

PHD PROJECT DESCRIPTION

(4000 characters max., including the aims and work plan to be published online)

Project title: Multiomics analysis of liver transcriptome to determine the influence of supplementary diets of medicinal herbs

1.1. Project goals

General hypothesis arises from that hepatoprotective medicinal herb {Andragraphis Paniculata (AP), and Silybum marianum (SM)} having a beneficial effect on liver cells. Proposed PhD Project research hypothesis assumes that hepatoprotective herbs 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. 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 and metabolomics analyses are the key to a better understanding and elucidation of the hepatoprotective molecular mechanism of the medicinal herbs. Young pigs with a highly reactive organism are the best model to evaluate hepatoprotective action.

Scientific goals (aims) of the project: Specific and operational goals (WP-1 to WP5) of the PhD project are: (i) to arrange and accomplish feeding experiments on piglets under monitored conditions, and constant veterinary control, along with periodic evaluation of blood morphometric, haematological and biochemical parameters, and final histopathological evaluation of the liver (WP1); (ii) to verify the hepatoprotective effect of AP and SM medicinal plant extracts on the liver function (WP2); (iii) to identify the differentially expressed (DE) genes using advanced bioinformatics tools (WP3); (iv) to identify the role of key genes in biological processes and metabolic pathways altered by herbal extracts, (WP4); (v) to explain the molecular mechanism of the metabolic changes, in vivo in porcine liver, and blood plasma, (WP5); and finally to consolidate coordinated multi-level analysis efforts, by integrating experimental data for comprehensive bioinformatics assessment of WPs.

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1.2. Outline

Some crucial liver dysfunctions can be attenuated by bioactive components present in medicinal herbs that may be useful as feed/diet supplements. Among many natural bioactive components present in the medicinal herbs viz., *Andragraphis Paniculata* (AP), and *Silybum marianum* (SM), showed the potential hepatoprotective properties. However, there still is a lack of comprehensive explanation of the mechanism of their action on the molecular level and their contribution in known biological processes. The "diet-oriented problems" became particularly noticeable as a significant issue to animal health and animal production as well as to human health. Therefore, a multi-level molecular research on hepatoprotective bioactive components is of great importance for indicating their adequate supplementation for restoring or securing disturbed liver cells metabolism caused by contaminated food, protecting from the development of more severe metabolic disorders, tumor, cancer development, and also impaired immune response in cirrhosis associated immune dysfunction.

Justification for tackling a specific scientific problem: Dietary action of a potential medicinal herbs AP and SM will be investigated - to examine their hepatoprotective effects in growing piglets. The use of pig model is justified by many similarities with human at the level of anatomy, physiology, and metabolism. Especially for our purpose, which is the generation of biological knowledge of the early (initial) stages of metabolic changes, those animals are a far better animal model than generally used laboratory animals such as mice, which have a different metabolism. This project aims to clarify the molecular mechanisms of hepatoprotection actions of herbs extracts. This type of research is not possible to perform on humans. Due to the ethical issues regarding to induce an unhealthy status in humans and compare the metabolism before and afterwards. Thus, it is possible in such studies to induce the different metabolic activities mimicking the human liver metabolic health status. In combination with bioinformatics and systems biology methodologies, modern multi-Omics tools allow for profound investigation of unknown gene-protein-metabolite interaction in biological processes. Transcriptomics, proteomics and metabolomics result in the generation of "big data", creating databases storing considerable knowledge for further meta-analysis and clinical studies.

1.3. Work plan

The planned research is intended to answer the following questions on wide multilevel analysis of piglet liver: What are the primary biological processes and pathways disturbed by intake of SM herbs, and low dose of AFB1 levels in the diets of young pigs? What are the primary biological processes and metabolic pathways influenced by supplementation of hepatoprotection action of SM herbs extract in the diet of young pigs? How does the various level of hepatoprotective SM herbs supplementation affect liver detoxification capacity? Which are similar and different points/ways of actions of SM hepatoprotective herbs? How does the SM herb diet and AFB1 contaminated diet affect the liver mitochondrial state?

