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Integrating omics data to investigate molecular responses to diverging environmental conditions in livestock

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Introduction

Environmental adaptation is a fundamental process ensuring species persistence. It is increasingly recognized that adaptive responses are driven by multiple factors, including genetic, epigenetic, and metabolic changes. While genetic variation underlies long-term evolutionary adaptation, non-genetic variation—particularly epigenetic modifications—can occur over short time scales through a process known as acclimation. This process depends on phenotypic plasticity, defined as the ability of a phenotype to respond to environmental changes (Ghalambor *et al*., 2007).

The molecular basis of phenotypic plasticity, which enables organisms to cope with contrasting environmental conditions, represents a major challenge in modern livestock and agricultural research. Species or populations exhibiting higher plasticity are thought to be more resilient and therefore better able to respond to climatic perturbations (Sgrò *et al*., 2011). Recent advances in omics technologies are beginning to shed light on the complex interactions among different biological layers. Epigenetic mechanisms, such as DNA methylation, can repress or enhance transcriptional pathways, with downstream effects on proteomic and metabolomic profiles.

Although substantial progress has been made—particularly in medical research and model organisms—the role of multi-omics interactions in environmental adaptation and acclimatization remains poorly understood in livestock species. In this study, we provide an integrated multi-omics perspective (RRBS, RNA-seq, proteomics, metabolomics, and lipidomics) to investigate molecular responses in animals translocated from stable housing to mountain pasture conditions.

Materials & Methods

Experimental design and sampling. Twenty individuals of the Pezzata Rossa Italiana (PR) cattle breed and twenty individuals of the Massese (MS) sheep breed were sampled in spring (T0; June). Subsequently, half of the individuals from each population were translocated to mountain pasture environments (>1,800 m a.s.l.). Specifically, ten PR individuals were moved to Alpine pastures, while ten MS individuals were moved to Apennine pastures. During late summer (September) all individuals either remained at stable conditions (T1s) or moved to the mountain Alpine/Apennine pasture (T1a) have been resampled. Blood samples were immediately frozen for RRBS and RNA-seq analyses, while an aliquot was centrifuged to obtain serum for proteomic, metabolomic, and lipidomic analyses.

Omics data preparation. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen), and total RNA was extracted using the NucleoSpin RNA Blood Kit (Macherey-Nagel). RRBS libraries were prepared using the Methylation MagPrep kit, which converts

unmethylated cytosines to uracil, while RNA-seq libraries were generated using a standard mRNA library preparation kit. All libraries were sequenced on the SURFSeq5000 (Genemind) NGS platform.
Untargeted metabolomic and lipidomic analyses, as well as next-generation proteomics, were performed using liquid chromatography–tandem mass spectrometry (LC-MS/MS).

Data analysis and integration. RRBS reads were mapped and methylation calls were performed using Bismark (Krueger & Andrews, 2011), and differentially methylated sites were identified using the methylKit R package (Akalin *et al*., 2012). RNA-seq reads were mapped and annotated using STAR (Dobin & Gingeras, 2015), and differential gene expression analysis was conducted with edgeR (Chen *et al*., 2025).
All omics datasets were integrated using the mixOmics R package (Rohart *et al*., 2025), applying the DIABLO (Data Integration Analysis for Biomarker Discovery using Latent Variable Approaches for Omics Studies) framework to maximize covariance across datasets while balancing discrimination and integration. Gene Ontology (GO) enrichment and pathway analyses were subsequently performed on the most significant variables.

Table 1. Summary of differential methylation (RRBS) and differential gene expression (RNA-seq) analyses in Massese sheep and Pezzata Rossa Italiana cattle.

