DairyUP
Unlocking potential

Final Report

P2 Unlocking the potential of the cow



This project was led by Ian Lean from Scibus

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

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CONTENTS

Executive Summary **5**

Project Overview **7**

Abbreviations **8**

Project Background and Rationale **11**

Project Objectives **12**

Subproject P2a Cow Longevity – i. Dairy UP Database **13**

Subproject P2a Cow Longevity – ii. Retrospective Study Series **15**

Increased parity is negatively associated with survival and reproduction in different production systems 15

Associations of parity with health disorders and blood metabolite concentrations in Holstein cows in different production systems 18

Subproject P2a Cow Longevity – iii. Body Condition Score Series **20**

Holstein dairy cows lose body condition score and gain body weight with increasing parity in both pasture-based and total mixed ration herds 20

Associations among body condition score, body weight, and serum biochemistry in dairy cows ... 21

Subproject P2a Cow Longevity – iv. Lipidomic series **24**

Glycerophospholipids in dairy cow health and longevity: a review 25

A large multisite lipidomic investigation of parity and aging in dairy cows 27

Confinement and pasture-based dairy herds differ in plasma lipid profiles 29

A large, multisite investigation into the lipidomics of survival in dairy cows 31

Do housed cows have greater metabolic-age than pasture-based cows based on lipid profiles? 33

Subproject P2a Cow Longevity – v. Housing System Series **35**

Reproduction, mastitis and lameness in housed and pasture-based systems: associations with parity 35

Effects of a bacterial and enzymatic product on physical and chemical properties of bedding and cow preference in compost bedded pack barns 38

Subproject P2b – Early Alert Systems **40**

Subproject P2c – Milk as a Diagnostic Fluid **41**

Editorial: Milk as a Diagnostic Fluid 41

Milk as an indicator of dietary imbalance 43

Milk as diagnostic fluid for udder health management 45

Milk as a diagnostic fluid to monitor viral diseases in dairy cattle - 46

Subproject P2d Heat Stress **47**

Effects of changes in total mixed ration nutrition and maximum temperature humidity index (THI) on dairy cow milk yield 47

Subproject P2e Calves/Heifers - i. Lifetime Different Diet Effects in Steers 49

Different lifetime dietary strategies affect carcass characteristics and rumen function in Holstein steers - doi.org/10.1071/AN25153 49

Subproject P2e Calves/Heifers - iii. Calf Performance 51

Milk consumption and behavior of calves in automated calf feeders as early indicators of weaning liveweight 51

Subproject P2e Calves/Heifers - iv. Feedlot Adaptation 53

Feedlot adaptation: characterising rumen fermentation profiles that determine success or failure 53

Subproject P2f The Infectome 55

Next-generation detection in bovine respiratory and enteric diseases: metagenomic and amplicon sequencing insights into microbial diversity 55

Integrated Metatranscriptomic Profiling of Enteric Pathogens in Bovine Neonatal Diarrhea 58

Bovine Respiratory Disease in NSW Dairy Calves- Understanding Respiratory Viruses, Bacteria, and Calf Immune Responses Across NSW Dairy Farms 63

The molecular epidemiology of rotaviruses in NSW dairy calves, Australia 67

Bovine Respiratory Disease Study – Mesonivirus Findings 69

Whole-genome surveillance of Escherichia coli from healthy and scouring dairy calves across New South Wales: a matched case-control study 72

Detection of blaCTX-M genes in Escherichia coli in dairy calves in Australia. 74

Subproject P2g Heifers Early Calving 77

Meta-analysis of the effects of age at first calving on production outcomes, calving difficulty, and reproduction in dairy heifers 77

Age at first breeding in Holstein dairy cattle: Effects on age at first calving, production, dystocia, health and reproduction in primiparous heifers 79

Age at first breeding in Holstein dairy cattle: Associations of hormone, biochemical and protein measures - *In Review* 81

Project-wide Dissemination 83

Annexes 86

Conclusion and Recommendations 93

Executive Summary

Dairy UP Project 2 was a broad, integrated research program designed to improve the health, productivity, welfare and longevity of Australian dairy cattle. The project addressed these outcomes across the life course of the animal, from calf and heifer development through to mature cow survival, reproduction, disease risk and the impact of production systems. Across seven subprojects, that each focused on improving dairy cattle health, productivity, welfare, longevity and resilience. The work combined large-scale farm data, prospective field studies, retrospective analyses, diagnostic reviews, molecular technologies and nutrition research.

A central achievement of Project 2 was the development and use of the Dairy UP database, which provided a high-integrity data platform for research, farm reporting and future industry decision-making. The database integrated herd records, milk production, health, reproduction, cow movement, environmental and herd test information, and now contains approximately 200,000 lactation records across 30 farms. This data infrastructure supported several studies within the cow longevity, facility design and heifer rearing programs and demonstrated the value of well-audited, farm-derived data for both research and practical farm management.

The cow longevity studies on body condition score, body weight, blood metabolites and lipidomic profiles provided new insights into the biological changes that occur as cows age. These findings highlighted potential pathways, including omega fatty acid biology, that may contribute to differences in health, reproduction and survival among cows.

Project 2 also addressed system-level changes in the Australian dairy industry. Comparisons between pasture-based and housed or confinement systems indicated that both systems can support strong health and reproductive outcomes, although differences in diet, housing, management and lipid profiles may create different biological risk profiles. The project also examined the effects of nutrition and heat load on milk production, showing that dietary changes may have delayed effects on milk yield and that heat stress remains an important constraint on productivity.

Other subprojects extended the program beyond mature cow longevity. Work on milk as a diagnostic fluid reviewed the growing potential for milk-based monitoring of nutrition, udder health and infectious diseases. Early alert systems were explored as a mechanism to improve preparedness for animal, plant and environmental health risks. Calf and heifer studies examined early-life nutrition, calf performance, rumen adaptation, feedlot outcomes and age at first breeding. The infectious disease project used advanced sequencing technologies to improve understanding of respiratory and enteric pathogens in dairy calves and to strengthen future disease surveillance.

Specific outcomes for the projects include;

- A clear understanding of the increased risks of disease and metabolic disorder with increased parity of cows. This will provide farmers and advisors with clarity around the increased risks with increased parity of cows.
- Identifying, that there are marked differences in lipid profiles with age of cows and that cows in housed facilities have older profiles than those at pasture.
- These differences suggest means by which the problems of aging might be modified with lipid supplementation.
- Cows lose body condition but gain weight as they age, indicating a pathway to reducing risks of problems in older cows.

- We identified differences in herd removals between housed and pastured herds which will help farmers and advisors understand the benefits and risks from different systems.
- We identified methods to improve bedding management in compost bedded pack barns.
- An early alert system for bovine ephemeral fever was established.
- Milk has great value as a diagnostic fluid and is under-utilised by the Australian industry, Milk urea nitrogen will be very valuable to routinely report.
- An important finding was to quantify the lagged influence of diet on milk production highlighting that nutritional responses to diet occur over days to weeks.
- Diets differing in formulation provided the potential to integrate dairy calves into the beef supply chain but with different rumen lifetime adaptations.
- Greater milk consumption in early life (up to day 5) had substantial effects on weaning weight of calves.
- Holstein cattle achieved excellent feedlot performance compared to beef breeds. High performing cattle at feedlot induction were higher performing overall compared to those with lesser early performance.
- Metagenomic and amplicon sequencing methods will change understandings and improve accuracy of veterinary diagnostic testing.
- Calf diarrhoea is rarely caused by a single pathogen and is substantially explained by the farm environment. This reinforces the importance of good husbandry for farmers and advisors.
- Kobuvirus was identified as an emerging pathogen associated with diarrhoea.
- Pestivirus, while of moderate prevalence, was a substantial pathogen. This can be controlled with vaccination.
- Insights were gained on host responses to potential pathogens providing avenues for future control of disease without using antimicrobial treatments.
- Similarly respiratory disease risks were dominated by farm environment. Rhinitis-viruses and Torovirus were identified as pathogens. Again, disease occurs as result of interactions among potential pathogens.
- Host responses to pathogens were an important part of the pathogenesis of the disease.
- Reducing the age at first mating of heifers weighing >330 kg to 10 to 13 months as opposed to mating at >13 months resulted in > 800 L greater milk production in the first lactation as a result of more heifers entering and remaining in the herd. This has implications for costs of rearing and green-house gas footprint.
- Heifers with higher blood free fatty acids had a lesser risk of being actively cycling indicating the importance of a positive nutritional plane near mating.

Collectively, Project 2 delivered new knowledge, research infrastructure and practical insights to support a more resilient Australian dairy industry. The findings provide evidence to guide farm management, veterinary advisory services, future research priorities and industry decision-making as dairy systems become larger, more data-rich and more diverse in feeding and housing practices.

