PHD PROJECT DESCRIPTION

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

Project title: Transcriptome Analysis of Hepatoprotective Herbal Supplementation and circRNA-Mediated Liver Response in Aflatoxin-Challenged Pigs as an animal model (in-vivo)

1.1. Project goals

Proposed PhD Project research hypothesis assumes that crucial liver dysfunction 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., Andrographis paniculata (AP: Andrographolide), Silybum marianum (SM: Silymarin), showed the potential hepatoprotective properties. However, there is still a lack of a comprehensive explanation of the mechanism of their action on the molecular level and their contribution to known biological processes. In the proposed PhD project, a comprehensive genome-wide transcriptomic analyses, including ribo-depleted total RNA sequencing and circRNA profiling, along with validation through RT-PCR experiments will be carried out. The planned research is intended to answer the following questions on a wide multilevel analysis of piglet liver:

- What are the genome-wide transcriptomic alterations, as determined by ribo-depleted total RNA sequencing, in response to AFB1 exposure and herbal treatment?
- How is the hepatic circRNA expression profile modulated under conditions of AFB1-induced toxicity and subsequent herbal intervention?
- Which candidate genes and circRNAs exhibit significant differential expression, and can these findings be independently validated via quantitative RT-PCR analysis?
- What biological pathways and molecular networks are implicated in the hepatoprotective effects mediated by the herbal compounds?
- How do the transcriptomic and circRNA expression changes integrate to elucidate the molecular mechanisms underlying the protection against AFB1 toxicity?
- How do novel circRNAs coordinate with microRNAs and mRNAs to regulate gene expression networks and molecular pathways involved in liver function and response to aflatoxin and herbal supplementation?

• Can integrated analysis of circRNA, microRNA, and mRNA data identify novel regulatory interactions and potential biomarkers relevant to hepatoprotection and liver health?

Scientific goals (aims) of the project: Specific and operational goals (WP-1 to WP5) of the PhD project are:
WP1: Conducting feeding experiments on weaners fed with high and low doses of feed supplemented with different hepatoprotective herbs. WP1 includes the following research tasks: Experimental animal selection and pre-trial health screening; Procurement of herbs, formulation of experimental diets; Aflatoxin screening and contamination control measures; Herb supplementation trial, sample collection
WP 2: Isolation of Total RNA, rRNA Depletion, and Genome-wide circRNA and Total RNA Sequencing of Porcine Liver
WP 3: Construction of pipeline of circRNA-seq data and Genome wide circRNA profiling from circRNA-seq Data of porcine liver.
WP 4: Bioinformatics Analysis and Functional Characterization of Porcine Liver Omics Data
WP 5: Validation experiments to assess changes in the expression of selected genes using the RT-PCR method in relation to the obtained results of circRNA-seq.

1.2. Outline

Despite stringent agri-food production controls, accidental consumption of naturally contaminated food persists. Both human diets and livestock feed can contain harmful contaminants. Mycotoxins pose significant risks, impairing animal performance and causing liver damage. AFB1 is the most toxic aflatoxin, affecting key food products like maize and peanuts. Maize is vital for global animal nutrition. Pigs exposed to high aflatoxin levels exhibit severe aflatoxicosis and reduced weight gain. Chronic exposure to lower AFB1 levels impairs pig metabolism. Even brief mycotoxin exposure can lead to serious liver damage and productivity loss. Some crucial liver dysfunction 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., Andrographis paniculata (AP: Andrographolide), Silybum marianum (SM: Silymarin), or Curcuma longa L (CL: Curcumin) showed the potential hepatoprotective properties. However, there is still a lack of a comprehensive explanation of the mechanism of their action on the molecular level and their contribution to known biological processes. The proposed project refers to a healthy diet-oriented experiment based on hepatoprotective medicinal herbs on the liver. Moreover, the "diet-oriented problems" became particularly noticeable as a significant issue to human health. In addition to lung disease, severe cases showed varying degrees of liver damage. Since chronic liver disease (CLD) is associated with immune dysregulation and inflammation, it is not surprising that CLD patients may be at a higher risk of adverse effects. Therefore, 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 AFB1 contaminated food, protecting from the development of more severe metabolic disorders, tumor, cancer development, and also impaired immune response in cirrhosis associated immune dysfunction.

1.3. Work plan

The planned research will be carried out feeding experiment 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. The feeding experiment starts with one week post weaning piglets (n=48). Experiment design will be assigned as control (n=4), contaminated with AFB1(n=4), and three general experimental groups supplemented with AP, SM, and CL subdivided into 12 groups (n=4) with different levels of herb extracts without and with simultaneous administration of AFB1. The optimal dosage levels for aflatoxin B1 (AFB1), and high level group will include high doses AP (40 mg/kg), SM (120 mg/kg), and CL (120 mg/kg) in a balanced mixture dietary food were already established for our proposed project experimental design. At the end of the experiment, animals will be slaughtered, and liver and other metabolomics tissue samples will be collected. After feeding experiments, animals will be slaughtered, liver and other tissue samples will be collected, frozen in liquid nitrogen for all multilevel analyses of Ribo- depleted total RNA sequencing and circRNA profiling including Differentially expressed circRNAs (DE-circRNAs) will be determined between experimental

groups using statistical tools optimized for RNA-seq data (e.g., DESeq2 or edgeR), with FDR correction for multiple testing. Lastly, the bioinformatics, statistical analysis and dissemination of results will be done. At this stage, 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.