Abstract (ENG)

Thesis title: Dietary effect of polyunsaturated fatty acids on the porcine liver by analyzing the differential gene expression and weighted gene co-expression network analysis of miRNA data in Polish Landrace and Polish Landrace x Duroc pigs.

Background: The objective of the research is to investigate how omega-6 and omega-3 polyunsaturated fatty acids (PUFA) affect liver transcriptome activity in pigs. The study aims to analyze the alterations in hepatic gene expression, co-expression and metabolic pathways linked to pig genotype. Although the impact of these fatty acids on potential health outcomes has been settled, their action on the whole transcriptome level remains unclear. The researchers plan to vary the pigs' diet with different amounts of omega-6 and omega-3 PUFAs and then use RNA sequencing to examine changes in liver gene expression. By exploring how dietary omega-6 and omega-3 PUFAs affect the transcriptome and gene co-expression in pigs, the study could provide valuable information for understanding the health effects of these fatty acids and optimizing pig nutrition and production.

Methods: The NGS based small RNA sequencing (miRNA-Seq) of porcine liver transcriptome was performed on 6 Polish Landrace (PL) pigs, and 6 PL x Duroc (PLxDuroc) pigs using MiSeq Illumina platform (ICNT, UMK, Torun). The miRNA-Seq reads were mapped onto the miRBase v.22 using miRDeep2 was used. MiRDeep. Six differentially expressed miRNA (DE-miRNA) genes comparisons analysis between diets (n=2) and breeds (n=2) were performed to identify the upregulated and downregulated DE-miRNA genes of porcine liver. The co-expression analysis of porcine hepatic miRNA was performed using weighted gene co-expression network analysis (WGCNA) R/Bioconductor R package. The biological interactions between gene networks and metabolic pathways of DE miRNA genes were performed using ClueGO v 2.2.0 Cytoscape v. 3.1.0 software, and the GO-BiologicalProcess-EBI-UniProt-GOA database for human genes.

Results: Using MiSeq Illumina platform, the NGS experiment generated 12 fastq files of porcine liver miRNA reads, ranging from the lowest number of 135842 and highest number of 4018483 sequence reads. The lowest and highest GC content were 46% and 49%. Mapping results revealed the matrix of 535 miRNAs reads for 12 samples which were later utilized in DEGs and WGCNA analysis of porcine liver transcriptome representing Polish Landrace (PL) purebred and Polish Landrace x Duroc (PLxDuroc) crossbred pigs. The miRNA DEGs experiment was divided into four groups representing, control diet and PUFAs diets in PL pigs, and control diet and PUFAs diets in PLxDoruc pigs. A total of six miRNA DEGs comparisons were performed. In first comparison between control and PUFAs diets of PLxDuroc pigs, 1256 DE-miRNA transcript were identified that commonly sheared in both control vs PUFAs diets of PLxDuroc pigs. By comparing the control vs PUFAs diets in PLxDuroc pigs, 13 significant ($p < 0.05$), 5 upregulated ($log2FC > 2$) and 10 downregulated ($log2FC > 5$) DE-MiRNA transcripts were identified. In the Second comparison between control diets of PL and PLxDuroc, 1060 breed-specific DE-miRNA transcript were identified that commonly sheared in control diet of both PL and PLxDuroc pigs. By comparing the control vs PUFAs diets in PLxDuroc pigs, 10 significant ($p < 0.05$), 21 upregulated ($log2FC > 3$) and 31 downregulated ($log2FC > 5$) DE-MiRNA transcripts were identified. In the third comparison between control diet of PLxDuroc pigs and PUFAs diet of PL pigs, 1380 DE-miRNA transcripts were identified that commonly sheared in both control and PUFAs diets of PL and PLxDuroc pigs. By comparing the control diet of PLxDuroc vs PUFAs diet in PL pigs, 54 significant ($p < 0.05$), 29 upregulated ($log2FC > 2$) and 64 downregulated ($log2FC > 5$) DE-MiRNA transcripts were identified. In the fourth comparison between control diet of PL pigs and PUFAs diet of PLxDuroc pigs, 696 DE-miRNA transcripts were identified that commonly sheared in both control and PUFAs diets of PL and PLxDuroc pigs. by comparing the control diet of PL pigs vs PUFAs diet in PLxDuroc pigs, 56 significant ($p < 0.05$), 5 upregulated ($log2FC > 2$) and 10 downregulated ($log2FC > 5$) DE-MiRNA transcripts were identified. In the fifth comparison between PUFAs diets of PL and PLxDuroc pigs, 986 DE-miRNA transcripts were identified that commonly sheared the PUFAs diets in both PL and PLxDuroc pigs. by comparing the PUFAs diets between PL vs PLxDuroc pigs, 27 significant ($p < 0.05$), 5 upregulated ($log2FC > 2$) and 10 downregulated ($log2FC > 5$) DE-MiRNA transcripts were identified. In the sixth comparison between control vs PUFAs diets of PL pigs, 1019 diet-specific DE-miRNA transcripts were identified that commonly sheared in both control vs PUFAs diets of PL pigs. By comparing the control vs PUFAs diets in PL pigs, 34 significant ($p < 0.05$), 29 upregulated ($log2FC > 2$) and 27 downregulated ($log2FC > 5$) DE-MiRNA transcripts were identified.