Project Overview

Item	Description
Project Title	Unlocking the potential of the cow
Funding Body	Dairy UP
Dairy UP Project	Project 2
Project Duration	4 years
Subprojects	P2a: Cow longevity P2b: Early alert systems P2c: Milk as a diagnostic fluid P2d: Heat stress P2e: Calves/Heifers P2f: Infectious disease P2g: Heifers early calving
Lead Organisation	Scibus
Project Lead	Ian Lean
Key Collaborators	University of Sydney, DPIRD, DataGene, Charles Sturt University, AgriBio, MLA

Abbreviations

100DICR — 100-day in-calf rate;
ACF — Automated calf feeders;
ADG — Average daily gain;
AFB — Age at first breeding;
AFC — Age at first calving;
ALA — α-linolenic acid;
AMR — Antimicrobial resistance;
ANOVA — Analysis of variance;
ATGL — Adipose triglyceride lipase;
BCS — Body condition score;
BHB — β-hydroxybutyrate;
BHG — British high-bodyweight gain;
BHV-1 — Bovine alphaherpesvirus-1;
BLG — British low-bodyweight gain;
BoRVA — bovine rotavirus A;
BRD — Bovine respiratory disease;
BSI — Bloodstream infection;
BTM — Bulk tank milk;
BW — Body weight;
BWT — Birth weight;
CBP — Compost-bedded pack;
CDP — Cytidine diphosphate;
CDS — Phosphatidate cytidylyltransferase;
CEPT — Choline/ethanolamine phosphotransferase;
CI — Confidence interval;
CL — Corpus luteum;
CON — Control;
CP — Crude protein;
CTP — Cytidylyltransferase;
DAGK — Diacylglycerol kinase;
DGAT — Diacylglycerol acyltransferases;
DHA — Docosahexaenoic acid;
DIM — Days in milk;
DM — Dry matter;
DPIRD — Department of Primary Industries and Regional Development;
ECC — Chromogenic E. coli/coliform media;
EHG — European high-bodyweight gain;
ELG — European low-bodyweight gain;
ELISA — Enzyme-linked immunosorbent assay;
EMAI — Elizabeth Macarthur Agricultural Institute;

EPA — Eicosapentaenoic acid;
ESBL — Extended-spectrum β-lactamase;
FFA — Free fatty acids;
FT-MIR — Fourier-transform mid-infrared;
GC — Gain category;
GPAT — Glycerol-3-phosphate acyltransferase;
HCW — Hot carcase weight;
HHG — Holstein high-bodyweight gain;
HLG — Holstein low-bodyweight gain;
HNBRED — Hazard of not being bred;
HOUSED — Housed or housing-based dairy system;
HPREG — Hazard of pregnancy;
HR — Hazard ratio;
IGF — Insulin-like growth factor;
IGFBP-6 — Insulin-like growth factor-binding protein 6;
LA — Linoleic acid;
LAMP — Loop-mediated isothermal amplification;
LASSO — Least absolute shrinkage and selection operator;
LI — Lactational incidence;
LPAAT — Lysophosphatidic acid acyltransferases;
LPC — Lysophosphatidylcholine;
MALDI-TOF — Matrix-assisted laser desorption/ionisation time of flight;
MLA — Meat & Livestock Australia;
MS — Mass spectrometry;
NCBI — National Center for Biotechnology Information;
NDF — Neutral detergent fibre;
NDICP — Neutral detergent insoluble crude protein;
NEFA — Nonesterified fatty acids;
NFC — Non-fibre carbohydrates;
NSW — New South Wales;
OPAL — Odds of becoming pregnant in a lactation;
O-PLS — Orthogonal partial least squares;
PAP — Phosphatidate phosphatase;
PASTURE — Pasture-based cows or pasture-based system;
PC — Phosphatidylcholine or Principal component;
PCR — Polymerase chain reaction;
PE — Phosphatidylethanolamine;
PEMT — Phosphatidylethanolamine-N-methyltransferase;
PI — Phosphatidylinositol;
PIS — Phosphatidylinositol synthase;
PL — Phospholipids / glycerophospholipids;
PLD — Phospholipase D;

PMT — Phosphoethanolamine methyltransferases;
PREG1 — Pregnancy to first breeding;
PS — Phosphatidylserine;
PSD — Phosphatidylserine decarboxylase;
PSS — Phosphatidylserine synthase;
RNA — Ribonucleic acid;
RVA — Rotavirus A;
SA — South Australia;
SCC — Somatic cell count;
SD — Standard deviation;
SE — Standard error;
SM — Sphingomyelin;
ST — Sequence type;
THI — Temperature-humidity index;
TMR — Total mixed ration;
TRT — Treatment;
UTI — Urinary tract infection;
VFA — Volatile fatty acid;
VIC — Victoria;
VLDL — Very-low-density lipoproteins;
VP — Viral protein;
VP7 — Viral protein 7;
WWT — Weaning weight.

Project Background and Rationale

The Australian dairy industry is changing rapidly. Herds are larger, production systems are becoming more diverse, and many farms are increasingly reliant on digital records, and automated technologies. At the same time, the industry faces persistent challenges including reproductive inefficiency, disease, cow removal, heat stress, calf health, and the need to maintain social licence across both pasture-based and more intensive dairy systems.

Dairy farms now generate large amounts of information, but much of this information remains under-used. Data may be inaccessible, inconsistently recorded, fragmented across software platforms, or insufficiently audited for research and decision-making. Project 2 responded to this challenge by developing a high-integrity database that could integrate data from herd management software, milk processors, weather stations, herd testing and farm records. This provided a platform for research-grade analyses and for returning useful information to producers and advisors.

Cow longevity was a central focus because improving lifetime productivity requires cows to remain healthy, fertile and productive across multiple lactations. Previous industry and research data have consistently indicated that older cows experience greater risks of disease, reproductive failure and removal. However, the biological reasons for these changes remain incompletely understood. Project 2 therefore examined parity-associated changes in reproduction, health, body condition, body weight, blood biochemistry and lipidomic profiles, with the aim of identifying practical management opportunities and biological pathways that may support improved longevity.

The project also recognised that longevity and productivity are influenced by the broader system in which cattle are managed. Australian dairy farming includes both pasture-based and increasingly housed or confinement-based systems. These systems differ in diet, housing, climate exposure, labour structure and management opportunities. Understanding the strengths, limitations and biological consequences of these systems is essential for supporting industry adaptation and maintaining public confidence in dairy production.

Beyond mature cow performance, Project 2 addressed early-life development, milk-based diagnostics, infectious disease surveillance, early warning systems and age at first breeding. These areas are linked by a common need for earlier, more accurate and more practical information to guide decisions.

Project Objectives

This report presents each subproject individually, including the background rationale, objectives, methods, key findings and implications for industry. While each subproject has distinct aims, the combined program addressed the common goals of:

- Building on the strong links between Scibus, NSW DPIRD, the University of Sydney, private and other research providers to produce a program of research designed to unlock the potential of Australian cattle and to enhance their well-being, health, welfare and 'happiness', while reducing the environmental footprint: that is, to enhance our social licence.
- To generate evidence that helps Australian dairy farms improve animal health and productivity while adapting to changes in herd size, data availability, climate risk, disease pressure and production system design.
- Develop new data analytical strategies to determine the effects of novel interventions on health, reproduction and production.
- Use a network of monitored individual farms in NSW, VIC and SA to develop 'co-operative' models for data- and knowledge sharing and innovative, farmer-orientated, extension activities.
- Demonstrate and quantify the potential to increase productivity in commercial farms through advanced systematic, data-based management of cattle through nutrition, genetics, environmental and health management.

Subproject P2a Cow Longevity – i. Dairy UP Database

Summary

A core component that facilitated much of the work was the development of a database that would provide farm data of high integrity. We recognized that the current industry databases had substantial flaws and that a different structure was required to provide data suitable for decision making and evaluation. This required the integration of disparate sources of data including herd records, data from herd recording, environmental data and milk production and health records recorded on farm-based software often associated with milk meters. This innovative database developed by Drs Golder, Sheedy and Lean with the assistance of DataGene. This development was highly regarded by the panel conducting the mid-term review of DairyUP.

There are currently 200,000 lactational records on the database across 30 farms. The database has contributed substantially to all prospective studies within Project 2a, production of highly regarded farm reports and, subject to ongoing development and funding, plans to expand and support future projects (*Figure 5.a*).

Background

The Australian dairy industry generates an enormous amount of data. Most of these data are under-utilised. If these can be accessed and presented in usable, farmer-friendly forms then they can be capitalised on to improve the resilience of the dairy industry.

Missed opportunities for data driven decisions occur because (1) some data are never accessed from the source ie farm computers, (2) data are of too poor quality to be of use, (3) data are collated and held in disaggregated institutions, (4) data are not in an easily usable form, and (5) useful integrations and interpretations of data do not make it back to the source farm. Therefore, practice-change does not occur.

These reasons are exacerbated by Australian dairy farms using a variety of herd management software programs; some farms use more than one software program. The number of software programs available to assist with specific farm management practices such as total mixed ration feeding and fertility, as well as the core herd management software program are increasing. These generate large amounts of data that does not always integrate into the main herd management software, further stalling effective data utilisation. Data entry and data definitions are different for each of the herd management software programs. The Dairy UP database acquires data from both opportunistic and targeted sources to provide a platform to offer new and important insights.

Methods

In brief, data is collected from multiple sources, including herd management software, milk factory, weather station data and herd test centres. Data includes treatment and health, reproductive, cow movements, cow exits, daily milk or feed, local weather, herd test data and herd level descriptors. To help ensure high data quality, the Dairy UP database is dictated by a comprehensive list of rules and requirements outlined in Business Requirements Documents. Errors or unusual data are flagged for manual examination and correction as required. This auditing process often involves clarification through direct consultation with farm managers.

