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Detection of 70 kDa heat shock protein in the saliva of dairy cows

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This Research Communication describes, for the first time, the detection of HSP70 in saliva of dairy cows. Thermal stress is a major environmental stress that limits animal growth, metabolism, and productivity. The cellular response to heat stress involves the synthesis of heat shock proteins (HSPs), presumably to protect the functional stability of cells at increasing temperatures. HSP70 has been found to be present in cattle blood serum and may also be present in other secretory fluids, such as saliva, as already observed in humans. The aim of this study was to detect heat shock protein HSP70 in bovine saliva. Saliva samples were taken from higher- ($n = 5$) and lower milk producing ($n = 5$) Holstein-Friesian cows in summer and in winter for the detection of HSP70. HSP70 concentrations were assayed using the ELISA technique. Salivary HSP70 concentrations ranged from 0·524 to 12·174 ng/ml in cows. Higher salivary HSP70 concentrations were significantly associated with higher milk production and higher environmental temperature, but not with rectal temperature.

Keywords: heat shock proteins, dairy cow, saliva.

The adverse effects of heat stress in dairy cows have been extensively studied in the past decades. Heat stress is a common cause of decreased production, reproductive disorders, increased mortality and changes in behaviour (West, 2003; Collier et al. 2006; Allen et al. 2015; Slimen et al. 2016). The body reacts to heat stress at different levels. At the cellular level, the production of heat shock proteins (HSPs) plays a major role in the short- and long-term acclimation to heat stress. As stress chaperones, heat shock proteins are most likely to protect cells from external stressors, such as thermal or oxidative damage. The 70 kDa molecular weight (HSP70) is one of the most studied HSPs and its function has been described in several stress situations (Daugaard et al. 2007). HSPs are found mainly inside the cells (in the cytosol, nucleus, lysosomes, mitochondria and endoplasmatic reticulum, Daugaard et al. 2007) and a free fraction is present in blood in very low concentrations that can be detected and quantified (Gaughan et al. 2013). The plasma levels of HSP70 have been related to heat stress in cattle (Gaughan et al. 2013) and this protein has also been reported to be present in human saliva (Fábián et al. 2003). Though the composition of the saliva of ruminants is different from that of humans (Lamy & Mau, 2012), the findings of Fábián et al. (2003), presenting evidences of salivary HSP70 in humans, lead us to assume that heat shock proteins may be also present in the saliva of cows. There is a limited number of studies in animals and to our knowledge, the presence of HSP70 in cattle saliva has not been reported until now. We hypothesised that HSP70 is present in cattle saliva in detectable amounts and that it may be positively correlated with rectal temperature.

Materials and methods

Five high-producing (lactation 2·3 ± 0·5, DIM: 115·8 ± 47·4, milk yield (kg): 22·1 ± 2·53) and 5 lower-producing (lactation 2·1 ± 0·4, DIM: 112·0 ± 46·3, milk yield (kg): 15·9 ± 1·73)

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from a dairy farm in the Alentejo region, Portugal, were assigned for study. The cows were kept in similar housing and feeding conditions and had no previous lameness, mastitis or other health issues to minimise any confounding factors that could influence HSP70 production other than the presence of heat stress. Saliva samples were collected from the cows on two consecutive days in summer (average daily temperature: 32·8 °C, average daily black globe temperature in shade: 26 °C) and winter (11 and 8 °C, respectively). At the time of saliva sampling, rectal temperatures and respiratory rates were measured to evaluate heat stress. Salivette cotton rolls (Sarstedt GmbH, Germany) were used for saliva sampling. The Salivettes were kept on ice from the time of sampling until processing in the laboratory where the samples were centrifuged at 3000 rpm for 5 min. The two saliva collections from each period were pooled for analysis, and the pooled samples were stored at −20 °C until processing. For the quantification of HSP70, an ELISA kit (SEA873Mi, Cloud-Clone Corp, USA) was used, according to the manufacturer's recommendations. A standard curve, prepared with the standard provided with the kit was run in the microplate. Samples and standards were run in duplicate without dilution. The intra-assay precision was assessed by running the same sample is five different wells, and resulted in a coefficient of variation (CV) of 3.6%. Inter-assay precision was not calculated, authors relied on manufacturer's instructions on sensitivity and precision (inter-assay CV<12%).