General work plan and methodology: The planned research will be carried out on weaners (females). Animals will be kept with mothers up to the 28th day of life. Then, will be weaned and fed a basal (control) diet for seven days. Afterwards, the in-vivo experiment in piglets will be started in two stages for a period of 4 weeks. In the first stage of in-vivo pilot study, the various doses of AP and SM herbs will be tested in piglets for establishing its influence on health status and liver functions based on control and experimental group with diet supplementations of AP and SM herbs through 4 weeks. In each week, the biochemical analysis of blood and liver function test (LFT) (Alanine transaminase -ALT, Alkaline phosphatase -ALP, Albumin and total protein, Aspartate transaminase -AST, Bilirubin, Gamma-glutamyltransferase -GGT, Prothrombin time -PT) will be performed. The experiment design comprised of experimental groups supplemented with SM herbs diet (n=12), AP herbs (n=12), and control (n=12). After feeding experiments, animals will be slaughtered, liver and other tissue samples will be collected, frozen in liquid nitrogen for all multilevel analyses of transcriptomic, metabolomics, and selected molecular tests. Lastly, the bioinformatics, statistical analysis and dissemination of results will be done. Finally, the integration of bioinformatics pipelines and statistical analysis will be done, to a final evaluation: herbs bioactive component – gene –protein pathway interaction, SM herb protective action on base biological processes.

1.4. Literature (max. 7 listed as a suggestion for a PhD candidate preliminary study)

1. Pareek CS, et al. (2025). Identification of trait-associated microRNA modules in liver transcriptome of pig fed with PUFAs-enriched supplementary diet. *J Appl Genet.* 2025 May;66(2):389-407. <http://doi:10.1007/s13353-024-00912-w>
2. Nalpadan, et al. (2026). Transcriptional insights into aflatoxin B1 induced hepatotoxicity and comparative effects of medicinal herbs in pigs. *BMC Vet Res.* 22: 53. <https://doi.org/10.1186/s12917-025-05270-1>,
3. Kibitlewska, et al. (2026). Hepatoprotective Potential of Curcumin in the Prevention of Liver Dysfunction in a Porcine Model. *Nutrients*, 18: 408. <https://doi.org/10.3390/nu18030408>,
4. Sharma, et al. (2025) Hepatoprotective Effect of Silymarin Herb in Prevention of Liver Dysfunction Using Pig as Animal Model. *Nutrients*, 17: 3278. <https://doi.org/10.3390/nu17203278>. <https://www.mdpi.com/2072-6643/17/20/3278>;
5. Lichwiarska et al. (2025). Impacts of *Andrographis paniculata* supplementation on health and productivity in monogastric farm animals: A Comprehensive Review. *Animal Nutrition*. <https://doi.org/10.1016/j.aninu.2025.07.004>
6. Chałaśkiewicz et al. (2025) Impact of Aflatoxins on the Digestive, Immune, and Nervous Systems: The Role of Microbiota and Probiotics in Toxicity Protection. *International Journal of Molecular Sciences*, 26(17), 8258. <https://doi.org/10.3390/ijms26178258>
7. Kępka-Borkowska et al. (2025) Current Approaches to Aflatoxin B1 Control in Food and Feed Safety: Detection, Inhibition, and Mitigation. *Int J Mol Sci.* 2025 Jul 7;26(13):6534. <http://doi:10.3390/ijms26136534>

1.5. Required initial knowledge and skills of the PhD candidate

The PhD Candidate should acquire the master's degree in the field of veterinary science (M.V. Sc.), Animal science, animal biotechnology and bioinformatics, and biomedical sciences (M. Sc.). Laboratory skill in the field on molecular biology, and computer skill in analysing the multi-omics data.

1.6. Expected development of the PhD candidate's knowledge and skills

The PhD candidate can expect the scientific skill and knowledge in the field of multi-disciplinary topics including, Animal science, veterinary science, animal biotechnology and bioinformatics, and biomedical sciences.