A specific goal of the Dairy UP P2a project was to investigate longevity of dairy cattle, which necessitates the clear understanding of cow exit reasons. The national database was not fit for this specific purpose due to limited culling definitions and extensive missing data. A report by Workie et. al. (2021) indicated that the most common reasons for Australian dairy cow culling was 'other reasons not reported' (37.4%). The Dairy UP database requires each exited cow to have up to three culling or mortality reasons recorded, using an expanded list of 50 exit reasons. Currently there are 29,542 sold animals with only 29 (0.1%) listed as 'sold – unknown reason'. Without the detailed culling data, data on reproduction and health will be less valid.

Implications

The value of an Australian database with research-grade data integrity is demonstrated through the Project P2a studies discussed later in this document. Comparisons between pasture-based dairy production and intensively housed systems indicate that both approaches can achieve similar health and reproductive outcomes. These findings provide important evidence to support the social licence of intensification within the Australian dairy industry. Outcomes from Dairy UP 1.0 have been widely disseminated through consultants, media releases, social media, domestic and international conference presentations, scientific publications, roadshows, industry field days, summary documents,

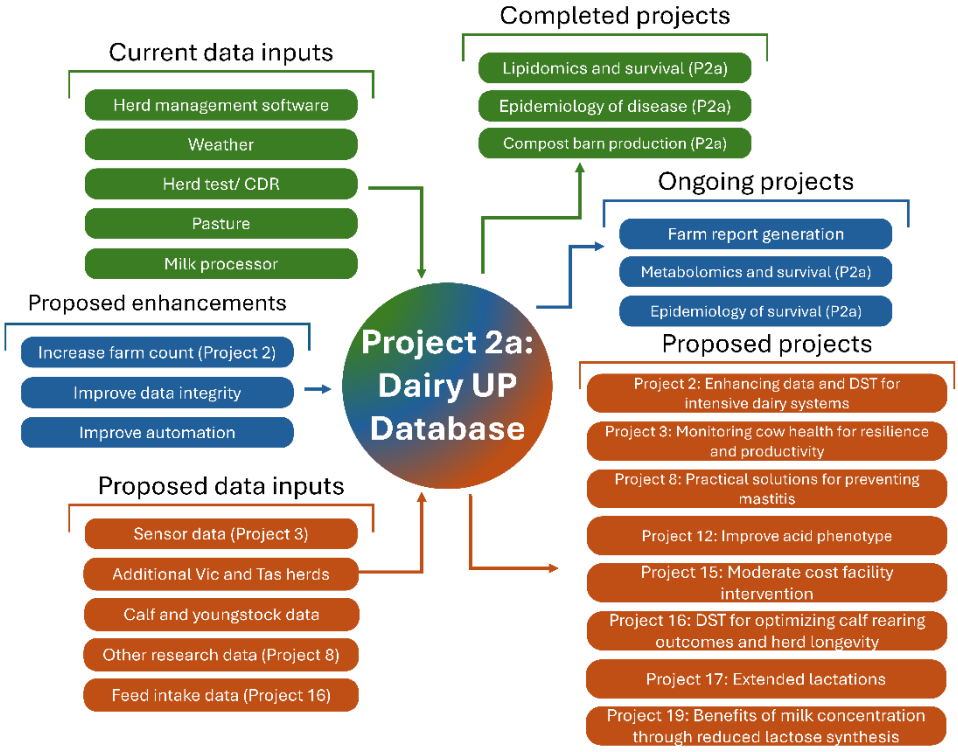


Figure 5.a: Schematic of the current and proposed data inputs, proposed enhancements, completed, ongoing and proposed projects linked to Project 2a – I Dairy UP database

and direct email communication.

Subproject P2a Cow Longevity – ii. Retrospective Study Series

Background

There is a need to ensure that we unlock the potential of the cows to produce by providing interventions to allow them to be productive, healthy and reproductively successful. Further, there is a need to understand the implications of changes in production systems to identify strengths and weaknesses of different systems and to provide social license for different production systems.

Increased parity is negatively associated with survival and reproduction in different production systems

doi.org/10.3168/jds.2021-21672

Authors

I.J. Lean, H.M. Golder, S.J. LeBlanc, T. Duffield, J E.P. Santos

Background

We conducted a retrospective meta-analysis based on individual cow data to assess the associations of parity, level of production, and pasture-based or intensively fed systems with fertility. Our goal was to provide understandings of the role of parity in risks for removal and reproductive failure. We hypothesized that risks of reproductive failure would increase with parity but not differ with production system.

Methods

Multilevel models were used to evaluate the fixed effects of parity, milk, milk solids, milk fat and protein percentage and yield, and production system [intensively fed (n = 28,675) or predominantly pasture fed (n = 4,108)] on reproductive outcomes.

Findings

The outcomes were the hazard of not being bred (**HNBRED**), hazard of pregnancy (**HPREG**), pregnancy to first breeding (**PREG1**), and odds of becoming pregnant in a lactation (**OPAL**). The 32,783 cows were in 13 studies conducted in Australia (14.6% of cows), Canada (2.4% of cows), and the United States (83.0% of cows). There were 38.5% of cows in the sample in parity 1, 27.3% in parity 2, 16.7% in parity 3, 9.0% in parity 4, and 8.6% in parity ≥5. Compared with cows of parity 1, parity ≥5 cows had a greater HNBRED [hazard ratio (**HR**) = 2.45], lesser HPREG (HR = 0.73), and reduced OPAL (odds ratio = 0.36). However, the parity ≥5 cows had similar PREG1 to other parities except for parity 1. This suggests the possibility of a higher proportion of subfertile parity ≥5 cows than for other parities. Associations between parity and reproductive measures were influenced by the different milk production measures, indicating that milk yield and milk component percentages and yields modified the odds or hazards of successful reproduction. All milk production measures had quadratic associations with OPAL, indicating that either low or high production or concentration of solids within a cohort reduced OPAL. This reduced OPAL reflected a greater HNBRED for lower milk yield and milk protein and fat yielding cows. Both milk yield and milk protein percentage had quadratic associations with HPREG. When centered milk yield was categorized into quartiles, small differences in HPREG existed. A more marked association of milk protein percentage occurred with HPREG, with optimal HPREG at approximately 0.5% above group mean milk protein percentage. Milk fat percentage (HR = 0.901), fat yield (kg/d; HR = 0.78), protein yield (kg/d; HR = 0.71), and milk solids yield (kg/d; HR = 0.84) were all linearly associated with reduced HPREG.

Difference in production systems did not have substantive effects on PREG1 but did for HNBRED, HPREG, and OPAL. Estimates of associations of parity with reproductive outcomes HNBRED, HPREG, and OPAL were influenced by milk and milk solids yield; older cows had markedly lower reproductive outcomes. Interestingly, for PREG1, there were few differences among parities and differences were less influenced by milk yield and constituent measures.

Implications

The marked associations of parity with removal for all reasons, deaths and culling, and reductions in HNBRED, HPREG, and OPAL indicate a need to focus on the physiological changes with parity to produce better strategies to support optimal longevity of cows.

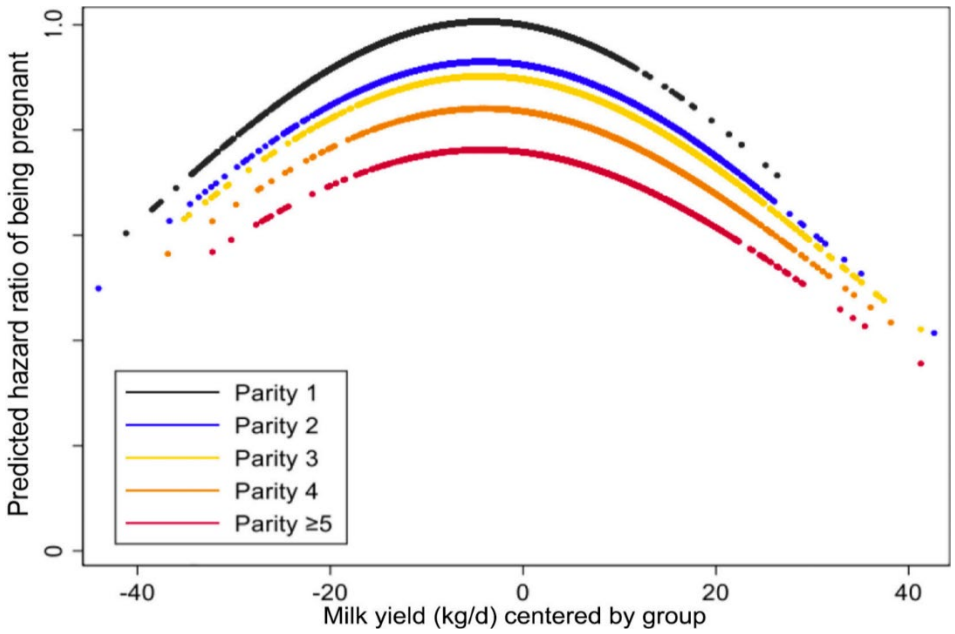


Figure 6.a: The association of milk yield (kg/d) centered in group within herd at approximately 70 DIM on the x-axis with the relative predicted hazard of being pregnant on the y-axis. The data are from 20,071 cows. Based on the model that contains parity, study year, and production system, all parity groups differed in probability or hazard of pregnancy (HPREG). Cows in parity 1 had the greatest daily probability or HPREG, and parity ≥5 had the least probability of pregnancy.

