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S4-P7 miRNA profiling in extracellular vesicles reveals host stress and dietary effects in ewes challenged by water restriction and *Haemonchus contortus*

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Water scarcity and rising temperatures caused by climate change threaten extensive and semi-extensive sheep production systems in the Mediterranean basin. This study aimed to evaluate the influence of the stress tolerance and sainfoin supplementation on miRNA profile of extracellular vesicles (EVs) from gastrointestinal fluids of ewes subjected to water restriction and *Haemonchus contortus* infection. EVs were isolated from abomasal gastric fluid of 28 Rasa Aragonesa ewes, classified as high (HT; n=14) or low tolerance (LT; n=14) to water and heat stress based on the neutrophil-to-lymphocyte ratio. Half of the ewes in each group received sainfoin pellets (S; n=14, 7 HT-S and 7 LT-S ewes), while the rest received a total mixed ration as control (C; n=14, 7 HT-C and 7 LT-C ewes) for 58 days. Small RNA-seq libraries (TruSeq Illumina) were processed by adapter trimming and quality filtering, retaining reads of 15-40 nucleotides (sRNAtoolbox). Reads were aligned to the sheep genome (Rambouillet 1.0) and miRNA precursors from miRbase using Bowtie. Mapping to *H. contortus* miRNA precursors was also performed. Differential expression analysis was conducted using DESeq2 ($|\log_2FC| > 1$; FDR < 0.05) via sRNAtoolbox. Fifty-six ovine and 72 *H. contortus* known miRNAs were identified. No significant changes were found in parasite-derived miRNAs. However, 6, 2, and 4 miRNAs were differentially expressed in comparisons HT-S vs HT-C, HT-S vs LT-S, and HT vs LT, respectively. These findings suggest that EV miRNAs are modulated by stress tolerance and diet, supporting further research into host-parasite interactions under combined environmental and biotic stress.