Final Report

P9 Designer Milk

Department of Primary Industries and Regional Development

This project was led by Juan Gargiulo from the Department of Primary Industries and Regional Development

Dairy UP (Phase 1) was a $16 million, five-year industry driven project with a portfolio of 10 research, development and adoption projects collectively aiming to realise three primary objectives:

- Increase Productivity and Profitability by unlocking the potential of milk, the cow and water,
- De-risking the industry and
- Developing new markets.

A key part of Dairy UP was a coordinated network of partner farms across New South Wales (and beyond) providing valuable insights into real world application of new practices, including the challenges and benefits of new innovative technologies.

Dairy UP made a big contribution to dairy research and development rejuvenation, (attracting new researchers, PhD students and Industry investment).

Dairy UP was funded through the Australian and NSW Government's Bushfire Industry Package – Sector Development Grant (BIP-SDG) program, with cash co-contributions from Dairy Australia, The University of Sydney's Dairy Research Foundation, Local Land Services, Norco, Leppington Pastoral Co Ltd and Dairy NSW; and in kind contributions by all the above organisations plus NSW DPI (Biosecurity and Food Safety; Agriculture), Scibus, Australia Fresh Milk Holding Ltd, Dairy Connect and NSW Farmers.

This project was delivered jointly by University of Sydney's Dairy Research Foundation, Scibus, Department of Primary Industries and Regional Development.

Proudly funded by

Delivery organisations

Additional program supporters, collaborations or partnerships

Charles Sturt University | DairyBio | DataGene | Eagle Direct | Entegra | Macquarie University | NSW EPA | smaXtec | UC Davis | University of Technology Sydney

Contents

1. Executive Summary

The Australian dairy industry is under growing pressure to produce more efficiently, manage climate risk, and reduce waste. The P9 "Designer Milk" project was funded under the Dairy UP program to address three specific gaps across the supply chain, each with direct relevance to Australian producers and processors.

P9a — Less lactose, less water, more milk solids: Milk is approximately 87% water, and it is the sugar lactose that draws most of that water into the udder. Cows that synthesise less lactose while maintaining equivalent milk solids output (fat + protein), can effectively produce more valuable milk per litre — reducing transport, processing costs, and resource use. Despite this potential, no large-scale Australian dataset existed to determine whether such cows exist in meaningful numbers or whether the trait could be selected for. Analysis of over 30,000 NSW cows identified a group of 80 individuals that consistently produced equivalent milk solids output with around 15% less lactose and water volume. Heritability estimates suggest the trait has a meaningful genetic component, indicating that selection is feasible. The potential scale of impact is substantial: a 1% reduction in lactose across the national herd could reduce milk transport volumes by around 80 million litres annually — saving an estimated 2,700 truck trips and 20,000 litres of water per farm per year. Though the underlying molecular mechanisms remain to be confirmed, the work establishes an evidence-based foundation for a genetic selection program.

P9b — Milk as an indicator of heat load: Heat stress (HS) costs the Australian dairy industry millions of dollars each year in lost production, poorer reproduction, and compromised animal welfare. Yet most monitoring approaches rely on environmental measures that do not directly reflect what is happening inside the animal. This subproject evaluated heat shock protein 70 (HSP70), a stress-response protein naturally present in milk, as a non-invasive indicator of cellular HS measurable during routine herd testing. Reliable, low-cost measurement methods were validated, and sample handling requirements were established. A key finding is that milk HSP70 responds to HS with a delay of approximately 33–44 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 HS monitoring framework.

P9c — Adding value to dairy food waste (DFW): More than 1,300,000 tonnes or ~16% of milk produced in Australia is lost across the supply chain as food waste, representing a significant economic and environmental cost to the industry. While the potential to convert DFW into value-added products through microbial fermentation has been demonstrated internationally, the waste streams most amenable to this approach in the Australian context, the biological pathways best suited to them, and the most commercially relevant bioproducts had not been systematically evaluated. This subproject addressed those gaps by mapping Australian DFW streams and identifying manufacturing by-products — particularly cheese whey and whey permeate — as strong candidates for scalable bioproduction, with microbial biomass and derived products representing the most immediate valorisation opportunities. As a proof of concept, a food-grade yeast strain was successfully engineered to grow on lactose as its sole carbon source and produce pro-vitamin A, demonstrating that the biological pathway is viable. Significant technical and economic development is required before commercial application; however, the work establishes a clear foundation and a defined set of next steps toward scalable bioproduction from Australian DFW.

Collectively, the Designer Milk project generated 6 peer-reviewed publications in high-impact international journals, one PhD thesis and one Honours thesis. The project reached audiences of over 2,700 people across more than ten conference presentations in Australia and internationally, and engaged the broader industry through podcasts, media articles and farmer meetings. The three subprojects offer evidence-based, interconnected pathways for improving dairy productivity, animal welfare, and environmental sustainability — and together provide a sound basis for continued investment in applied dairy research in NSW.

2. Project Overview

Item	Description
Project Title	Designer Milk
Funding Body	Dairy UP
Dairy UP Project	P9
Project Duration	2022-2026
Lead Organisation	NSW Department of Primary Industries and Regional Development (NSW DPIRD)
Project Lead	Dr. Juan Gargiulo
Key Collaborators	The University of Sydney (USYD); Macquarie University (MCU); University of California, Davis (UC Davis)

3. Abbreviations

ADSA — American Dairy Science Association
AGF — Australian Genome Foundry
CV — Coefficient of Variation
DFW — Dairy Food Waste
DIM — Days in Milk
ELISA — Enzyme-Linked Immunosorbent Assay
HS — Heat Stress
HSP70 — Heat Shock Protein 70
JDS — Journal of Dairy Science
JDS Comms — Journal of Dairy Science Communications
LALBA — Alpha-lactalbumin
LP — Lactose Percentage
LY — Lactose Yield
MCU — Macquarie University
MSY — Milk Solids Yield (kg fat + kg protein)
MSY:LY — Milk Solids Yield to Lactose Yield Ratio
NSW DPIRD — NSW Department of Primary Industries and Regional Development
SCC — Somatic Cell Count
THI — Temperature–Humidity Index
UC Davis — University of California, Davis
USYD — The University of Sydney

4. Project Background and Rationale

The Australian dairy industry faces increasing pressure to improve productivity, sustainability, and resilience. Rising input costs, climate variability, tightening environmental regulation, and competitive global markets are pushing producers and processors to find new ways to extract more value from existing resources.

Dairy UP was established to accelerate applied research that directly addresses some of these pressures. Within this context, the P9 "Designer Milk" was funded to explore three interconnected opportunity areas: improving the efficiency of milk composition, enhancing the tools available for animal health monitoring, and recovering value from DFW streams. These areas were selected because they span the full supply chain, from cow genetics and on-farm management through to processing and waste, and because each represented a gap between existing research knowledge and practical industry application in the Australian context. Key strategic alignments of each subproject with the priorities of Dairy UP, Dairy Australia, and NSW DPIRD are summarised below.