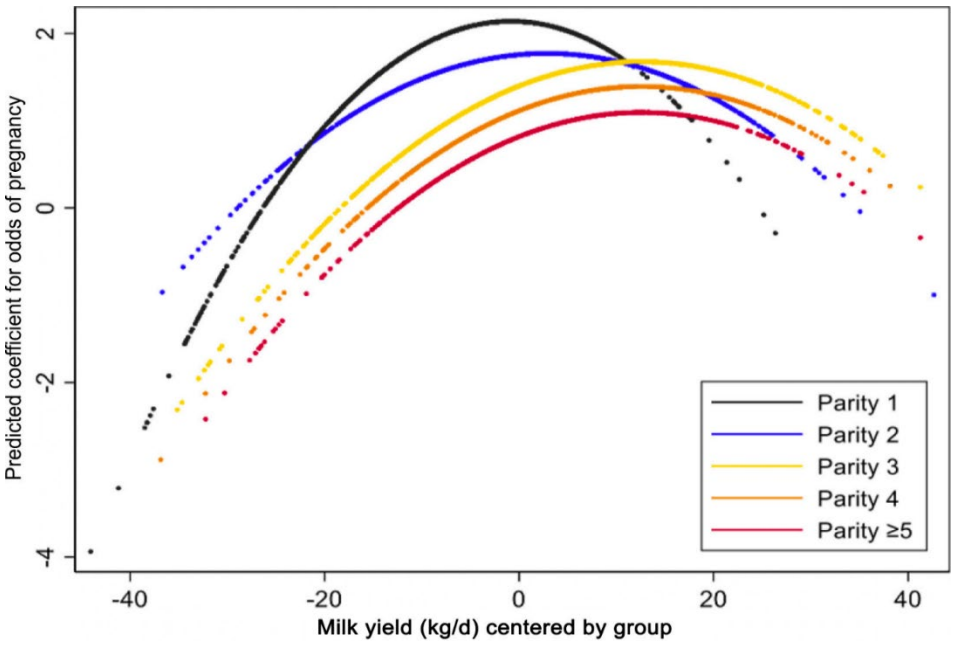


Figure 6.b: The association of milk yield (kg/d) centered on group within herd at approximately 70 DIM with the predicted coefficient of odds of becoming pregnant in a lactation (OPAL). The data are from 28,071 cows. The x-axis is milk yield centered by group, and the y-axis is the predicted coefficient of odds of pregnancy at first insemination. Based on the model that contains parity and production system and the interaction of parity with production system, all parities differed, with cows in parity 1 having the greatest odds and parity ≥5 having the least odds of pregnancy in the lactation.

Associations of parity with health disorders and blood metabolite concentrations in Holstein cows in different production systems

doi.org/10.3168/jds.2021-21672

Authors

I.J. Lean, S.J. LeBlanc, D.B. Sheedy, T. Duffield, J.E.P. Santos, H.M. Golder

Background

The associations among diseases and parity are important, there is a need to identify potential risk factors that may influence these associations. The pathogenesis of the increased risk of removal for reproductive failure and differences in odds or risk of disease with parity, as well as differences in blood metabolites with parity, may indicate aspects of metabolism that alter with parity

Methods

Data were obtained from studies in Australia, Canada, and the United States using individual cow data from 28,230 Holstein cows to evaluate associations between parity and disease. Our goal was to develop understanding of disease risks for cows of differing parity.

Findings

Parity ≥5 represented 2,533 cows or 9.0%, parity 4 was 9.8% (2,778), parity 3 as 19.0% (5,355), parity 2 as 28.1% (7,925), and parity 1 was 34.1% (9,639) of the sample. Of these cows, 15.5% were in Australia, 14.7% in Canada, and 69.8% in the United States. Lactational incidence (**LI**) risk of clinical hypocalcemia increased with parity from 0.1% for parity 1 to 13% for parity ≥5 cows. The marked increase suggests profound differences in metabolism with increased parity. The LI of clinical mastitis was 17.4%. The odds of mastitis increased with parity to 2.5 times greater in parity ≥5 than in parity 1. The LI of lameness increased with parity; specifically, the odds of lameness was 5.6 times greater for parity ≥5 than parity 1. Dystocia incidence was 8.7% and greatest for parity 1 cows. The LI of retained placenta was 7.4% and increased with parity, with the odds for parity ≥5 2.3 times greater than for parity 1. The LI of metritis was 10% and of endometritis 14%, with the greatest odds in parity 1. The LI of clinical ketosis was 3.3% with a marked increase in odds with parity. The prevalence of subclinical ketosis was 26.8% with only cows in parity 1 having lower odds than other parities. Parity ≥5 cows had greater odds (odds ratio = 1.7) of respiratory disease than parity 1 cows, which were lesser than other parities.

Metabolite concentrations were evaluated in 5,154 Holstein cows in the precalving, calving, and immediate postcalving data sets. Metabolic measures near peak lactation provided 1,906 observations. Concentrations of β-hydroxybutyrate (**BHB**) and nonesterified fatty acids increased with parity on d 1 to 3 of lactation and at peak lactation. On d 1 to 3 after calving differences in glucose, nonesterified fatty acids, and BHB indicated a greater reliance on mobilized lipid to export energy to peripheral tissues as BHB for greater parity cows. Differences in concentrations among parity groups were marked at times, for example >0.20 mM in Ca for parity 1 and 2 to parity ≥5 and >0.33 mM for all older parities compared with parity 1 for P on the day of calving. The marked increase suggests profound differences in metabolism with increased parity are probably influenced, in part, by increased production. We found marked differences in concentrations of metabolites with parity that are consistent with reduced reproduction, health, and body condition for higher parity cows. These unfavourable differences in metabolism in Ca, P, glucose, and cholesterol concentrations for higher parity cows also complement the often-substantial differences in disease risk with parity and suggest a need to carefully consider the parity structure in study design.

Implications

Managers and advisors will need to consider methods to reduce risk of health disorders tailored to cows of different ages.

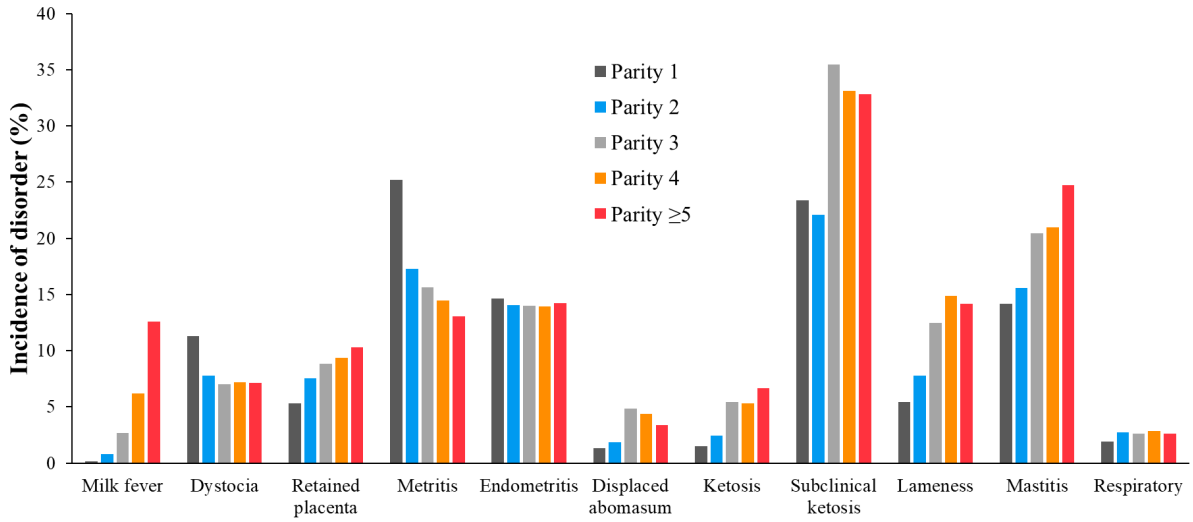


Figure 6.c: Incidence of clinical and subclinical disease by parity

Subproject P2a Cow Longevity – iii. Body Condition Score Series

Background

The accessible metrics of body condition score (**BCS**) and body weight (**BW**) are important in assessing the health of dairy cows. During the transition from late-pregnancy to peak-milk production, cows mobilise their stored energy and protein reserves to meet the homeorhetic demands of milk production. However, excessive mobilisation has been associated with increased disease incidence, reproductive inefficiencies, poor cow welfare, and is observed more often in high parity cows. Although BCS and BW are the most commonly used metrics to assess body tissue reserves, their correlation is considered only low to moderate, and is influenced by differences in visceral adipose tissue, stage of lactation or pregnancy, water intake, gut fill, and parity. To address this limitation a novel combined BCS – BW classification scheme is introduced and explored.

Holstein dairy cows lose body condition score and gain body weight with increasing parity in both pasture-based and total mixed ration herds

doi.org/10.3168/jdsc.2022-0246

Authors

I.J. Lean, D.B. Sheedy, S.J. LeBlanc, T. Duffield, J.E.P. Santos, H.M. Golder

Background

Body condition scoring (BCS) and body weight (BW) are observations associated with labile tissue reserves, health, and reproduction efficiency of dairy cows.

Methods

The effect of parity (1 through to ≥5) and feeding system (pasture-based and total mixed ration [**TMR**]) on BCS and BW were evaluated utilizing raw data sets from 16 retrospective studies that totalled 24,807 Holstein cows across 3 nations (Australia, Canada, and the United States). Linear regression models were used to investigate the 5 outcome variables of precalving BCS, peak milk BCS, change in BCS from precalving to peak milk, and peak milk BW and their respective associations with parity and feeding system. To help control for the influence of calendar time, study treatment protocols when applicable, and genetic change, all outcome variables were center-transformed around each study group mean.

