Salivary Heat Shock Proteins as Potential Biomarkers of Stress in Cattle

Among the non-invasive mean of evaluating stress, salivary cortisol has been the widely used one. Nevertheless, it is known that cortisol amounts can vary due to different stressful and physiological factors, and that they mainly reflect acute rather than chronic stress. Preliminary results from Portuguese partners (not published) suggest that salivary cortisol is not appropriate to evaluate moderated heat stress. Consequently, the search for other salivary biomarkers is warranted. Due to the expertise of Hungarian partner in dairy cattle heat stress and the expertise of Portuguese partner in salivary biomarkers, this STSM is intended to evaluate the potential of salivary heat shock proteins (HSPs) as stress markers in dairy cattle. During 15 days, saliva obtained in an *in vivo* assay carried out in Hungary will be analysed for the presence and amounts of HSPs 60, 70 and 90, using methodologies such as ELISA and Western blotting.

A heat stress period of 5 days was followed by a period of 5 days when barn cooling techniques had been used to eliminate heat stress. Altogether 16 healthy dairy cows in the mid-lactation were involved in the experiment. The same cows were used in the two periods of the experiment to evaluate the effects of heat stress and the effects of cooling on the animals. Salivary samples were taken into Salivette tubes (Sarstedt, Germany) once a day, at 16.00 pm on each day of the experiment. The samples were immediately frozen, and stored at – 20 °C.

Heart rate and heart rate variability was also recorded continuously during both period, by recording interbeat intervals (IBIs) with the Polar Equine RS800 CX heart rate receiver and a Polar Equine T56H transmitter (Polar Electro Oy, Finland), with two integrated electrodes and a specific transmitter. The Kubios HRV software (version 2.2, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) will be used for HRV analysis. Besides time domain measures [HR and vagal tone parameter root mean square of successive differences (RMSSD) between the successive IBIs] frequency-domain indices of HRV will also be calculated. IBI data are subjected to Fast Fourier Transformation of power spectrum analysis. Spectral parameters include the normalized power of the high-frequency band (HF) for detecting

tendencies in vagal activity and the relative power of the low frequency (LF) component and HF (LF/HF ratio), which is indicative for the sympathovagal balance.

Beside the biological samples and HRV, behaviour (time of lying and standing and rumination activity) was also evaluated by using HOBO Pendant G data loggers (Onset Co, USA), and SCR Heatime HR LD system respectively.

To evaluate the heat stress, environmental temperature and relative humidity was monitored in every 10 minutes during the 10 days period by using a Testo 175 H1 data logger (Testo AG, Germany).

Saliva samples will be analysed by using ELISA and Western blot methods, as mentioned above, at the Institute of Mediterranean Agricultural and Environmental Sciences (ICAAM) at the University of Evora, Portugal.