Differential methylation analysis in Massese				
Condition	Tot. methylated sites	Filtered sites	Up regulated	Down regulated
T0 vs T1s	71,427	70,615	0	0
T0 vs T1a	159,163	149,972	31	81
Differential methylation analysis in Pezzata Rossa Italiana				
Condition	Tot. methylated sites	Filtered sites	Up regulated	Down regulated
T0 vs T1s	12,847	12,779	1	0
T0 vs T1a	24,801	24,601	0	2
Differential expression analysis in Massese				
Condition	Initial genes	Filtered genes	Up regulated	Down regulated
T0 vs T1s	56,874	13,598 (23.91%)	3,354 (24.67%)	2,790 (20.52%)
T0 vs T1a	56,874	13,598 (23.91%)	4,354 (32.02%)	3,556 (26.15%)
Differential expression analysis in Pezzata Rossa Italiana				
Condition	Initial genes	Filtered genes	Up regulated	Down regulated
T0 vs T1s	37,599	12,945 (34.43%)	1,153 (8.91%)	621 (4.80%)
T0 vs T1a	37,599	12,945 (34.43%)	320 (2.47%)	365 (2.82%)

Results & Discussion

Differential methylation (RRBS) and gene expression (RNA-seq) results for both PR and MS breeds are summarized in Table 1. In the MS breed, both analyses revealed a higher number of differentially methylated sites and differentially expressed genes in animals translocated to mountain pasture conditions (T0–T1a). In contrast, no significant differences were observed between conditions in the PR breed. This pattern may reflect a predominant seasonal effect influencing methylation and gene expression in PR animals.
Integration of the five omics layers (epigenomics, transcriptomics, proteomics, metabolomics, and lipidomics) using the DIABLO pipeline identified 515 and 580 variables maximizing discrimination among conditions in MS and PR breeds, respectively (Fig. 1A,B). Pairwise correlations among omics blocks for the selected variables showed high Pearson correlation coefficients, ranging from 0.89 to 0.95 in MS and from 0.89 to 0.92 in PR (Fig.

1C,D). Notably, integrated data visualization confirmed that PR individuals were primarily discriminated by sampling season (T0 vs. T1a/T1s), whereas MS individuals showed clear separation according to environmental conditions (T0/T1s vs. T1a). Based on this result, variables displaying strong positive or negative correlations (higher than 0.9) were further investigated in the MS breed to identify molecular pathways associated with environmental adaptation. GO enrichment analysis revealed significant molecular function terms related to oxygen carrier activity (GO:0005344). Pathway analysis highlighted enrichment in immune-related pathways, including the complement cascade, innate immune system processes, and oxygen transport pathways such as erythrocyte oxygen uptake and O_2/CO_2 exchange. The observed changes probably reflect a rapid adaptive response to altitude. Similar evidences have been reported in different livestock species including cattle, sheep and camels (Wang *et al*., 2021; Yan *et al*., 2024; Gera *et al*., 2025) in which animals exposed to high-altitude exhibit enhanced oxygen transport capacity, with increased abundance of several hemoglobin subunits, supporting improved oxygen supply and stress tolerance.

Figure 1. Circos plot showing highly positively and negatively correlated features among epigenomics, transcriptomic, proteomics, metabolomics and lipidomics datasets (with a correlation cutoff of 0.9) for PR A) and MS B). Inter-omics correlations of individuals (upper diagonal plot) displaying the first component in the five omics datasets and the Pearson correlation (lower diagonal plot) between each omics dataset for PR C) and MS D).

Conclusions

This study provides an integrated view of the molecular mechanisms underlying environmental adaptation in two livestock breeds exposed to contrasting farming conditions. By adopting a multi-omics approach, we demonstrate that the response to environmental change is breed-specific and reflects different levels of phenotypic plasticity. The Massese breed exhibited a clear molecular response to translocation to mountain pasture, with coordinated changes across epigenomic, transcriptomic, proteomic, and metabolomic layers.

Integrated data analysis, based on cross-layer concordance, enabled the identification of correlated features across datasets related to oxygen transport and immune response, supporting functional adaptation to high-altitude conditions. In contrast, the Pezzata Rossa Italiana breed showed molecular differences mainly associated with seasonal variation rather than environmental translocation, indicating a more limited plastic response to pasture conditions. However, the partial overlap between seasonal and environmental variables represents a potential confounding factor that should be considered when interpreting these differences. Although the relatively small sample size may limit statistical power and the generalizability of the findings, the consistency observed across multiple omics layers strengthens the robustness of the detected patterns.

This work underscores the value of integrative multi-omics approaches for studying adaptation and acclimatization processes in livestock species and provides novel insights relevant to sustainable animal management under changing climatic conditions. Future studies involving larger sample sizes, improved control of confounding factors and deeper functional validation will be essential to clarify causal relationships among omics layers and their role in adaptive responses.

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