Findings

Including feeding system as a covariate improved model fit for most outcome variables; however, the relative effect size of parity was generally much greater than feeding system effect size. Parity 2 cows had the lowest precalving BCS of −0.087 [95% confidence interval (**CI**): −0.107, −0.065] less than the mean, whereas parity 1 cows had the greatest, 0.068 (95% CI: 0.043, 0.092) above mean, regardless of feeding system. Peak milk BCS overall decreased with increasing parity (parity 1 to parity ≥5: −0.13, 95% CI: −0.19, −0.08) and BCS change during the transition period monotonically decreased with increasing parity (parity 1 to parity ≥5: −0.22, 95% CI: −0.26, −0.17). Peak milk BW monotonically increased with increased parity (parity 1 to parity ≥5: 114 kg, 95% CI: 104, 125). A waffle plot was used to present the proportions of cows, by parity, that were partitioned into “low BCS and low BW,” “low BCS and high BW,” “high BCS and low BW,” or “high BCS and high BW” groups. Cows were assigned either a high or low status by being above or below their specific centered study group means, respectively. Considering a null hypothesis of 25% per

BCS-BW category, there was a striking change in category from parity 1 cows that were predominantly in the "high BCS and low BW" category (61.2%) to parity ≥5 cows that were predominantly in the "low BCS and high BW" category (55.5%).

Implications

The study supports studies showing increased weight and change in BCS with increased parity. We highlight the associations among production system, BCS, BW, and parity.

Associations among body condition score, body weight, and serum biochemistry in dairy cows

doi.org/10.3168/jds.2024-25425

Background

Body condition score and BW yield insights into body tissue reserves and diet, and serum biochemical measures reflect the metabolic status of cows. Associations between body composition measures and biochemistry are unclear and investigation may reveal important information on the metabolic and physiological status of cattle with varying levels of labile tissue reserves.

Methods

Cohorts of 739 nonlactating, late-pregnancy, dry cows (26.9 d prepartum, standard deviation [**SD**] = 12.4) and 690 peak-milk cows (58.0 DIM, SD = 14.5) were selected by stratified (parity: 1, 2, 3, >3) random sampling from 30 farms (15 pasture, 15 TMR) in this cross-sectional study. Eleven analytes were collected, analyzed, and standardized within group (cohort/breed per farm). Mixed linear models for BCS and BW were specified, with the random effect of group. A 6-point, unordered, categorical body-group classification that combined BCS (greater, equal to, or less than group median; as high, median, or low BCS) and BW (greater or less than group median; as high or low BW) was

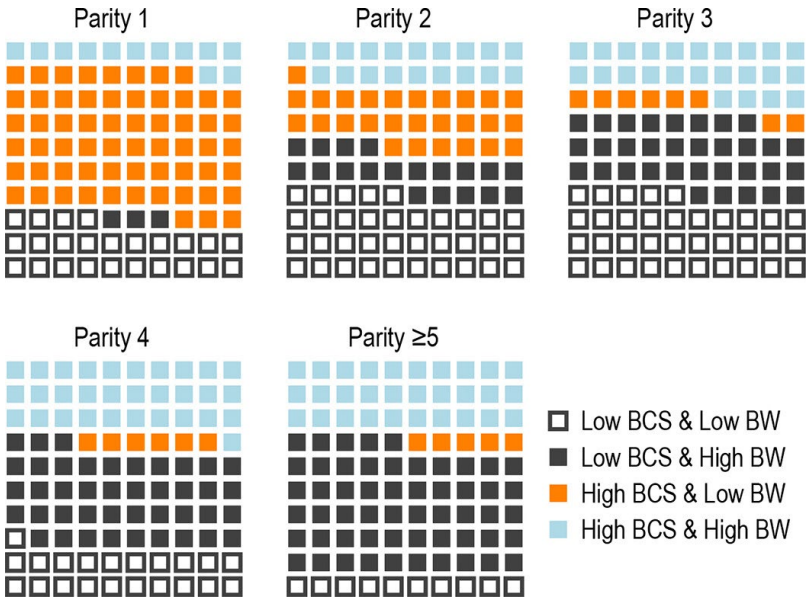


Figure 7.a: Proportion of cows in each category of BCS (low BCS ≤ group mean, high > group mean) and BW (low BW ≤ group mean, high > group mean) by parity at peak milk. Indicating a shift from High BCS and Low BW (orange square) in young cattle to Low BCS and High BW in older cattle (black square).

analyzed by polytomous logistic regression. Effect sizes are listed for a 1 SD increase in the specified analyte, keeping other covariables at their mean value.

Findings

Dry BCS was positively associated with albumin (0.075 BCS ± 0.014 standard error [**SE**]), urea (0.038 BCS ± 0.014 SE), and glucose (0.052 BCS ± 0.014 SE), and negatively with the interaction between cholesterol and days precalving. Dry BW positively associated with albumin (11.03 kg ± 2.48 SE) and negatively with cholesterol (−8.47 kg ± 2.57 SE). Peak-milk BCS was positively associated with albumin (0.47 BCS ± 0.015 SE), BHB (0.048 BCS ± 0.015 SE), and glucose (0.051 BCS ± 0.015 SE). Peak-milk BW was positively associated with albumin (6.94 kg ± 2.35 SE) and negatively with Ca (−7.02 kg ± 2.33 SE). Increasing BW and decreasing BCS was associated with increasing parity, except in dry second-parity cows that had low BCS. The dry polytomous model associated a 1 SD increase in albumin with a 4.89% ± 1.56 SE decreased risk of being low BCS/low BW and 5.87% ± 1.46 SE increased risk of high BCS/high BW. Risk change associated with 1 SD of glucose was −5.61% ± 1.58 SE for low BCS/high BW and 3.17% ± 1.58 SE for high BCS/high BW. For the peak-milk cohort, change in risk was associated with albumin for low BCS/low BW −3.67% ± 1.56 SE, low BCS/high BW −3.22% ± 1.53 SE. Risk change with 1 SD of BHB was −3.36% ± 1.47 SE for median BCS/low BW, 2.86% ± 1.44 SE for high BCS/low BW, and 2.69% ± 1.37 SE for high BCS/high BW. Risk of low BCS/low BW was greatest in second-parity cows, and high BCS/high BW was greatest in dry cows with greater than third parity and third-parity cows in peak milk. There were no interactions between parity and analytes.

Implications

Albumin was consistently and positively associated with body measurements and milk production parameters, and milk production was negatively associated with BCS. We speculate that the observed associations between metabolites that reflect protein and energy metabolism, including albumin, glucose, urea, cholesterol, BHB, and calcium, within a cohort and body tissue reserve measures (BCS and BW) may indicate that protein metabolism importantly differs among cattle. Further investigation into albumin and protein metabolism may identify biological pathways that support high milk production while reducing BCS-related diseases and loss.

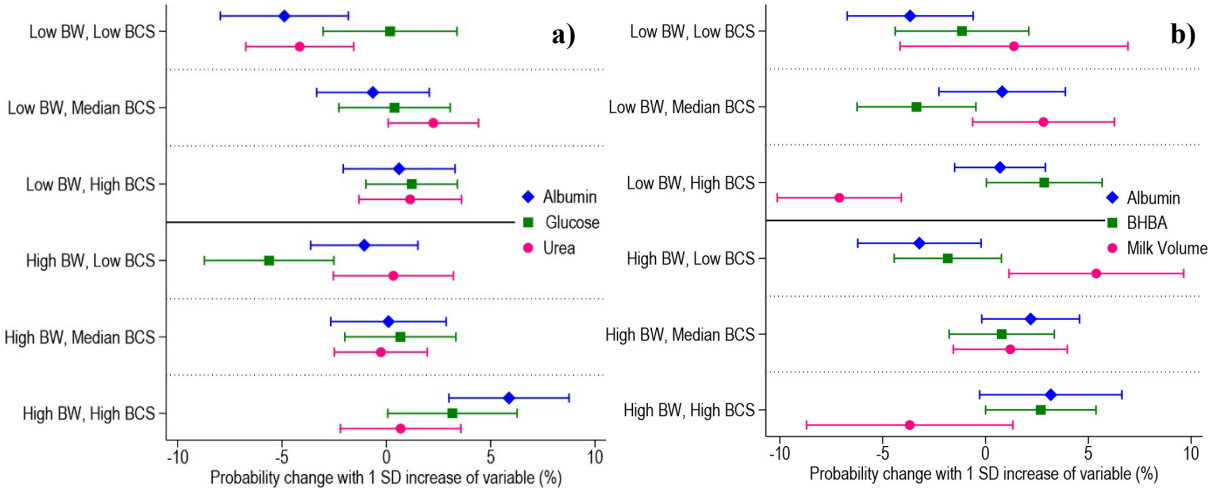


Figure 7.b: The change in probability of being in a specific body condition group associated with a 1-SD change in the shown variable for cows in a) the dry-cow cohort and b) the peak-milk cohort, with all other variables in the multivariable polytomous model held constant at their mean values. The error bars are 95% CI

Integration of body condition score and weight

These two papers introduced and refined a novel metric of considerable importance. We identified only weak associations between BCS and BW and noted that the labile reserves of tissue may differ greatly between cows with high BCS but low BW and those of similar BCS and high BW. Given that the novel metric was associated strongly with parity, metabolite concentrations and milk production, this metric will be used in future research and to more precisely understand body tissue reserves.

