Final Project Summary

P9b: Milk as an Indicator of Heat Load

May 2026

Dr Rezaul Rakib completed his PhD on a project to investigate the potential to use milk samples as an indicator of heat load.

Milk samples can be collected during routine milking without additional animal handling or discomfort. This offers a non-invasive and convenient alternative to testing though blood and saliva. There's also less room for error or subjectiveness compared to visually assessing a herd for heat load.

This project investigated the potential to use the level of the 'heat shock protein' HSP70 in milk as an indicator of heat load in dairy cattle. HSP70 is a stress-response protein that is naturally present in milk.

Key findings

The research found that milk HSP70 levels reflect heat load with a lag of approximately 33–50 hours, meaning it reflects prior or cumulative cellular stress rather than current conditions.

During sustained heatwave conditions it showed strong diagnostic performance, though its discriminatory power was more limited outside extreme heat events and may be influenced by health or physiological status.

Further validation across herds and seasons is needed, but the work positions milk HSP70 as a promising complementary tool within a broader framework for monitoring heat load. Potential applications include monitoring heat load at the individual cow level, at the herd management level and at the processor level. Additionally, it has the potential for remote monitoring and could be a service offered by milk processors.

Getting more from milk

Milk underpins the dairy industry. Getting more from the precious commodity has to potential to increase its value throughout the entire supply chain.

The Dairy UP team investigated novel ways to get more value from milk.

There were three elements to this research:

- P9a: Producing Milk with Less Lactose
- P9b: Milk as an Indicator of Heat Load
- P9c: Adding Value to Dairy Waste

This document provides an overview of P9b.

More info

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Research and results

The project developed and validated reliable, low-cost measurement methods and sample handling protocols. It also characterised seasonal variation and quantified lag times relative to physiological heat stress measures.

Analytical validation
An "in-house" ELISA test was adapted to detect HSP70 in milk, blood and saliva samples. The method was initially developed by Dr Indunil Pathirana in a collaboration with the Dairy Research Foundation.

ELISA is a laboratory testing technique that detects certain proteins, antibodies, hormones and much more. This ELISA test is cost effective and detects a broader range of HSP70 than commercial kits.

The research found that the ELISA test is reliable way to measure milk HSP70. Strong agreement was demonstrated between the in-house competitive ELISA and commercial ELISA across all sample types (saliva, blood and milk). This confirmed that the ELISA test is a feasible, accurate and affordable option for routine monitoring of heat load from milk samples.

Sample handling and storage
Milk samples were processed, either skimmed before storage or skimmed after storage and stored under controlled conditions (room temperature, refrigeration, freezing, refrigeration + preservative). HSP70 stability was assessed across multiple time points.

The research found that sample handling conditions materially affect HSP70 detection accuracy. Skimming milk before storage and holding samples refrigerated or frozen for up to three days preserved HSP70 integrity.

Storage at room temperature or with bronopol preservative reduced accuracy, with direct implications for how samples should be managed in commercial herd-testing workflows.

Seasonal variation
HSP70 concentrations were measured in milk, blood, and saliva across contrasting seasons and linked to temperature humidity index (THI), milk yield, and milk composition. Statistical models were used to quantify environmental and physiological influences.

The research found that HSP70 concentrations vary substantially between cows and across seasons. Levels were highest in summer and lowest in winter, consistent with seasonal variation in heat load.

Milk HSP70 showed greater variation between samples than blood or saliva, indicating that results from milk are more variable and need to be interpreted with this in mind.

Test sensitivity
Simultaneous measurements of temperature humidity index (THI), rectal temperature, infrared thermography (IRT), and reticulo-rumen temperature were collected to compare the sensitivity and responsiveness of milk HSP70 with established heat stress indicators. Time-lagged correlations (0–48 h) were calculated.

