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# MAPOWANIE SZLAKÓW ZALEŻNYCH OD MIRNA W WĄTROBIE ŚWIŃSKIEJ: PODEJŚCIE SYSTEMOWE DO TOKSYCZNOŚCI AFLATOKSYNY B1 I HEPATOPROTEKCJI

# MAPPING MIRNA-MEDIATED PATHWAYS IN THE PORCINE LIVER: A SYSTEMS APPROACH TO AFLATOXIN B1 TOXICITY AND HEPATOPROTECTION

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**BACKGROUND**

Aflatoxins—particularly aflatoxin B1 (AFB1)—are hepatotoxic and hepatocarcinogenic mycotoxins produced by certain *Aspergillus* species (e.g., *A. flavus*, *A. parasiticus*) that cause health detriments in livestock due to consumption of contaminated feed. While known to cause hepatic damage, the underlying microRNA (miRNA)-mediated molecular mechanisms of AFB1 toxicity and the protective effects of herbal extracts are not fully elucidated. This study aims to investigate the effects of AFB1 and medicinal herb dietary supplements (*Andrographis paniculata, Silybum marianum, and Curcuma longa*) on the porcine liver's miRNA expression profile to identify and map the miRNA-mediated pathways involved in toxicity and hepatoprotection.

**MATERIALS AND METHODS**

Commercial crossbred TN70 pigs (Norsvin Landrace × Large White; n = 44) underwent 7 days of adaptation before allocation to six treatments: (1) AFB1 + DMSO (120 µg/kg BW; n = 6), (2) *Andrographis paniculata* (30 mg/kg BW; n = 10), (3) *Silybum marianum* (90 mg/kg BW; n = 10), (4) *Curcuma longa* (90 mg/kg BW; n = 10), (5) DMSO (n = 4), and (6) Control (n = 4). Medicinal herbs were administered from day 42, while AFB1 was administered from day 56 until the end of the experiment on day 70. Liver samples were collected, snap-frozen, and stored at −80°C. Total RNA was isolated using a standard RNA extraction protocol; integrity was verified by Agilent Bioanalyzer (RIN > 7). Small-RNA libraries were prepared, size-selected to 145–160 bp, and quantified by Bioanalyzer. Raw data underwent quality control to remove low-quality and adapter-trimmed reads. Subsequent bioinformatics analysis, including differential expression analysis and pathway enrichment, was performed to identify and map the miRNA-mediated pathways.

**RESULTS AND DISCUSSION**

The small RNA-Seq analysis is anticipated to reveal a distinct differential expression of miRNAs in the livers of pigs exposed to AFB1 compared to the control group. These aberrantly expressed miRNAs are likely to be associated with key biological processes such as inflammation, oxidative stress, apoptosis, and lipid metabolism, which are hallmarks of AFB1 hepatotoxicity. The dietary supplementation with *Andrographis paniculata, Silybum marianum*, and *Curcuma longa* is expected to significantly alter the miRNA expression profile, counteracting the effects of AFB1 exposure. Through target gene prediction and pathway enrichment analysis, we anticipate identifying specific signaling pathways regulated by these miRNAs. These findings will provide a systems-level understanding of how herbal extracts modulate the miRNA landscape to exert their hepatoprotective effects and offer valuable insights into the molecular mechanisms of AFB1 toxicity.

**CONCLUDING REMARKS**

Small RNA-seq of livers from AFB1-exposed piglets showed robust differential miRNA expression versus controls. *In silico* target prediction and KEGG enrichment indicated involvement of inflammatory signaling, apoptosis, oxidative stress, and lipid metabolism pathways. Diets containing *Andrographis paniculata*, *Silybum marianum*, and *Curcuma longa* attenuated AFB1-associated miRNA shifts toward control profiles. Enrichment analyses implicated cancer-related miRNA modules, with ssc-miR-26a, ssc-miR-29c, ssc-miR-122, ssc-let-7f, ssc-miR-181b, ssc-miR-28, ssc-miR-19, ssc-let-7c, ssc-miR-103, and ssc-let-21 emerging as candidate regulators of detoxification and cellular stress responses.

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