Subproject P2a Cow Longevity – iv. Lipidomic series

Background

Reducing the risk of adverse health and reproductive events as cows age could provide, producers with options to shift herd management strategies towards a lower replacement rate and extended longevity. However, while substantial collective research efforts have focused on individual diseases and reproductive challenges, there have been no similar effects into investigating the broader biological changes that occur with the aging process in dairy cattle. This lack of research interest is somewhat surprising given the consistent associations between parity and incidence of diseases and reproductive failure. Understanding the biological underpinnings of aging in cows may reveal novel targets for intervention that allow cows to maintain their health and productivity as they age.

A compelling platform for investigating the biology of dairy cow aging is lipidomics, the large-scale study of lipids. Continued advances in laboratory analytical technology now allow precise quantification of very-low abundance, polar and non-polar lipids that facilitate the identification of clinically relevant bioactive lipids. With lipidomics now an accessible research tool, there have been an increase in cow lipid metabolism studies, reflected by recent reviews on topics of sphingomyelin and ceramide metabolism, omega fatty acids, and the endocannabinoid system. Lipidomics is also being used to study healthy aging and age-related diseases in humans. Given these developments, lipidomics was considered an appropriate analytical platform to investigate the biological changes associated with cattle aging and longevity.

Glycerophospholipids in dairy cow health and longevity: a review

doi.org/10.1017/S0022029926102222

Authors

D.B. Sheedy, H.M. Golder, S.C. Garcia, I.J. Lean

Summary

This review examines the role glycerophospholipids (**PL**) in dairy cow health, with specific focus on phosphatidylcholine (**PC**), phosphatidylethanolamine (**PE**), phosphatidylinositol (**PI**) and phosphatidylserine (**PS**). Increasing parity of cows is associated with lower concentrations of plasma PL that contain very long-chain omega-3 fatty acids, including docosahexaenoic acid and eicosapentaenoic acid, which are precursors for prostaglandin synthesis, and have anti-inflammatory roles. Low concentrations of these PL could plausibly contribute to the increased risk of disease, reproductive failure and mortality in older cows. The bioavailability and metabolism of fatty acids may differ among supplements that are predominately neutral lipids, such as triacylglycerol-rich oils, and those bound to PL including pasture, whole or ground oilseeds and fish meal. Hepatic lipidosis can occur during the transition period if there is insufficient very-low density lipoproteins (**VLDL**) production in the liver to transport lipids into blood circulation. The PC are the primary PL of VLDL and are produced by two main pathways in the liver, the cytidine diphosphate-choline pathway that uses choline as a substrate, and the PE N-methyltransferase pathway that uses PE and methyl-donors as substrates. Co-supplementation strategies that target both pathways may increase PC production over a one-pathway supplementation strategy. The PIs are phosphoinositides precursors, which have broad physiological roles including regulating inflammatory processes and may offer targets for novel treatment and management of disease. Both the PI and PE are precursors to endocannabinoids, important regulators of energy metabolism, immune function and reproduction in mammals. Early findings on the endocannabinoid system in transition dairy cows yielded results that diverge from non-ruminant models. The PS expression on cytoplasmic membranes signals apoptosis, coagulation and contributes to sperm–oocyte recognition. As lipidomic diagnostics become increasingly available, understanding the metabolism of PL will continue to develop and promises to offer novel strategies for optimising cattle health and longevity.

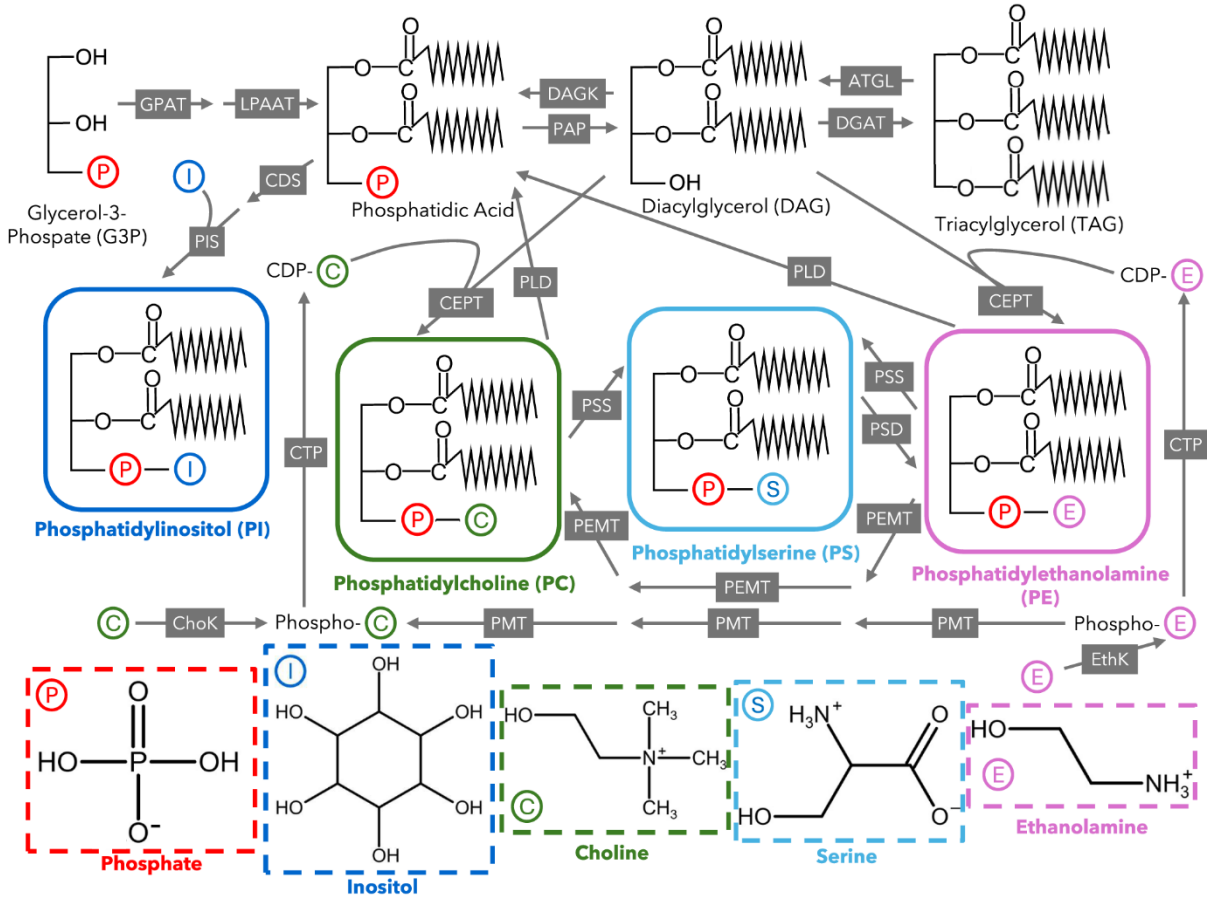


Figure 8.a: Glycerophospholipid metabolism depicting the most common pathways for production of phosphatidic acid, phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylethanolamine (PE). Enzymes are in grey boxes; fatty acyl chains are simplified as wavy chains esterified to the glycerol backbone. Phosphate and the specific head groups are depicted by the circled colored letter described in the bottom of the figure. Intracellular locations and lysophospholipids are not shown. ATGL – adipose triglyceride lipase; CDP – cytidine diphosphate; CDS – phosphatidate cytidylyltransferase; CEPT – choline/ethanolamine phosphotransferase; ChoK – choline kinase; CTP – cytidylyltransferase; DAGK – diacylglycerol kinase; DGAT – Diacylglycerol acyltransferases; EthK – ethanolamine kinase; GPAT – glycerol-3-phosphate acyltransferase; LPAAT – lysophosphatidic acid acyltransferases; PAP – phosphatidate phosphatase; PEMT – phosphatidylethanolamine-N-methyltransferase; PIS – phosphatidylinositol synthase; PLD – phospholipase D; PMT – Phosphoethanolamine methyltransferases; PSD – phosphatidylserine decarboxylase; PSS – phosphatidylserine synthase.

A large multisite lipidomic investigation of parity and aging in dairy cows

doi.org/10.3168/jds.2024-25578

Background

Efforts to optimize the longevity of dairy cows are hindered by the increased risk of adverse health events, culling, or dying on farm with increased parity. Lipidomics provides a platform to help identify important biomarkers and biological pathways associated with increased parity and associated aging.

Methods

A large multisite (15 pasture-based, 15 TMR farms) cross-sectional study collected plasma samples from nonlactating, late pregnant, dry cows (n = 696, ~27 d prepartum) and peak milk cows (n = 796, ~58 DIM) in a disproportionate stratified random sampling frame (parity: 0, 1, 2, >2 for dry cows; 1, 2, 3, >3 for peak milk cows). A total of 185 lipid species, comprising the lipids classes of phospholipids, sphingomyelins (SM) and triacylglycerols, were quantified in a targeted, liquid chromatography-MS approach. Dry and peak milk cohorts were analyzed separately throughout.