Subproject	Dairy UP Priorities	Dairy Australia Strategic Plan 2030	DPIRD RD&E Strategy 2025–2030
P9a Less Lactose, Less Water, More Milk Solids	Productivity & profitability — unlock the true potential of NSW dairy systems; developing value-add dairy products	Priority 2.2 — Building herd productivity: genetic gain; nutrition efficiency; higher milk income	Future-ready production — driving innovation in established industries; strengthening supply chains; circular economy
P9b Milk as an Indicator of Heat Load	De-risking — new tools to better prepare dairy businesses for future challenges (climate)	Priority 2.3 — Adapting to a changing operating environment: tools to adapt to a changing climate; data-driven decision making	Climate resilient primary production - Resilience and improved management to extreme weather
P9c Adding Value to Dairy Food Waste	Developing new markets — greater economic contribution through value-adding; improved long-term food security of NSW	Priority 3.1 — Advancing industry sustainability: coordinated action on emerging issues; industry sustainability framework	Future-ready production — driving innovation in established industries; circular economy and sustainability in primary industries

Dairy UP Priorities; **DA Strategic Plan 2030**; **NSW DPIRD RD&E Strategy 2025-2030**

5. Project Objectives

The project aimed to generate applied, industry-relevant knowledge to improve the efficiency, sustainability, and resilience of the Australian dairy supply chain. The specific objectives were to:

- **Improve milk production efficiency** by quantifying variation in lactose synthesis and identifying cows capable of producing equivalent milk solids with reduced lactose and water volume (i.e., concentrated milk).
- **Develop novel indicators of animal stress and welfare** by evaluating milk-based biomarkers, particularly heat shock protein 70 (HSP70), as non-invasive tools for monitoring heat stress (HS) in dairy cows.
- **Identify and develop pathways for dairy food waste (DFW) valorisation** by characterising Australian waste streams and assessing their suitability for conversion into value-added products through microbial fermentation.
- **Generate integrated datasets and analytical frameworks** linking production, genetics, environment, and physiology to support future research and industry application.
- **Translate research findings into practical insights** for producers, processors, researchers and industry stakeholders through targeted dissemination and engagement activities.

6. Subproject P9a — Less Lactose, Less Water, More Milk Solids

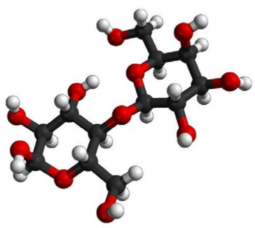
Juan Gargiulo (Research Scientist, NSW DPIRD); Russ Hovey (Prof. Animal Science, UC Davis); Yani Garcia (Prof. of Dairy Science and Dairy UP Program Leader, USYD); Rezaul Rakib (PhD Student, USYD).

6.1 Background

Milk lactose is the primary carbohydrate in bovine milk and plays a central role in regulating milk volume by generating an osmotic gradient that draws water into the mammary gland (Figure 1). Given that milk is approximately 87% water, even small changes in lactose synthesis can substantially influence milk volume. Across breeds, higher concentrations of milk components are typically associated with a proportional reduction in milk volume, often resulting in lower total daily fat and protein yields. However, some cows may exhibit reduced lactose synthesis while maintaining total kilograms of fat and protein which could improve dairy efficiency. Such animals would likely have lower energy requirements, reduced water transport and processing costs, and enhanced resource use efficiency across the supply chain.

Previous research demonstrated that the administration of exogenous glucocorticoid (dexamethasone) can reduce lactose synthesis and water volume by approximately 45% without affecting fat and protein output (Shamay et al., 2000). This response has been linked to the downregulation of α-lactalbumin (LALBA), a key enzyme in lactose synthesis (Sadovnikova et al., 2022). These findings suggest that reduced-lactose milk production has a biological basis, can vary among individuals, and may be heritable.

Despite this evidence, large-scale data characterising natural variation in lactose production across Australian dairy herds remain limited. The factors regulating lactose synthesis under commercial conditions have not been systematically evaluated in the Australian context, and no validated phenotypic or genetic framework currently exists to identify and select animals with improved lactose efficiency.



Lactose is the main milk sugar and a key determinant of milk volume. Lactose concentration is relatively stable

Some species produce milk with lower lactose and higher fat and protein content

Glucocorticoids reduced lactose synthesis and milk volume (~45%), associated with changes in gene expression of key mammary gland proteins

Figure 1. Milk lactose is the primary determinant of milk volume and is relatively conserved across cows; however, substantial variation exists across species and cattle breeds, and lactose synthesis can be experimentally manipulated, resulting in marked changes in milk volume, and concentration of milk solids.

6.2 Objectives

- Quantify variation in lactose yield (**LY**) and lactose percentage (**LP**) in NSW dairy herds.
- Characterise associations between lactose traits and key variables, including milk yield, milk solids yield (**MSY,** kg fat + protein), cow characteristics, and environmental factors.
- Identify cows with high MSY relative to lactose output and characterise the genetic and phenotypic attributes.

6.3 Materials & Methods

- **Data sources:** The analysis used almost 400,000 herd test records from 33,200 cows across 85 NSW herds (2008–2022), including milk yield, milk composition (lactose, fat, protein), reproductive performance, and breed. Supplementary datasets included sire genetic values and local meteorological data**.** Records were collated, cleaned, and merged with genetic and climate datasets.
- **Statistical analysis:** Linear mixed models were used to assess the contributions of parity, stage of lactation, year, season, climate indices (THI), and sire genetics to variation in LY, LP, and MSY. Phenotypic correlations between lactose traits and other production variables were also estimated.
- **Identification of high-efficiency cows:** Cows were ranked by their milk solids yield to lactose yield ratio (MSY:LY), a metric aiming to capture the efficiency of milk solids production relative to lactose output. An enhanced methodology was subsequently developed, incorporating lactation curve modelling and adjustment for parity, days in milk, and mastitis events, to enable more precise and equitable comparisons across animals.

6.4 Key Findings

- **Substantial phenotypic variation exists across the population.** LY, LP, and MSY varied considerably across cows and herds, confirming that meaningful phenotypic diversity exists within the commercial population, a necessary precondition for selection.
- **Breed influences lactose production traits:** On average, Holsteins produced the highest LY and MSY, but had the lowest MSY:LY ratio (~1.50) (Figure 2 a-d). In contrast, Jerseys had the lowest LY and MSY, yet achieved the highest MSY:LY ratio (~1.75), indicating that, they produce milk solids more efficiently relative to lactose and water output. Crossbred animals showed intermediate values for most traits. LP was broadly similar across breeds (~4.95 - 4.98%).
- **Stage of lactation and parity are significant determinants.** LY and LP peaked in early lactation and declined progressively thereafter. LY increased with lactation number while LP decreased (Figure 2e-h).
- **Lactose is negatively associated with heat load and somatic cell count (SCC).** A negative phenotypic correlation was observed between LY and the temperature-humidity index (THI, $r = -0.20$), and between LP and SCC ($r = -0.30$), consistent with known effects of HS and mammary health on lactose synthesis.
- **Lactose traits have a meaningful genetic component.** Heritability estimates for LY and LP ranged from 0.24 to 0.33, indicating that a substantial proportion of phenotypic variation is attributable to genetic effects. This is a critical finding, as it supports the feasibility of genetic selection for improved lactose efficiency.

