Transport and stress: bio-loggers and miRNAs to measure the adaptive physiological response in lambs

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Transportation is one of the most stressful conditions for farm animals [1]. Handling and management of lambs during transport are challenges that perturb homeostasis. The consequent adaptive response with the hypothalamic-pituitary-adrenal axis (HPA) activation attempts to restore balance and welfare conditions. To date, innovative approaches to defining the welfare state of food-producing animals are linked with the digital revolution that allows quantifying animal welfare using parameters analysis obtained by sensors. On the other hand, from molecular biology comes identifying new molecules as biomarkers. Indeed, the HPA axis is promptly activated by stress stimuli modifying behavior, physiological parameters, and molecule expression, such as the microRNAs (miRNAs). These molecules play a role in the post-transcriptional gene regulation of several cellular processes. Expressed by tissues, miRNAs are released in body fluids and therefore called circulating miRNAs (c-miRNAs) [2]. They can be used as minimally invasive biomarkers by changing their profile under physiological, pathological, and psychological conditions [3].

Plasma and saliva samples of fourteen lambs, implanted with subcutaneous temperature (BT) and heart rate (HR) bio-loggers (DST micro-HRT, Star Oddi, Iceland), were collected five times/animal: 2 PRE (T0-24h; T1-4h before loading) and 3 POST (T2- immediately after unloading; T3-4h; T4- 24h) a transport of 75 min.

Salivary cortisol concentration was determined with an enzyme immunoassay. Based on their involvement in the adaptive response, 17 c-miRNAs were selected from literature and extracted from plasma and saliva. C-miRNAs' expression analysis was performed in real time q-PCR. BT (°C) and HR (bpm) data were analyzed with the Star-Oddi Mercury software.

Cortisol analysis showed a significant higher concentration immediately after the unloading procedure (T2) (ANOVA one-way test; $p<0.05$) when compared with T0, T1, T3 and T4 identified as basal undisturbed points (rest time). At T2, lambs presented a significant drop of BT (38.72±0.01) ($P<0.05$) and a peak of HR (155.14±5.44) ($p<0.05$).

Out of the 17 c-miRNAs analyzed, 5 were found expressed in plasma and saliva matrices of all sampling points. MiR-17, -23a, and -27a were differentially expressed in T2 only in saliva samples, while miR-24 in both matrices.

Lambs presented the same BT at basal points (mean 39.02±0.19), reaching a significant drop at T2 (38.72±0.3) ($P<0.05$), while they reached the maximum HR (bpm) during loading (150.09±4.89) and unloading procedures (T2: 155.14±5.44).

Physiological data obtained by innovative technologies like bio-loggers combined with classical and potential molecular biomarkers are useful for identifying the highly stressful time points during common farm procedures.

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