71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

DIFFERENTIAL MICRO-RNA EXPRESSION IN DARK-CUTTING BEEF SAMPLES AT EARLY POST-MORTEM STAGES

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I. INTRODUCTION

High stress situations before or during slaughter disrupt muscle tissue homeostasis, alter *post-mortem* biochemical processes and compromise meat quality. This can lead to a quality defect known as dark cutting or DFD (dark, firm, and dry) meat. DFD is characterized by a high pH at 24 hours *post-mortem* (pH_{24}) due to a rapid depletion of muscle glycogen reserves. Stress conditions may induce changes in gene expression [1], potentially mediated by microRNAs (miRNAs), small non-coding RNA molecules that regulate gene expression in various cellular processes and contribute to cellular homeostasis [2]. Therefore, miRNAs may play a role in the development of DFD meat. Previous studies have identified several miRNAs with differential expression at 24 hours *post-mortem* in DFD beef, suggesting their involvement in the development of this quality defect [3]. The aim of this study was to analyze the evolution of miRNAs expression in DFD and normal beef (CONTROL) at earlier stages of *post-mortem* conversion of muscle into meat (2h to 24h), in order to identify biomarkers for the early detection of this quality defect.

II. MATERIALS AND METHODS

Biopsy samples (10 g) were collected from the *Longissimus thoracis et lumborum* (LTL) muscle at the 13th rib level of the left half of 90 carcasses from yearling bulls of the "Asturiana de los Valles" breed at 2, 8, and 24 hours *post-mortem* (*pm*) and stored at -80°C. At 24h *pm*, pH was measured at the same location where the biopsies were taken, identifying 5 carcasses as DFD (pH_{24} > 6.2) and 5 as CONTROL (normal pH_{24}: 5.4 – 5.6). Total RNA was isolated using the miRNeasy Mini Kit (QIAGEN). One hundred nanograms of total RNA were used to generate miRNA NGS libraries with the QIAseq miRNA Library Kit (QIAGEN). Primary analysis was performed using CLC Genomics Server 23.0.5 with the “QIAseq miRNA Quantification” workflow, mapping reads to miRBase (v22). Reads were processed by trimming, filtering by length, and deduplicating using unique molecular identifiers (UMIs). Differential expression analysis was performed using the “Empirical analysis of DGE” algorithm, based on the Exact Test for two-group comparisons from the EdgeR Bioconductor package. A variance stabilizing transformation was applied to the raw count matrix using the DESeq2 *vst* function.

III. RESULTS AND DISCUSSION

Analysis of the sequencing data identified 1028 miRNAs in the LTL muscle, of which eight showed significant differential expression (False Discovery Rate (FDR) p-value < 0.05) in the DFD group compared to the CONTROL. All were upregulated in DFD, except *bta-miR-499*, which was downregulated.

Table 1 – Significant differentially expressed miRNAs between DFD and CONTROL beef at 2, 8 and 24 hours *post-mortem*

Time	Name	Log_2 fold change	p-value	FDR p-value	Bonferroni
2h	*bta-miR-2332*	1.722	2.41E-05	1.52E-02	1.52E-02
	bta-miR-2411-3p	2.128	6.33E-05	2.01E-02	4.01E-02
8h	*bta-miR-2332*	1.781	1.03E-06	3.76E-04	6.37E-04
	bta-miR-11972	5.046	1.27E-05	2.31E-03	7.82E-03
	bta-miR-12030	2.467	2.04E-04	2.47E-02	1.25E-01
	bta-miR-142-3p	1.479	4.10E-04	3.73E-02	2.52E-01
24h	*bta-miR-11972*	5.389	1.46E-10	9.06E-08	9.06E-08
	bta-miR-11975	3.709	6.26E-05	1.58E-02	3.88E-02
	bta-miR-499	-0.854	7.65E-05	1.58E-02	4.74E-02
	bta-miR-12023	4.249	1.52E-04	2.35E-02	9.41E-02

Two miRNAs, *miR-2332* and *miR-11972*, were overexpressed in DFD beef at different *pm* time points. *miR-2332* showed significant overexpression at 2 and 8 hours *pm* (log_2 fold change > 1.7, both time points) while *miR-11972* exhibited the highest overexpression (log_2 fold change > 5) at 8 and 24 hours. Although *miR-11972* has been detected in LTL muscle of cattle, its biological functions remain largely unknown, mainly linked to cell cycle regulation, endothelial cell migration and cytoskeleton organization [4]. *miR-2332* has been reported as overexpressed in *Bos indicus* blood during summer [5] and positively correlated with heat shock protein-related genes (HSF1, HSP60, HSP70, and HSP90) in heat-stressed buffalo heifers [6], suggesting a role in thermoregulation and stress response. Notably, *miR-2332* and *miR-2411-3p* (differentially expressed at 2 hours *pm* in this study) were previously identified as potential DFD biomarkers at 24h *pm*; their differential expression was first detected through sequencing and then validated by RT-qPCR [3]. *miR-2411-3p* downregulates genes associated with oxidative stress and apoptosis [3], which means that *miR-2332 and miR-2411-3p* may contribute to altered stress regulation in DFD beef by modulating HSPs expression and limiting apoptosis, potentially influencing the development of meat quality defects. Principal Components Analysis was performed to assess the discriminative power of the eight differentially expressed miRNAs at 2, 8, and 24 hours *pm*. The results revealed a clear separation between CONTROL and DFD samples, indicating the potential of these miRNAs as early biomarkers of DFD beef.

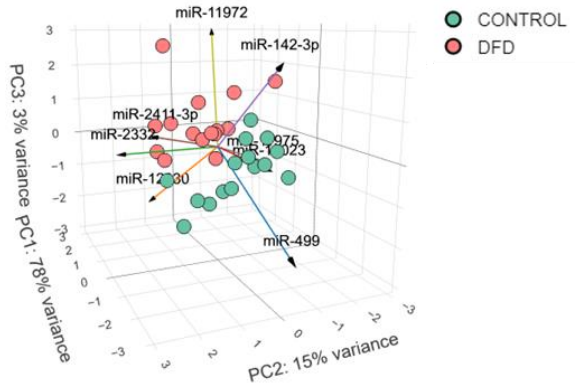


Figure 1. Biplot of the first three principal components, explaining 96% of the variance. Points represent sample scores, while lines indicate variable loadings. CONTROL samples are shown in green and DFD samples in red.

IV. CONCLUSION

This study identified 8 differentially expressed miRNAs in DFD beef at early *post-mortem* stages. While the functions of most of them remain unclear, the involvement of *miR-2332*, *miR-11972* and *miR-2411-3p* in cell stress response, muscle metabolism and apoptosis, suggests a significant role in DFD development, highlighting potential molecular mechanisms underlying meat quality defects and paving the way for early detection strategies.

ACKNOWLEDGEMENTS

This work was funded by MICIN/AEI/10.13039/501100011033 and ERDF ‘a way making Europe’ under project PID2021-123933OR-C31. L.G-B. (PRE2019-091053) N.A-I. (PRE2022-102928) and J.M.P-P. (PRE2022-104089) grants funded by MCIN/AEI/10.13039/501100011033 and FSE ‘investing in your future’. Authors are members of the Consortium cROS (IDI/2024/000719) and Consortium NySA-SERIDA (IDI/2024/000764) financed by Asturias Government, SEKUENS and ERDF.

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