- **A cohort of high-efficiency cows was identified.** Eighty cows were identified that consistently produced milk with a high MSY:LY ratio, delivering equivalent MSY output to the broader population with approximately 15% less lactose and water volume (Table 1). These animals were descendants of 13 sires spanning Jersey, crossbred lines and Holsteins, suggesting that the relevant genetic variation is already present within the commercial population.
- An enhanced methodology for identifying high-efficiency cows was subsequently developed and validated on a Dairy UP dataset, identifying a cohort of 90 cows. Blood and milk samples have been collected from some of these animals. DNA and milk LALBA concentrations are being analysed to identify genetic variants associated with the high-efficiency phenotypes.

Table 1*. Predicted means for cows with high milk solids yield to lactose yield ratio (MSY:LY) and the rest of the population for the main variables analysed.*

Variable	High MSY:LY cows (n = 80)	Rest of population (n = 33,200)	Difference (%)
Milk Yield (kg/cow/d)	20.36*	23.97	-15
Lactose Yield (kg/cow/d)	0.98*	1.19	-18
Fat Yield (kg/cow/d)	1.01*	0.96	+5
Protein Yield (kg/cow/d)	0.75*	0.8	-6
Milk Solids Yield (kg/cow/d)	1.76	1.76	0
Lactose (%)	4.81*	4.96	-3
Fat (%)	5.39*	4.14	+30
Protein (%)	3.89*	3.38	+15
Milk Solids (%)	9.29*	7.53	+23
MSY:LY	1.94*	1.53	+27
Somatic cell count (SCC, '000)	114*	91	+25

**groups are statistically different ($p < 0.05$)*

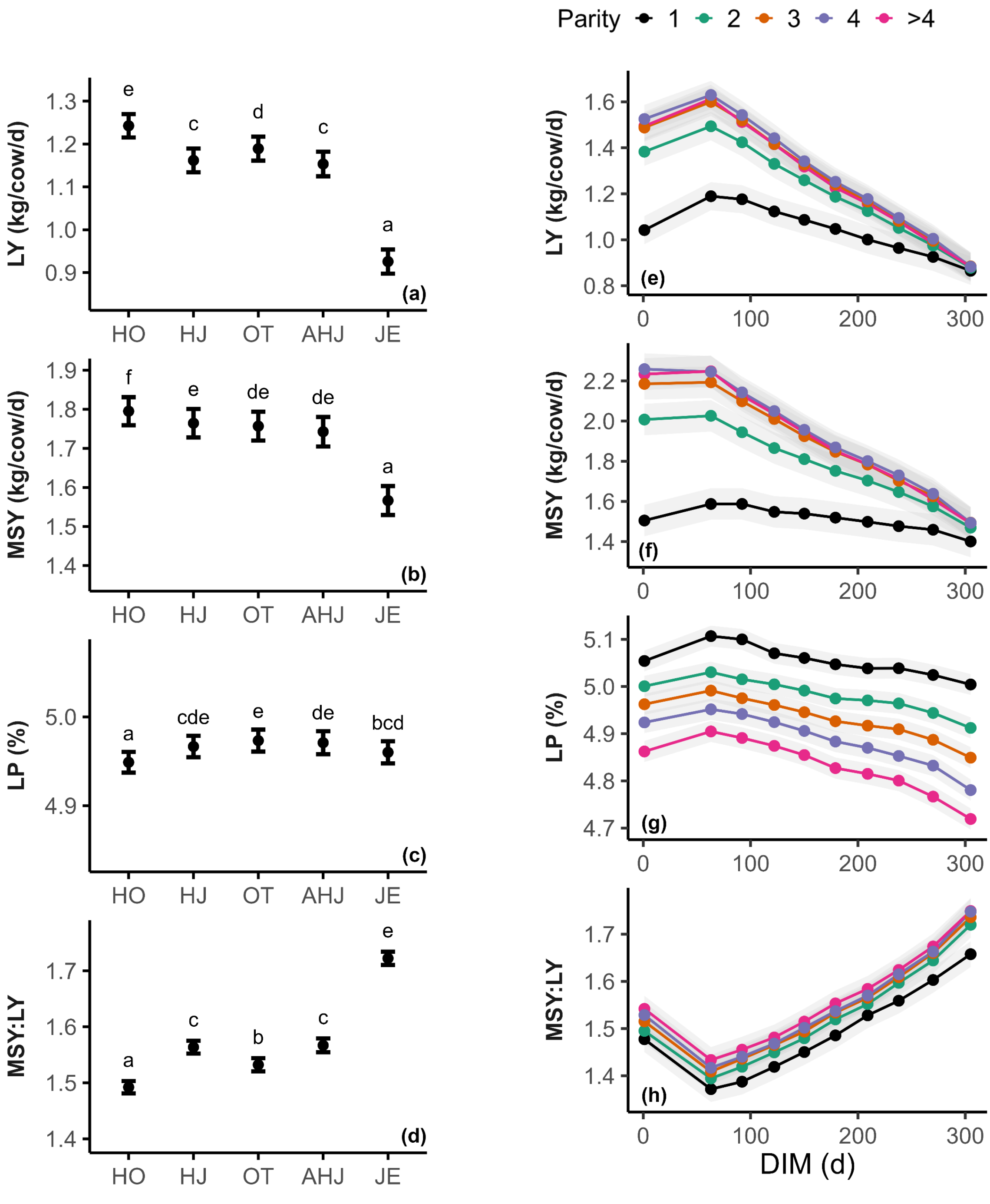


Figure 2. *Effect of breed* ***(a-d)*** *and parity and DIM* ***(e-h)*** *on key variables. HO = Holstein; HJ = Holstein × Jersey; OT = other; AHJ = Ayrshire × Holstein × Jersey; JE = Jersey. Means with different letters are significantly different (P < 0.05).*

6.5 Applications & Impacts

The findings have direct relevance across multiple levels of the dairy supply chain:

At the cow level, reducing lactose synthesis can lower the metabolic energy demand associated with milk production. For example, a 1% reduction in lactose output in a 7,000-litre cow would reduce glucose requirements by ~3.5 kg per lactation — representing a meaningful improvement in energy balance, particularly during periods of peak demand.

At the farm level, lower milk volume per cow may reduce milking frequency requirements in high-producing or automated milking systems, with associated benefits for labour and equipment utilisation.

At the processor level, a higher milk solids concentration reduces the quantity of liquid requiring transport and processing. A 1% reduction in lactose output across the national herd is estimated to reduce annual milk transport volumes by approximately 80 million litres, equivalent to approximately 2,700 truck trips or 800,000 kilometres travelled per year.