In WGCNA analysis, a total of 94 miRNAs counts were normalized using the "varinaceStabilizingTransformation" function from DESeq2 library, and soft threshold power $\beta=18$ was established to construct the gene network type of Topological Overlap Matrix (TOM). Based on the TOM the co-expressed miRNA with the highest interconnection were clustered into the modules which were represented by different colors. In WGCNA analysis, a total of 9 trait-associated modules that are significantly associated with the measured phenotypic traits were identified in PL and PLxDoruc pigs namely, meat color (a*), shoulder

subcutaneous fat thickness, conductivity 24 hours post mortem (PE24), and ashes, respectively. Trait-wise, large set of co-expressed miRNA of porcine liver were identified in these trait-associated significant modules (9, 7 ,2, and 8) affected by PUFAs diets in PL and PLxDoruc pigs. The mapping of fixed target miRNA genes result revealed that identified 44 miRNAs (out of 94 miRNA) had a 6719 statistically significant target genes with the target score >90. The highest number of target genes were found in ssc-miR-30e-5p (520) in magenta module, followed by ssc-miR-30b-5p (518) in pink module, and ssc-miR-30c-5p (518) in yellow module. The lowest number of target genes were found for ssc-miR-126-3p and ssc-miR-423-3p (1 each) in purple and brown modules.

Based on the identified trait-specific modules, the GO/pathway analysis were performed using the GO-BiologicalProcess-EBI-UniProt-GOA database for human genes. ClueGO analysis identified the highest number of GO/pathway specific term associated with miRNAs' target genes in green module (90). The second highest number of GO/pathway specific term were observed for target genes of yellow miRNAs (88). The lowest number of GO/pathway specific term were found for miRNAs' targets from clustered in modules: purple (16), turquoise (14), brown (6), and magenta (4).

Conclusions: Porcine hepatic miRNA gene expression profile database developed during the study will allow further investigation of relations between miRNA expression and porcine phenotypic traits. The study indicated the differences in miRNA expression between the types of diet and breeds. Furthermore, discovered modules in WGCNA show the strong interconnection between co-expressed miRNAs. Hub genes of discovered miRNAs clusters can be considered as predicted miRNA genes associated with PE24, meat color, Shoulder subcutaneous fat thickness, and ashes. Additionally, co-expressed miRNAs indirectly affect other functional pathways by regulation of it's target genes. Discovered target genes for miRNA clusters play significant roles in biological functions such as: i) muscle organ development, ii) different cellular processes and developments, iii) system development, iv) metabolic processes, v) muscle tissue development.

Keywords: fatty acids, PUFA, pig, liver, NGS, RNA-Seq, transcriptome, miRNA, DEG, WGCNA, bioinformatics

Mateusz Sakwinska
25.03.23