes are desirable with a combination of large-scale multi-Omics analyses. Transcriptomic analyses are the key to a better understanding and elucidation of the hepatoprotective molecular mechanism of action of the herbs SM.

1.2. Work

The planned research will be carried out by conducting feeding and NGS based transcriptome experiments in pigs. In the first stage, feeding experiments with SM herbs will be tested in pigs using control (n=12) and experimental groups (n=12) with diet supplementations of 7 g/kg per body weight of SM herbs through 4 weeks (Kropiwiiec-Domańska et al. 2022). In each week, the biochemical analysis of blood and liver function test (LFT) will be performed. In the second stage, one week post weaning piglets (n=12) will be assigned as control (n=12), and experimental groups supplemented with SM, (n=12) with different levels of herb extracts. At the end of two stages, animals will be slaughtered, liver tissue samples will be collected for all multilevel analyses of porcine transcriptome. Lastly, bioinformatics, statistical analysis and dissemination of results will be done.

1.3. Literature (max. 10 listed, as a suggestion for a PhD candidate): References:

- [1]. Jiang et al. *Andrographis paniculata* (Burm.f.) Nees and its major constituent andrographolide as potential antiviral agents. *J Ethnopharmacol.* 2021 May 23;272:113954.
- [2]. Rasool et al. Hepatoprotective Effects of Silybum marianum (Silymarin) and Glycyrrhiza glabra (Glycyrrhizin) in Combination: A Possible Synergy. *Evid Based Complement Alternat Med.* 2014;2014:641597
- [3]. Liu, et al. Non-antibiotic feed additives in diets for pigs: A review *Anim. Nutr.*, 4 (2018), pp. 113-125
- [4]. Ip et al. Non-alcoholic steatohepatitis and hepatocellular carcinoma: implications for lycopene intervention. *Nutrients.* 2013;6(1):124-62
- [5]. Szostak et al. Effect of a diet enriched with omega-6 and omega-3 fatty acids on the pig liver transcriptome. *Genes Nutr.* 2016;11:9.
- [6]. Ogłuszka et al. A porcine gluteus medius muscle genome-wide transcriptome analysis: dietary effects of omega-6 and omega-3 fatty acids on biological mechanisms. *Genes Nutr.* 2017;12:4

- [7]. Herosimczyk et al. 2017, Hepatic proteome changes induced by dietary supplementation with two levels of native chicory inulin in young pigs, *Livestock Science*, 203, 54-62.
- [8]. Ozgo et al. 2019, The current proteomic landscape of the porcine liver. *Journal of Physiology and Pharmacology*, 70, 369-387.
- [9]. Love et al. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.
- [10]. Langfelder, Horvath WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*. 2008 Dec 29;9:559.

1.4. Required initial knowledge and skills of the PhD candidate: English, Polish languages, Msc, MVsc, degree in the field of Veterinary science, medical science, and biological sciences.

1.5. Expected development of the PhD candidate's knowledge and skills: educational and scientific skills in the field of veterinary science, medical science, biological sciences.