At the environmental level, reduced lactose production lowers both the energy and water requirements associated with milk production and processing. For example, a 1% reduction in lactose is estimated to save approximately 20,000 litres of water per farm annually, with considerable cumulative benefit at national scale.

6.6 Key Outputs

- A high-quality, integrated dataset linking lactose production traits with genetics, environment, and production variables across 85 NSW commercial herds, the first such dataset compiled for the Australian dairy industry.
- Development and validation of an enhanced phenotypic methodology for identifying high-efficiency cows based on modelled lactation curves, adjusted for parity, stage of lactation, and health status.
- Peer-reviewed publication in the *Journal of Dairy Science (JDS)* characterising lactose variability and its determinants in Australian dairy herds.
- Conference presentations and industry reports disseminating findings to researchers, producer and processor audiences.

6.7 Future Research Opportunities

The identification of a cohort of cows consistently exhibiting high MSY:LY ratios, combined with moderate heritability estimates for lactose traits and previous experimental research, provides an empirical foundation for a genetic selection program. The leading hypothesis is that these animals may carry variation in regulatory regions governing LALBA expression, resulting in reduced lactose synthesis without affecting fat or protein secretion. Confirmation of this mechanism would represent a significant advance in understanding mammary gland function and could unlock a practical selection pathway for the Australian industry.

Priority areas for future research include:

- **Genetic marker discovery:** Genome-wide association analysis of high-efficiency cows to identify variants associated with elevated MSY:LY, as a basis for genomic selection tools.
- **Molecular characterisation:** Targeted investigation of LALBA and related lactation genes in sampled animals to confirm the proposed biological mechanism and define the molecular basis of the phenotype.

- **Early-life predictors:** Identification of markers measurable early in life that are predictive of long-term lactose efficiency, enabling earlier and more cost-effective selection decisions.
- **Economic and systems modelling:** Quantitative assessment of the farm- and industry-level impacts of selective breeding for this trait across different herd structures, breeds, and payment systems.

7. Subproject P9b — Milk as an Indicator of Heat Load

Juan Gargiulo (Research Scientist, NSW DPIRD); Rezaul Rakib (PhD student, USYD); Yani Garcia (Prof. of Dairy Science and Dairy UP Program Leader, USYD), Valeria Messina (Technical Specialist, USYD); Jade Nguyen (Honours student, USYD); Nicolas Lyons (Head of Science, DairyNZ); Indunil Pathirana (Prof. in Animal Science, University of Ruhuna).

7.1 Background

HS is a major constraint in Australian pasture-based dairy systems, reducing milk yield, reproduction, and animal welfare. Traditional HS indicators, such as the THI, rectal temperature or reticulorumen temperature, provide useful environmental and immediate physiological information but offer limited insight into cellular-level stress responses.

Heat shock protein 70 (HSP70), a conserved molecular chaperone, is rapidly upregulated during cellular stress and has recently been detected in milk, offering a potential non-invasive biomarker that can be incorporated into routine herd testing (Figure 3). Subproject P9b evaluated HSP70 as a practical indicator of cellular stress by validating analytical methods, defining sample-handling requirements, characterising seasonal variation, and quantifying lag times relative to physiological HS measures.

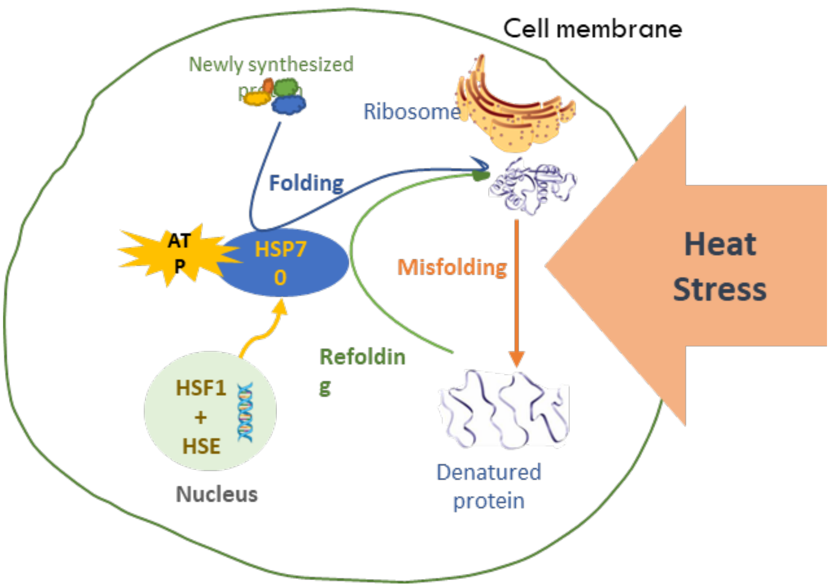


Figure 3. HPS70 is induced in response to heat stress (HS) to help stabilise and protect proteins, and has been previously measured in blood and saliva, and recently in detected in milk [adapted from Rakib et al. (2024)]

7.2 Objectives

- Review current knowledge of HS detection in dairy cows, focusing on the potential and limitations of HSP70 as a molecular biomarker.
- Validate reliable and cost-effective laboratory methods for detecting HSP70 in milk, blood, and saliva.
- Establish milk sample handling and storage protocols to ensure accurate HSP70 detection.
- Characterise between-cow and seasonal variation in HSP70.
- Compare milk HSP70 with environmental and physiological HS indicators.
- Quantify time lags between heat load and milk HSP70 response.
- Identify on-farm and industry applications for HSP70-based HS monitoring.

7.3 Materials & Methods

To address these objectives, the project undertook a series of coordinated studies:

- **Analytical validation**: A newly developed in-house competitive ELISA was benchmarked against a commercial ELISA across milk, blood, and saliva. Agreement, precision, working range, and cost were evaluated.
- **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.
- **Seasonal biological characterisation**: HSP70 concentrations were measured in milk, blood, and saliva across contrasting seasons and linked to THI, milk yield, and milk composition. Mixed-effects models were used to quantify environmental and physiological influences.
- **Comparative evaluation with physiological indicators**: Simultaneous measurements of THI, rectal temperature, infrared thermography, and reticulorumen temperature were collected to compare the sensitivity and responsiveness of milk HSP70 with established HS indicators. Time-lagged correlations (0–48 h) were calculated.
- **Heatwave trial:** A five-day heatwave event was monitored intensively to validate temporal behaviour, evaluate threshold values, and assess diagnostic performance using ROC analysis.

7.4 Key Findings

- **Milk HSP70 can be measured reliably using a validated, low-cost method.** Strong agreement was demonstrated between the in-house competitive ELISA and commercial ELISA across all matrices, confirming that accurate and affordable detection of HSP70 in dairy samples is feasible for routine application.
- **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.
- **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 exhibited greater coefficient of variation than blood or saliva (CV 54% vs. 34% and 32%, respectively), indicating a higher degree of biological variability in the milk fraction that must be accounted for in interpretation.
- **Milk HSP70 reflects prior heat exposure rather than current conditions.** Peak milk HSP70 concentrations occurred approximately 33–44 hours after peak THI (Figure 4) and 48–50 hours after peak reticulorumen temperature, confirming that the biomarker reflects cumulative cellular stress over the preceding one to two days rather than providing a real-time indicator of heat load. This temporal lag has direct implications for how and when the biomarker can be usefully applied in practice.

