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

Project title: Transcriptome analysis of the hepatoprotective effect of Andrographis paniculata herbs in the prevention of liver dysfunction

1.1. Project goals: Specific and operational goals (WP-1 to WP9) of the proposed PhD project are:

i. to conduct the feeding experiments (andrographis paniculata herbs) on pigs and laboratory examinations on blood morphometric, haematological and biochemical parameters, and histopathological evaluation of the liver (WP1);
ii. to verify the hepatoprotective effect of medicinal plant extracts ((andrographis paniculata herbs) on the liver function by transcriptome analysis (WP2);
iii. to identify and explain the impact of andrographis paniculata medicinal herbs in counteracting the hepatoprotective effect on porcine liver transcriptome, (WP3)
iv. Depleted total RNA-seq next-generation Genome sequencing (NGS) of liver tissues (WP4),

v. **identification of differentially expressed (DE) noncoding circular RNA (nc-circRNA) in context to therapeutic hepatoprotective action of AP in liver (WP5);**

vi. **identification of the role of key nc-circRNA genes in biological processes and metabolic pathways altered by herbal extracts, (WP6);**

vii. **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-WP6 (WP7).**

1.2. 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: Andrographis Paniculata (AP) in the porcine liver using the depleted total RNA-seq next-generation Genome sequencing (NGS) to identify the noncoding circular RNA (nc-circRNA). The circular RNAs (circRNAs) are a unique class of RNA molecule that are produced by a covalent linkage via back-splicing of linear RNA. The research hypothesis assumes that hepatoprotective herbs like AP 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. These types 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 identification of potential noncoding circular RNA (nc-circRNA) 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 AP.

1.3. **Work plan:** The planned research will be carried out by in vitro experiments in pigs according to WP1 to WP7 described in the project goals. In the first stage, feeding experiment with AP herbs will be tested in pigs using control (n=10) and experimental groups (n=10) with diet supplementations of 30, 60 and 120 µg/kg AP herbs through 4 weeks. 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=10) will be assigned as control (n=10), and experimental groups supplemented with AP, (n=10) with different levels of herb extracts. At the end of two stages, animals will be slaughtered, liver tissue samples will be collected for nc-circRNA NGS sequencing of liver transcriptome. Lastly, the bioinformatics, statistical analysis and dissemination of results will be done.

1.4. **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. SteinNon-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.

1.5. Required initial knowledge and skills of the PhD candidate: English, Polish languages, Msc, MVsc, degree in the field of Animal science (Zootechnic), Veterinary science, Medical science, and Biological sciences.

1.6. Expected development of the PhD candidate's knowledge and skills: educational and scientific skills in the field of Animal Science, Veterinary science, Medical science, biological sciences.