Findings

Variation in lipid profiles were mostly attributed to farm of origin (36%–41% of variation), with feeding system explaining 13% to 21% and parity explaining 6% to 9%, according to analysis of variance (**ANOVA**) simultaneous component analysis modelling. Multiple linear regression and orthogonal partial least squares (O-PLS) investigated the association of the lipid profile with age (d), whereas discriminant analysis compared first parity with >3 parity cows in O-PLS discriminant analysis, random forest, and support vector machine models. Rankings of the most important lipid species for each model type were compared. Phospholipids with 40 carbon atoms and 6 double bond equivalents (40:6) were consistently decreased with increasing parity and age across both dry and peak milk cohorts. These lipids most likely contained stearate (18:0) and docosahexaenoic acid (**DHA**, C22:6n-3), an n-3 fatty acid. Additionally, phospholipids with 40:5 and 38:6, lysophosphatidylcholine (17:0), SM(35:1), and SM(35:2) were commonly identified lipids that decreased in concentration with parity and age.

Implications

Docosahexaenoic acid has been associated with improved cattle health, reproduction, and milk production and quality. This study raises the hypothesis that reduced DHA levels in older cows may be an important factor increasing susceptibility to adverse health events, reduced reproductive performance, and herd removal. Studies that supplement DHA or its precursors can test this hypothesis and may be important in optimizing longevity of cows.

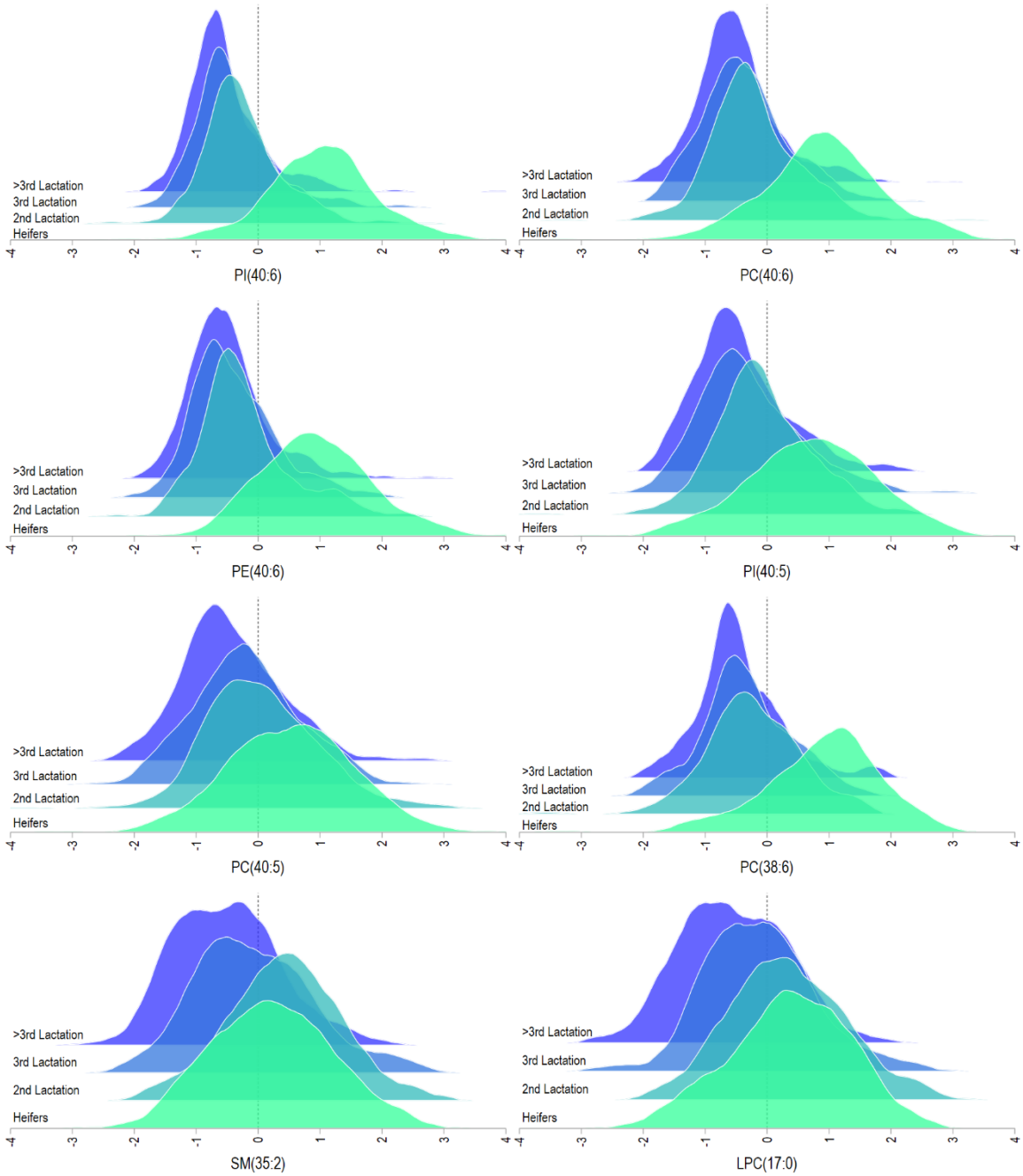


Figure 8.b: Specific lipid species concentrations stratified by parity groups. Presented lipids were identified as influential in differentiating parity and age. Data were centered and normalized on breed within farm (group) and cohorts of dry cows and peak milk cows. All figures contain both lactating and dry cow cohorts except PC(38:6) in the dry cohort only and LPC(17:0) and SM(35:2) in the peak milk cohort only.

Confinement and pasture-based dairy herds differ in plasma lipid profiles

doi.org/10.3168/jds.2025-26782

Authors

D.B. Sheedy, H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Hemsworth, D.E. Vincent, J.E. Pryce, I.J. Lean

Background

Dairy cow housing and management can be broadly described as either intensive confinement-based (**CONFINE**) or extensive pasture-based (**PASTURE**) systems. The diets between systems typically differ in their forage base, with CONFINE farms often utilizing maize silage in a TMR. Consequently, the lipid composition of diets differs between systems. The influence of housing system on blood lipidomics is currently unknown, but due to the bioactive role of lipids in influencing overall health and productivity, differences in diet may have consequences for reproduction, health, and aging of cows. The objective of this cross-sectional, multisite study was to investigate blood lipids and metabolites from cows in PASTURE and CONFINE systems, in the dry period (~27 d prepartum) and at peak milk (~58 DIM).

Methods

After exclusions, blood samples from 303 PASTURE and 398 CONFINE dry-period cows and 350 PAST and 431 CONFINE peak-milk cows from 15 PASTURE and 15 CONFINE farms were analyzed. A total of 185 lipid species (including glycerophospholipids, sphingomyelins, and triacylglycerols) were evaluated using targeted liquid chromatography-MS, as were 11 routinely measured metabolites. Dry and peak-milk cohorts were analyzed separately throughout. Lipids and metabolites associated with housing system were selected using a variable stabilization approach that was achieved by calculating the frequency of inclusion in categorical (housing system) penalized models using bootstrapping. Variables were retained if inclusion frequency exceeded a false-positive threshold. Five different statistical models were used with variable stabilization.

Findings

Dry cows in CONFINE systems had decreased globulin, urea, and glycerophospholipids associated with n-3 fatty acids. The highest total inclusion rates in the dry cohort were phosphatidylcholine (**PC**; 36:5), which mostly comprises palmitic acid (C16:0) and eicosapentaenoic acid (**EPA**; 20:5n-3), then phosphatidylethanolamine (**PE**; 38:5, 16:0/22:5n-3 or 18:0/EPA) and PC(34:3; 16:0/18:3 α-linolenic acid [**ALA**]). No lipids were increased in more than one stabilized model in CONFINE dry cows. Peak-milk CONFINE cows had increased glycerophospholipids associated with n-6 fatty acids. The highest total inclusion-rate lipids in the peak-milk cohort were phosphatidylinositol (PI; 38:3; 18:0/20:3n-6 dihomo-γ-linolenic acid), PC(34:2; 16:0/18:2 linoleic acid [**LA**]), PC(40:7; 18:2/22:5n-6), PC(34:1; 16:0/18:1), and PE(34:2; 16:0/LA). The CONFINE peak-milk cows also had decreased PC(34:3; 16:0/ALA).

Implications

This study identified specific lipids that were strongly associated with housing systems, findings that have not been reported elsewhere. Given the important biological functions of omega fatty acids, the pattern of glycerophospholipids with increased n-6 and decreased n-3 in CONFINE cows may indicate housing systems create different risk profiles for reproduction, health, and aging.