- **Diagnostic performance was strong during sustained heat events but more limited under typical seasonal conditions.** During a five-day 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 assessed in this study, including stage of lactation, lactation number, production level, and health status, which should be taken into account when interpreting results outside of extreme heat conditions.

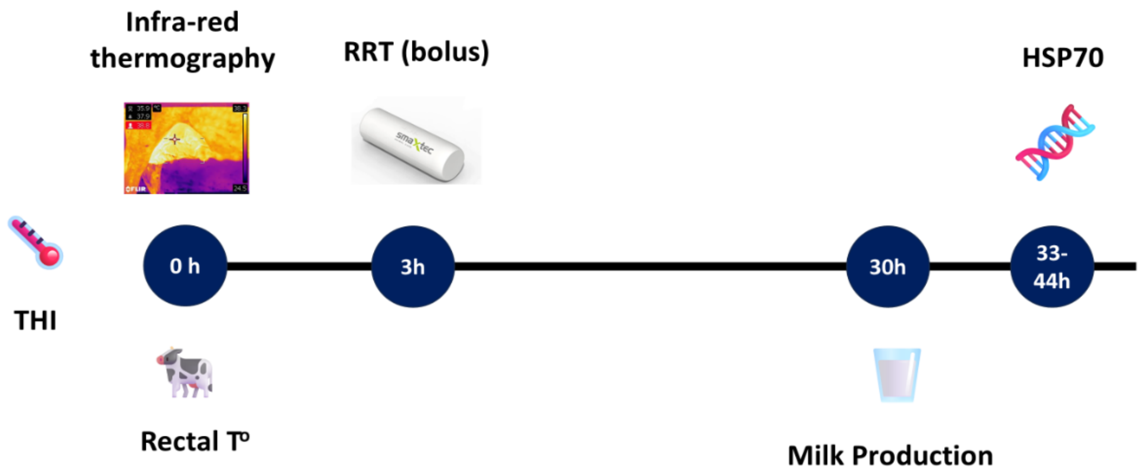


Figure 4. Comparison of heat stress (HS)detection methods and their lagged relationship with temperature-humidity index (THI)

7.5 Applications & Impacts

- **At the cow and farm level,** milk HSP70 provides a specific, quantitative indicator of cellular HS that can be collected during routine milking without additional animal handling or discomfort. Its delayed response profile, reflecting cumulative stress over the preceding 33–44 hours, makes it best suited to retrospective assessment of heat exposure 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 HS severity across cows and seasons in research and monitoring contexts, pending further field validation.
- **At the herd management level,** milk HSP70 measurement has potential for integration into herd testing, enabling surveillance of cumulative cellular stress at the herd level without additional labour requirements. It could also serve as an objective tool for evaluating the effectiveness of HS 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.
- **At the processor and industry level,** consistent and quantifiable HS monitoring can support processors in managing milk supply quality and variability and provides data to underpin sustainability and animal welfare reporting requirements increasingly expected by domestic and international markets.

7.6 Outputs

- Validation of a low-cost, in-house competitive ELISA for HSP70 detection in milk, blood, and saliva, benchmarked against a commercial assay — providing a practical analytical tool for adoption in research and industry settings.
- Establishment of evidence-based sample handling and storage protocols required for accurate milk HSP70 detection in commercial workflows.
- Characterisation of the temporal lag between heat load and milk HSP70 response, establishing the biomarker's behaviour relative to established physiological indicators across seasonal and acute heat conditions.
- Four peer-reviewed publications in the JDS and JDS Comms (Table 2), with two additional manuscripts under revision
- PhD thesis (Rezaul Rakib, USYD) documenting the full body of research conducted under this subproject.
- Honours thesis (Jade Nguyen, USYD) on "Assessing Alternatives to Rectal Temperature for Heat Stress Detection in Dairy Cattle"
- Presentations at major national and international scientific conferences, including the American Dairy Science Association Annual Meeting (USA, 2024 and 2025), the Australasian Dairy Science Symposium (New Zealand, 2024), and the Dairy Research Foundation Symposium (Australia, 2023 and 2025).

7.7 Future Research Opportunities

- Future studies should extend beyond HS alone to evaluate milk HSP70 as an integrative marker of overall physiological stress and health status in dairy cows, including potential associations with disease, ageing, mastitis, and immunological activation.
- Significant variations in HSP70 responses were observed among cows under similar environmental conditions, suggesting that animals may differ in how their cells respond to heat load. Further research is needed to determine whether these individual variations are consistent across lactations and environmental conditions, and to better understand the biological processes underlying them.

8. Subproject P9c — Adding Value to Dairy Food Waste

Juan Gargiulo (Research Scientist, NSW DPIRD); Briardo Llorente (Associate Prof. and Chief Scientist AGF, MCU); Yani Garcia (Prof. of Dairy Science and Dairy UP Program Leader, USYD); Ian Paulsen (Prof. and Director ARC Centre of Excellence in Synthetic Biology and AGF, MCU); Belinda Fabian (Business Manager AGF, MCU), Artur Wlodarczyk (Synthetic Biologist, MCU); Hugh Goold (Research Scientist, NSW DPIRD); Ken Garner (Manager Dairy, NSW DPIRD).

8.1 Background

Dairy food waste (DFW) represents a substantial and underutilised resource across the Australian supply chain, totalling 1,363,500 tonnes or ~16% of national milk production (Dairy Australia, 2024). Manufacturing is the dominant source, generating lactose-rich by-products, and process waste as inherent outputs of milk processing (Figure 5). Manufacturers spend approximately $967 million per year on waste management, with a further $786 million lost through finished products discarded across retail, food service, and households. Beyond these direct costs, DFW generates significant environmental burdens, including greenhouse gas emissions, water use, and waste disposal.