Data were analysed by one-way ANOVA, with a post-hoc Tukey-Hsu test for pairwise comparisons. Level of significance was set at $P < 0{\cdot}05$.

Results and discussion

Results for salivary HSP70 concentrations and physiological parameters are shown in Table 1.

Salivary HSP70 was successfully detected, and ranged in concentration ranged from 0·524 to 12·174 ng/ml. To our knowledge, this is the first study that demonstrates the presence of heat shock proteins in ruminant saliva. Salivary HSP70 concentrations were higher in summer than in winter in the high-yielding group (9·02 ± 2·75 *vs*. 2·54 ± 0·51 ng/ml. respectively; $P = 0{\cdot}042$). When comparing production levels, we found that HSP70 concentrations in summer were higher in high-producing cows, as compared to lower-producing animals (9·02 ± 2·75 *vs*. 2·34 ± 1·33 ng/ml, $P = 0{\cdot}04$). At the time of summer samplings, both low- and high-yielding cows were experiencing heat stress. Maximum rectal temperatures were higher in summer than in winter in both groups, but showed no difference between groups at either sampling. Our results show that salivary HSP70 levels are higher in a situation of high ambient temperature combined with increased productivity. Increase in the concentration of plasma HSP with heat stress has been reported in cows (Gaughan et al. 2013) and goats (Banerjee et al. 2014). However, salivary HSP70 concentrations in the low-producing group showed no difference between the time of heat stress and winter. Though experiencing a similar level of heat load, salivary HSP70 concentrations in the low-yielding group were lower than that of high-producing cows in summer. These interesting differences cannot be fully explained on the basis of our results. Yániz et al. (2009) reported an increased level of HSP70 in blood samples of cows as gestation progressed which was not affected by milk production, heat stress or chronic infection. Heat shock protein synthesis is induced by a variety of other factors, including hypoxia, viral infection, energy depletion, acidosis and reactive oxygen species (Kregel, 2002). Within the framework of this small-scale study, not all influencing factors could be controlled for. Yet, it can be speculated that the presence of higher extracellular HSP70 in the high-yielding group is due to differences in the energy status or as yet undefined changes in the passive or vesicular transport of HSPs (Ireland et al. 2007). This study highlights that the use of salivary HSP70 as a non-invasive biomarker may be a potential tool in further studies of thermal adaptation in cows.

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Table 1. Result for rectal temperature, respiratory frequency and salivary HSP70 concentrations in the high- and low-yielding groups of dairy cows

	Low-yielding		High-yielding	
	Summer	Winter	Summer	Winter
Rectal temp (°C)	38·87 ± 0·52	37·9 ± 0·43	38·77 ± 0·61	37·9 ± 0·54
Rectal max. (°C)	39·38 ± 0·27^{a}	38·27 ± 0·25^{b}	39·4 ± 0·27^{a}	38·58 ± 0·52^{b}
Resp. freq (1/min)	63·6 ± 8·36^{a}	32·5 ± 6·5^{b}	65·6 ± 11^{a}	32·7 ± 4·8^{b}
HSP70 (ng/ml)	2·34 ± 1·33^{A}	2·27 ± 0·35	9·02 ± 2·75^{aB}	2·54 ± 0·51^{b}

Descriptive statistics are based on mean ± SD of non-transformed data.
a, b: The different superscripts within a production group show significant difference ($P < 0{\cdot}05$).
A, B: The different superscripts between production groups in the same season show significant difference ($P < 0{\cdot}05$).

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