

Investigation of heat shock protein 70 for non-invasive detection of heat stress in dairy cows

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Heat stress (HS) negatively impacts dairy cows, leading to reduced milk production, impaired reproduction, and compromised welfare. The presence of heat shock protein 70 (HSP70) in bovine milk has been identified as a potential non-invasive biomarker for detecting HS in dairy cows. This study aims to evaluate various HS detection methods in dairy cows, focusing on the effectiveness of HSP70 as an indicator. Data were collected from 25 third-parity Holstein Friesian cows (211.8 ± 21.3 DIM) managed in a pasture-based system over 7 weeks at The University of Sydney's Dairy Farm, NSW, Australia. Rectal temperature (RT), infrared thermography (IRT), milk HSP70, milk yield, and milk composition were measured twice weekly, while reticulorumen temperature (RRT) was recorded every 10 min via bolus sensors, and environmental data were recorded every minute from the nearest weather station (Fig. 1). Pearson correlations and linear mixed models were used to evaluate the direct and lagged associations of RT, IRT, RRT, and milk HSP70 with the average temperature-humidity index (THI). Univariable and multivariable models with random effects for cow ID were compared to assess prediction accuracy. Results revealed that RT and IRT showed the strongest real-time associations with THI ($P < 0.001$; $R^2_c \approx 41\text{--}43\%$), while RRT also correlated significantly ($P < 0.001$; $R^2_c \approx 30\%$) but was weaker. Milk HSP70 was associated with THI at a 35-h lag with limited variance explained ($P < 0.001$; $R^2_c \approx 9\%$), indicating a delayed and supplementary response. Milk yield showed a negative association with THI from ~ 29 -h onwards ($P < 0.001$; $R^2_c \approx 33\%$), peaking at 48-h, whereas milk composition declined earlier at ~ 11 -h lags. Multivariable models identified RT and IRT as the most reliable real-time indicators, with RRT improving predictive accuracy, while milk HSP70 offered limited but complementary cellular-level insights. Integrating HSP70 with conventional methods may enhance HS prediction accuracy, support the selection of heat-tolerant cows, and improve dairy cow welfare under HS.



Fig. 1: Experimental design for physiological, environmental, and HSP70 biomarker measurements for heat stress detection in dairy cows.

Keywords: temperature humidity index, rectal temperature, infrared thermography, milk HSP70, sensor