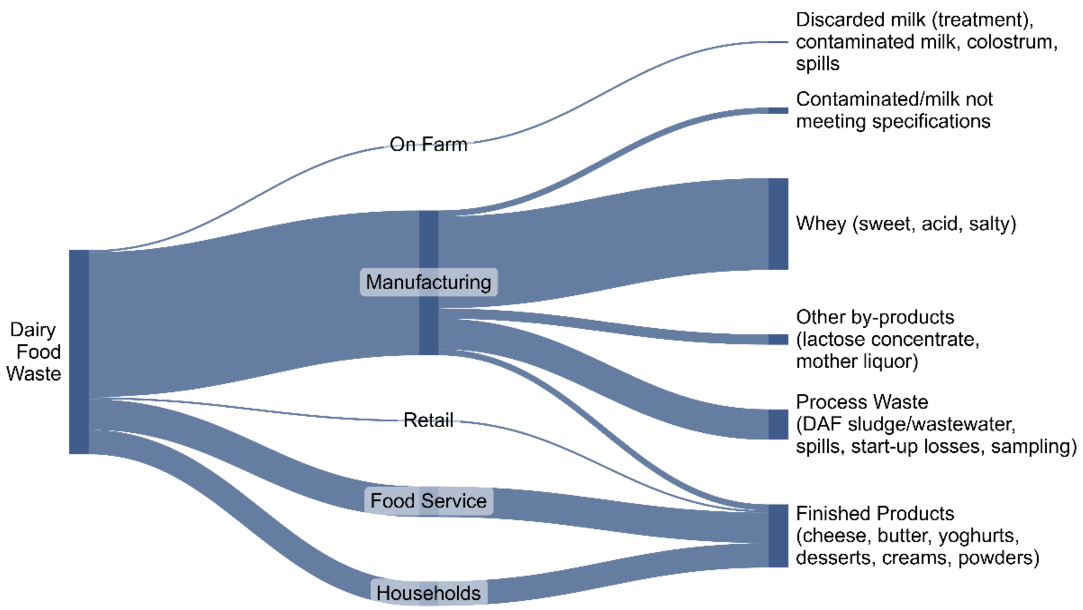


Figure 5. Dairy food waste across the supply chain [adapted from Dairy Australia (2023)].

Valorisation of DFW through microbial fermentation offers a pathway to recover value by converting these streams into useful products, including animal feed ingredients and other high value bioproducts, while reducing disposal costs and environmental impacts. This approach aligns with circular economy principles and has been demonstrated at laboratory scale internationally.

Despite this potential, the DFW streams most amenable to microbial fermentation in the Australian context had not been systematically evaluated. In addition, key biological and engineering constraints—particularly the inability of standard microbial strains to efficiently utilise lactose, the principal carbohydrate in DFW—had not been addressed in a food-grade production context. Limited proof-of-concept existed for producing value-added compounds from Australian DFW using engineered food-grade microorganisms. This subproject was designed to address these gaps.

8.2 Objectives

- Identify DFW streams in Australia that are most suitable for microbial fermentation
- Identify value-added compounds that can be produced from DFW
- Develop a food-grade yeast strain capable of growing on DFW and producing target compounds as a proof of concept.

8.3 Materials & Methods

- **Literature review and stakeholder consultation:** A comprehensive review was conducted to identify DFW sources, compositional variability, and their potential as substrates for microbial bioproduction. Industry stakeholders were consulted to prioritise feasible DFW sources and value-added products.
- **Biological and engineering strategies:** Approaches to overcome key constraints, particularly lactose utilisation and substrate variability, were evaluated.
- **Yeast engineering:** Synthetic biology and adaptive laboratory evolution were applied to engineer food-grade *Saccharomyces cerevisiae* capable of growing on lactose as the sole carbon source and producing pro-vitamin A as a proof of concept.

8.4 Key Findings

- **Microbial fermentation represents a viable and strategically relevant pathway for DFW valorisation.** A systematic review of the Australian DFW landscape confirmed that the sector produces waste streams well-suited to bioproduction in terms of volume, composition, and consistency, supporting the case for investment in this approach as a practical circular economy strategy.
- **Cheese whey and derived by products were identified as the most suitable streams for microbial valorisation.** These streams are produced in concentrated and relatively consistent volumes at manufacturing facilities, making them more amenable to scalable bioproduction than household or food service waste, which is compositionally variable and logistically dispersed. On-farm DFW is only ~1%, though a significant proportion comprises milk with antibiotics fed to calves, representing a potential risk and future opportunity for valorisation.
- **Microbial biomass and derived products represent the most immediate valorisation opportunity.** Analysis of potential bioproducts identified microbial biomass and its derivatives as the most scalable near-term opportunity, primarily for animal nutrition applications. Other compounds, including amino acids, organic acids, and enzymes, were identified as potential targets for higher-value applications requiring further technical development.
- **The inability of standard yeast strains to metabolise lactose is the principal technical constraint.** Conventional *S. cerevisiae* strains cannot efficiently utilise lactose. Alternative species, such as *Kluyveromyces marxianus*, offer partial solutions but present performance and regulatory limitations. Engineered S. cerevisiae strains can overcome this constraint but require further optimisation for industrial-scale efficiency.
- **A food-grade yeast strain capable of growing on lactose and producing pro-vitamin A was successfully engineered.** Using a combination of synthetic biology and adaptive laboratory evolution, the research team at MCU engineered a food-grade S. cerevisiae strain capable of growing on lactose as its sole carbon source and producing pro-vitamin A (Figure 6). The strain also demonstrated growth on 50% milk supplemented with lactose, confirming feasibility for DFW valorisation. While currently at laboratory scale, this establishes a functional platform for further development.

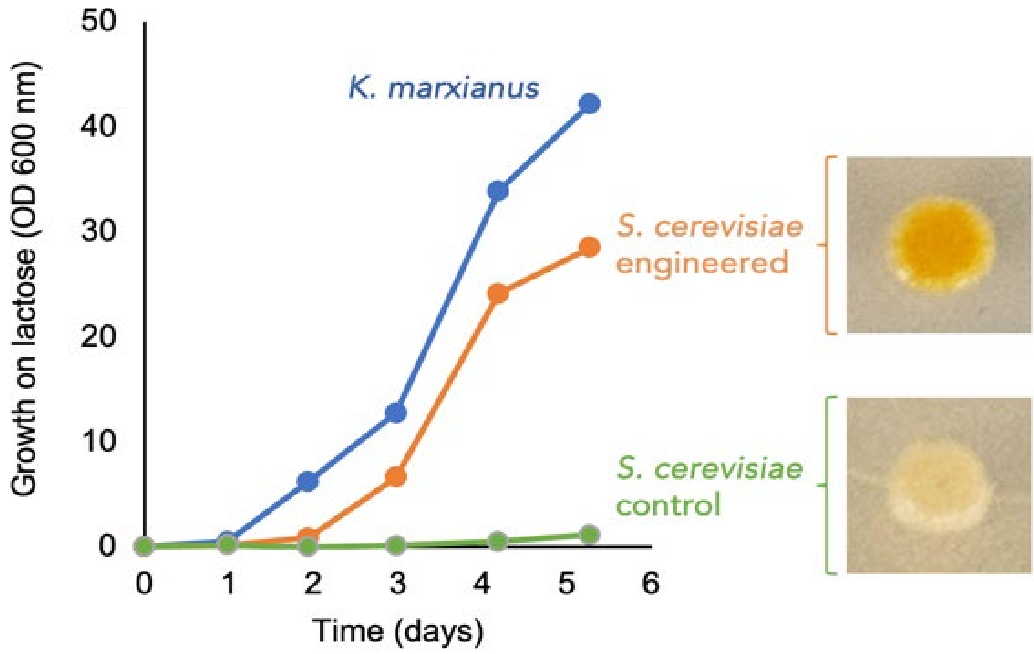


***Figure 6**. Engineered S. cerevisiae (orange) can grow on lactose and produces provitamin A, which gives the cells an orange colour. Kluyveromyces marxianus (blue), a yeast capable of growing on lactose is shown for comparison. Control, non-engineered S. cerevisiae (green) cannot grow on lactose and cells are white.*