The research found that milk HSP70 reflects prior heat exposure rather than current conditions. Peak milk HSP70 concentrations occurred approximately 33–44 hours after peak temperature humidity index and 48–50 hours after peak reticulo-rumen temperature. This confirmed that HSP70 levels reflect cumulative cellular stress over the preceding one to two days rather than providing a real-time indicator of heat load. This time lag has direct implications for how and when the HSP70 levels can be usefully applied in practice.

Heatwave trial
A five-day heatwave was closely monitored to understand how HSP70 levels changed over time, identify useful thresholds, and assess how accurately it can detect when cows are under heat stress.

The results show that diagnostic performance is strong during sustained heat events but more limited under typical seasonal conditions.

During the heatwave, a milk HSP70 threshold of approximately 550 ng/mL demonstrated good

diagnostic performance.

Under non-heatwave seasonal conditions, discriminatory power was more limited, consistent with the view that milk HSP70 functions most reliably as an indicator when cellular stress is both substantial and persistent.

HSP70 levels may also be influenced by other biological factors not considered in this study, including stage of lactation, lactation number, production level, and health status, which should be considered when interpreting results outside of extreme heat conditions.

Applications

These findings pave the way for future applications for monitoring heat load at the cow, herd and processor/industry level.

Cow/farm level
At the cow and farm level, milk HSP70 provides a quantitative indicator of heat load that can be collected during routine milking. It best suited to retrospective assessment of cumulative heat load rather than real-time intervention decisions. Its specificity to cellular stress, rather than environmental temperature alone, represents an advance over existing THI-based approaches, though its interpretation requires consideration of other factors known to influence HSP70 expression (e.g. stage of lactation or health status). The validated threshold of approximately 550 ng/mL provides an objective, repeatable basis for comparing heat stress severity across cows and seasons in research and monitoring contexts, pending further field validation.

Herd management
Milk HSP70 measurement has potential for integration into herd testing, enabling surveillance of cumulative heat load at the herd level without additional labour requirements. It could also serve as an objective tool for evaluating the effectiveness of heat stress mitigation strategies — such as cooling systems, shade provision, or adjusted milking schedules — providing evidence for both on-farm management decisions and industry-level welfare reporting.

Processor/industry application
Being able to consistently monitor heat load could help processors manage milk supply quality and variability. It could also be used to provide data to underpin sustainability and animal welfare reporting requirements which are increasingly expected by domestic and international markets.

Collaborators

The P9b project was a collaboration between Dairy UP researchers at the University of Sydney and NSW DPIRD and the University of Ruhuna, Sri Lanka.

This project involved a PhD study undertaken by Rezaul Rakib and an Honours thesis prepared by Jade Nguyen.

Related work

Other DairyUP projects investigated heat load from different angles:

- P2d: Heat stress
- P6: Cow response to climate extremes.

Read more

P9 final report

M.R.H. Rakib et. al. Skimming and storage factors affect the detection of heat shock protein 70 in raw bovine milk, JDS Communications, Volume 6, Issue 4, 2025, Pages 578-582.

M.R.H. Rakib et. al. Comparison between in-house competitive and commercial enzyme-linked immunosorbent assays for the detection of heat shock protein 70 in milk, blood, and saliva of dairy cows, Journal of Dairy Science, Volume 109, Issue 4, 2026, Pages 4596-4603

M.R.H. Rakib et. al. Detecting heat shock protein 70 in milk, blood, and saliva of dairy cows exposed to different seasonal conditions, Journal of Dairy Science, Volume 109, Issue 4, 2026, Pages 4647-4658

M.R.H. Rakib, et. al. Graduate Student Literature Review: Potential use of HSP70 as an indicator of heat stress in dairy cows—A review*, Journal of Dairy Science, Volume 107, Issue 12, 2024, Pages 11597-11610

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Delivery organisations

The University of Sydney – Dairy Research Foundation

NSW Government – Department of Primary Industries and Regional Development

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Dairy Australia

Partner organisations

NSW Farmers – Growing the best

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