8.5 Applications & Impacts

- **At the manufacturer level**, microbial valorisation of concentrated DFW streams — particularly cheese whey and whey permeate — provides a practical pathway to generate value-added products, reduce waste disposal costs, and create new revenue streams. For processors currently spending significant resources on waste management, even partial valorisation of these streams could materially improve the economics of waste handling.
- **At the environmental level**, converting DFW into useful products reduces the volume requiring disposal, lowering associated greenhouse gas emissions, water use, and environmental compliance costs. This approach directly supports the circular economy principles that are increasingly expected by regulators, retailers, and consumers in domestic and international dairy markets.
- **At the technical level,** the successful engineering of a food-grade yeast strain capable of lactose utilisation and pro-vitamin A production demonstrates that a key biological constraint can be overcome. This establishes a platform that can be extended to produce a broader range of target compounds.

The proof-of-concept demonstration of growth on lactose substrates and production of a nutritionally relevant compound provides the evidence base required to justify further investment in scale-up and techno-economic evaluation.

8.6 Outputs

- Systematic identification and characterisation of DFW streams suitable for microbial fermentation, with whey and derived by-products identified as primary candidates — providing an evidence base for industry investment decisions.
- Literature review characterising DFW valorisation opportunities and the biological pathways

available for their realisation.

- Engineering and characterisation of a food-grade S. cerevisiae strain capable of lactose utilisation and pro-vitamin A production, demonstrating proof-of-concept for value-added bioproduction from DFW.
- Established relationships with dairy manufacturers and industry stakeholders, facilitating access to dairy by-products and providing practical insight into supply chain constraints and potential commercial pathways.
- Compilation of historical datasets on milk composition, by-products, and waste streams that informed experimental design and provide a foundation for future techno-economic modelling.

8.7 Future Research Opportunities

This subproject established proof-of-concept for the valorisation of DFW through engineered yeast fermentation, confirmed the suitability of key Australian waste streams, and demonstrated that a major technical constraint—lactose utilisation by food-grade yeast—can be overcome. The work remains at laboratory scale, and translation to commercial application will require coordinated efforts in optimisation, scale-up, and economic validation.

Priority areas for future research include:

- **Techno-economic analysis:** Evaluation of production targets, input costs, and revenue potential for priority bioproducts (e.g. microbial biomass, pro-vitamin A) to define conditions for commercial viability.
- **Enzyme engineering for improved lactose assimilation:** Development of β-galactosidase variants with enhanced activity and stability under dairy waste conditions.
- **Strain optimisation:** Improvement of growth rates, product titres, and tolerance to substrate variability under industrial conditions.
- **Platform strain development:** Engineering strains for production of additional value-added compounds using the lactose-utilising platform developed in this project.
- **Pilot-scale validation:** Scale-up of promising strain–substrate systems in collaboration with industry partners to generate process and economic data required for commercial assessment.

9. Project-wide Dissemination

The project has produced one PhD thesis, one Honours thesis, and six peer-reviewed publications in high-impact international journals (JDS and JDS Comms), accumulating 34 citations to date (Table 2). The JDS, the leading journal in dairy science, has an impact factor of 4.4 and is ranked Q1 in its field, reflecting the quality and reach of the outlets targeted by this project.

Findings were also presented at multiple conferences and scientific events, including three major international forums, most notably the American Dairy Science Association (ADSA) Annual Meeting in the USA, as well as Australian and New Zealand venues (Table 3). These presentations collectively reached an estimated audience of over 2,700 people, spanning industry professionals, researchers and policymakers.

Beyond peer-reviewed and conference outputs, the project actively engaged internally and externally with industry and farming communities through a range of channels, including farmer meetings, webinars, podcasts, newsletters, and online articles. A list of these outputs is provided in Tables 3 and 4 (Annexes).

***Table 2**. Peer-reviewed publications in international journals.*

Author	Title	Journal	Year Published	Citations (to May 26)
Gargiulo (2026)	Invited Review: Turning Dairy Food Waste into Valuable Products via Yeast Bioproduction (In Press)	JDS	2026	-
Rakib et al. (2026a)	Detecting heat shock protein 70 in milk, blood, and saliva of dairy cows exposed to different seasonal conditions	JDS	2026	-
Rakib et al. (2026b)	Comparison between in-house competitive and commercial ELISA for the detection of heat shock protein 70 in milk, blood, and saliva of dairy cows	JDS	2026	-
Rakib et al. (2025)	Skimming and storage factors affect the detection of heat shock protein 70 in raw bovine milk	JDS Comms	2025	2
Gargiulo et al. (2025)	Sources of variation underlying the production of lactose by dairy cows	JDS	2025	7
Rakib et al. (2024)	Graduate Student Literature Review: Potential use of HSP70 as an indicator of heat stress in dairy cows—A review	JDS	2024	25

10. Conclusions and Recommendations

The P9 - Designer Milk project has delivered meaningful progress across three interconnected areas of dairy research, generating evidence-based findings with clear, still early-stage, pathways to industry application.

P9a demonstrated that substantial variation in lactose production exists across NSW dairy herds. The project identified a subset of cows capable of maintaining equivalent milk solids output with significantly lower lactose and water volume, with potential implications for transport, processing costs, and resource use efficiency. Heritability estimates indicate a genetic component to this variation, supporting selective breeding as a plausible long-term pathway for improving milk efficiency. However, the underlying genetic mechanisms remain unconfirmed, and further molecular research is required before practical selection tools can be developed.

P9b validated milk HSP70 as a measurable, non-invasive biomarker and established sample-handling requirements for reliable detection. However, its relationship with HS was not consistently strong, with concentrations influenced by additional biological and environmental factors, and a delayed response limiting its use for real-time monitoring. These findings highlight both the potential and current limitations of HSP70 as an indicator, indicating that further work is required to improve its specificity before integration into routine herd testing. Results suggest that HSP70 is better positioned as a complementary component within a broader, multifactorial biomarker framework for assessing HS and animal resilience.

P9c identified key DFW streams and prioritised bioproducts for valorisation through microbial fermentation. A proof-of-concept was established developing an engineered yeast strain capable of growing on lactose and producing pro-vitamin A, demonstrating the technical feasibility of this approach. The work remains at laboratory scale, and significant technical optimisation and economic evaluation are required before commercial application.

Across all three subprojects, the project has produced several peer-reviewed publications, engaged audiences across Australia and internationally, and built productive industry partnerships, laying a solid foundation for the next phase of research.

Recommendations for future investment should prioritise:

- Molecular studies to identify genetic markers associated with high-solids, low-lactose cows in P9a
- Field validation of HSP70 monitoring in commercial herd-testing workflows in P9b
- Techno-economic analysis and scale-up optimisation for dairy waste valorisation in P9c

11. Annexes

Table 3. Conference presentations, abstracts and other meetings

Authors	Title	Presentation Type	Conference /Event	Location	Year	Audience
Rakib, R.	Investigations of heat shock protein 70 for non-invasive detection of heat stress in dairy cows	Final PhD Presentation (oral)	SOLES HDR Showcase (USYD)	Sydney	**2025**	40
Rakib, R.	Milk speaks when cows are heat-stressed	Oral presentation	Thesis competition (USYD)	Sydney	**2025**	60
Rakib, et al.	Milk speaks when cows are heat-stressed	Abstract/oral presentation	DRF Symposium	Wollongong	**2025**	150
Gargiulo, J.	Unlocking the Potential of Milk	Oral presentation	DIAA Dairy Conference	Parramatta	**2025**	40
Gargiulo et al.	Factors influencing lactose production in dairy cows	Refereed abstract/poster presentation	ADSA Annual Meeting	Louisville, USA	**2025**	1,000
Rakib et al.	Potential of HSP70 as a non-invasive biomarker for detecting heat stress in dairy cows	Refereed abstract/poster presentation	ADSA Annual Meeting	Louisville, USA	**2025**	
Rakib et al.	Influence of management and storage factors on detection of HSP70 in raw bovine milk	Refereed abstract/oral presentation	ADSA Annual Meeting	Florida, USA	**2024**	1,000
Rakib et al.	Influence of management and storage factors on detection of HSP70 in raw bovine milk	Poster presentation (Intermediate PhD)	SOLES HDR Showcase (USYD)	Sydney	**2024**	100
Rakib et al.	Detection of HSP70 in milk, blood, and saliva of dairy cows exposed to different climatic conditions	Refereed abstract/Oral presentation	ADSS	Christchurch, NZ	**2024**	200
Rakib et al.	Can heat shock protein 70 be monitored in dairy cows under heat stress?	Oral presentation (Introductory PhD)	SOLES HDR Showcase (USYD)	Sydney	**2023**	40
Rakib et al.	Can heat stress be detected in the milk of dairy cows?	Oral presentation	DRF Symposium	Camden	**2023**	100
Gargiulo, J.	P9 – Designer Milk	Farmer meeting presentation	Dairy UP Roadshow	Bega and Cobargo	**2023**	30

Table 4. Technical reports, pamphlets, published material and other media engagements

Authors	Title	Place Published	Year
Gargiulo, J.	Producing milk with less water?	DPIRD Dairy Newsletter	2025
DairyNews Australia	Less water, more milk solids	DairyNews Australia	2025
Gargiulo, J.	Producing milk with less lactose - why?	The Business of Dairy Podcast	2025
Gargiulo, J.	HSP70 as an indicator of heat load	DPIRD Dairy Newsletter	2025
DairyNews Australia	Research aims to add value to dairy waste	DairyNews Australia	2024
Monks, L.; Gargiulo, J.; Hovey, R. & Garcia, S.	Producing milk with less water?	Dairy UP Website	2024
Monks, L.; Gargiulo, J.; & Llorente, B.	Adding value to milk waste	Dairy UP Website	2024
Gargiulo, J. & Llorente, B.	Revalorisation of dairy food waste	Dairy UP Webinar	2024
Monks, L.; Gargiulo, J.; Rakib R. & Garcia, S.	Milk as an indicator of heat load	Dairy UP Website	2023
Garcia, S. & Gargiulo, J.	Dairy UP - fast tracking the NSW dairy industry through R, D & E	The Business of Dairy Podcast	2023
Gargiulo, J.	New opportunities for milk?	Dairy UP Webinar	2023
Gargiulo, J.	More milk income from less milk volume? Exploring lactose variability in NSW herds to concentrate milk solids	Dairy UP Webinar	2023

12. References

Dairy Australia. 2023. Dairy sector food waste action plan. Accessed Oct. 27, 2025. https://dair-p-001.sitecorecontenthub.cloud/api/public/content/Dairy-Sector-Food-Waste-Action-Plan?v=8478bd66

Dairy Australia. 2024. Delivering on the Dairy Food Waste Action Plan. Accessed Oct. 27, 2025. https://dair-p-001.sitecorecontenthub.cloud/api/public/content/Delivering-Dairy-Food-Waste-Action-Plan?v=0d182afb

Gargiulo, J. I., S. C. Garcia, and R. C. Hovey. 2025. Sources of variation underlying the production of lactose by dairy cows. Journal of Dairy Science 108(4):4403-4421.https://doi.org/10.3168/jds.2024-25644

Gargiulo, J. I. L., B.; Fabian, B.; Wlodarczyk, A.; Goold, H.; Paulsen, I.; Garcia, S. C. . 2026. Invited Review: Turning Dairy Food Waste into Valuable Products via Yeast Bioproduction. Journal of Dairy Science (In Press).https://doi.org/10.3168/jds.2025-27900

Rakib, M. R. H., V. Messina, J. I. Gargiulo, N. A. Lyons, and S. C. Garcia. 2024. Graduate Student Literature Review: Potential use of HSP70 as an indicator of heat stress in dairy cows. Journal of Dairy Science 107(12):11597-11610.https://doi.org/10.3168/jds.2024-24947

Rakib, M. R. H., V. Messina, J. I. Gargiulo, N. A. Lyons, I. N. Pathirana, P. C. Thomson, and S. C. Garcia. 2025. Skimming and storage factors affect the detection of heat shock protein 70 in raw bovine milk. JDS Communications 6(4):578-582.https://10.3168/jdsc.2025-0759

Rakib, M. R. H., V. Messina, J. I. Gargiulo, N. A. Lyons, I. N. Pathirana, P. C. Thomson, and S. C. Garcia. 2026a. Detecting heat shock protein 70 in milk, blood, and saliva of dairy cows exposed to different seasonal conditions. Journal of Dairy Science 109(4):4647-4658.https://10.3168/jds.2025-27609

Rakib, M. R. H., V. Messina, J. I. Gargiulo, I. N. Pathirana, N. A. Lyons, and S. C. Garcia. 2026b. 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 109(4):4596-4603.https://10.3168/jds.2025-27811

Sadovnikova, A., S. C. Garcia, J. F. Trott, A. T. Mathews, M. T. Britton, B. P. Durbin-Johnson, and R. C. Hovey. 2022. Transcriptomic changes underlying glucocorticoid-induced suppression of milk production by dairy cows. Frontiers in Genetics 13.https://doi.org/10.3389/fgene.2022.1072853

Shamay, A., F. Shapiro, H. Barash, I. Bruckental, and N. Silanikove. 2000. Effect of dexamethasone on milk yield and composition in dairy cows. Annales de Zootechnie 49(4):343-352.https://doi.org/10.1051/animres:2000125