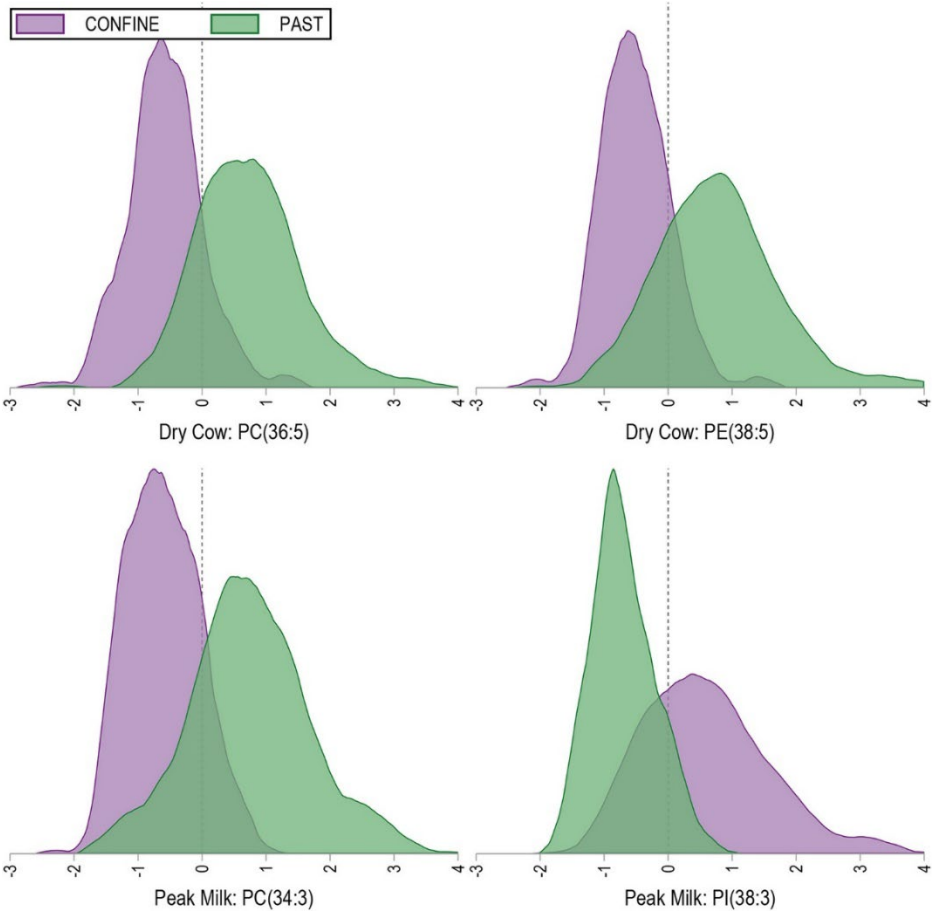


Figure 8.c: Kernel density estimation functions for the 2 most selected compounds associated with production system, from each cohort. PC(36:5), PE(38:5) and PC(34:3) are phospholipids associated with omega-3 fatty acids and PI(38:3) is associated with an omega-6 fatty acid. Compounds are standardized with a mean zero and SD of 1. CONFINE = confinement-based dairy system, according to lactating herd housing; PASTURE = pasture-based dairy system, according to lactating herd. Vertical dashed lines indicate the standardized mean value of zero.

A large, multisite investigation into the lipidomics of survival in dairy cows

doi.org/10.3168/jds.2025-27687

Authors

D.B. Sheedy, H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Hemsworth, D.E. Vincent, J.E. Pryce, I.J. Lean

Background

Identifying physiological determinants of dairy cow survival and their potential modulation by parity may reveal opportunities to improve herd health and longevity. This multisite, prospective, observational study investigated culling and mortality hazards using targeted lipidomic and standard metabolite assays.

Methods

Blood samples, stratified by parity, were collected from 2 cow cohorts (1) dry and (2) peak-milk, from across 29 commercial Australian farms (14 pasture-based, 15 confinement-based). There were 717 nonlactating, late-pregnant, dry cows (~27 d prepartum) and 794 peak-milk cows (~58 DIM) sampled. A total of 186 lipid species (including glycerophospholipids, sphingomyelin, and triacylglycerols) and 11 routinely measured metabolites were evaluated. Sample cows were followed for an average of 693 d and exit reasons recorded. Competing risk survival models were used to estimate the cumulative incidence of culling and mortality by parity and cohort. Blood analytes were autoscaled within cohort and farm, controlling for farm-level effect on metabolites. Survival analysis was performed using an adaptive least absolute shrinkage and selection operator (**LASSO**) Cox full likelihood model that explored associations among blood analytes and hazards of removal, with a shared frailty of farm (accounting for farm-level baseline hazards), and 2 removal outcomes considered: culling (censoring: death, cull from farm accident, end of follow-up) and mortality (censoring: cull, death from farm accident, end of follow-up). Separate models were used to estimate survival outcome by cohort and parity groupings (first, second and third, and greater than third) or with parity as a categorical covariate. Due to high correlations, analyte data were reduced to 25 clusters using Ward's hierarchical clustering criterion. Bootstrapping of the LASSO variable selection procedure identified clusters with high selection frequency for use in the final model. The hazards and cumulative incidence of culling and mortality increased with parity.

Findings

Glycerophospholipids with very-long-chain n-3 fatty acids were associated with reduced hazards of culling in parity 1 peak-milk cows (0.39 hazard ratio [**HR**]), whereas glycerophospholipids with n-3 α-linolenic acid were associated with reduced hazards of culling in parity >3 peak-milk cows (0.18 HR). Sphingomyelin with >C18 fatty acyl chains was associated with increased hazards of culling in parity >3 peak-milk cows (2.02 HR). Clusters containing the routinely evaluated analytes albumin, globulin, urea, magnesium, glucose, triglycerides, β-hydroxybutyric acid, bilirubin, and nonesterified fatty acid were associated with culling and mortality, consistent with their roles in health and reproduction. Lipids collected from dry cows were poor predictors of survival.

Implications

Many novel plasma lipid targets for future research into survival of cattle were identified, with proinflammatory lipid profiles associated with increased risk of culling and mortality.

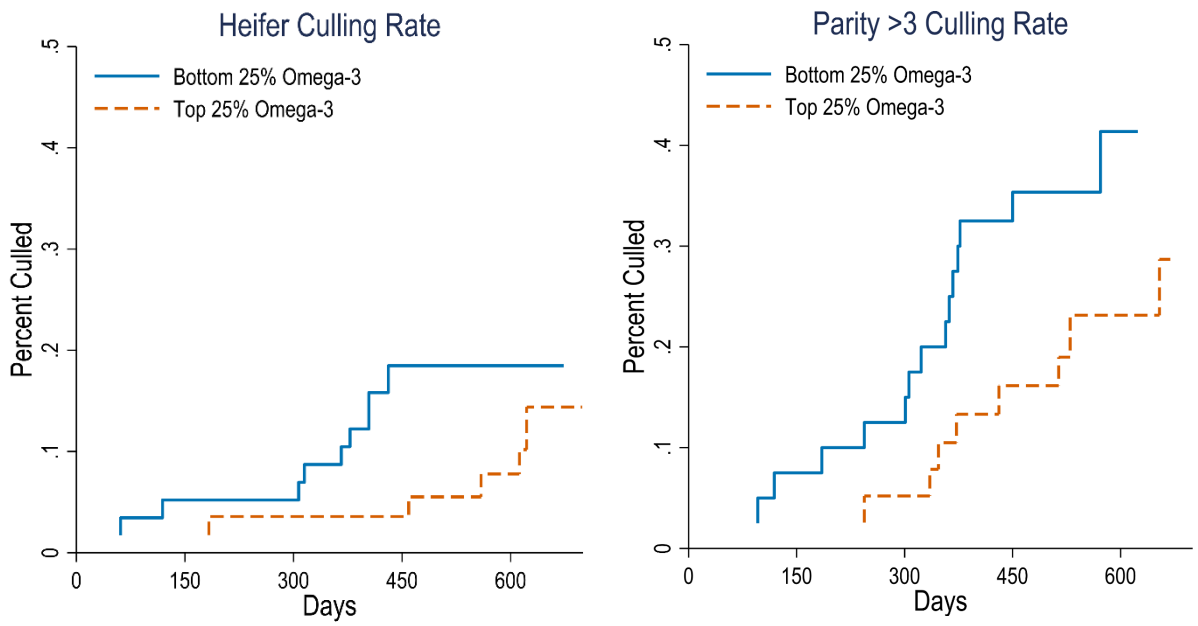


Figure 8.d: Predicted survival of heifers (left) and parity >3 (right) with low (blue, solid line) or high (orange, dashed line) plasma concentrations of phospholipids associated with omega-3 fatty acids. Cows with high omega-3 blood concentrations were predicted to survive in the herd by around 300 days compared to cows with low levels.

Do housed cows have greater metabolic-age than pasture-based cows based on lipid profiles?

Submitted to the Journal of Dairy Science Communications

Authors

D.B. Sheedy, H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Pryce, I.J. Lean

Background

Metabolic clock models use biomarkers to predict the metabolic-age of a subject and may infer information regarding their health. Plasma glycerophospholipids that contain omega-3 fatty acids are reduced with increased age of dairy cows and reduced in cows from TMR-fed, housed systems (**HOUSED**) compared to cows from extensive, pasture-based systems (**PASTURE**). We hypothesized that a lipid-biomarker metabolic clock model that was trained exclusively on either HOUSED or PASTURE cows would produce systematically biased estimates on test datasets comprised of cows from the alternate feeding system. If this were true, the results could infer different metabolic status between feeding systems.

Methods

Data from a previous study of 794 peak-milk cows (~58 DIM), stratified by parity, were sampled from 29 Australian farms (14 PASTURE, 15 HOUSED). A total of 186 lipid species (including glycerophospholipids, sphingomyelin, and triacylglycerols) were quantified. A prediction-oriented, 'cubist' machine learning algorithm was applied using training:test data splits of HOUSED:PASTURE and PASTURE:HOUSED.

Findings

The model trained on HOUSED cows had no bias in the training set (0.0 d, 95% CI: -2.3, 2.3), but the predicted age of PASTURE cows was 627 d younger than their true age (95%CI: -683, -571). The model trained on PASTURE-cows had no bias in the training set (0.0 d, 95%CI: -22.1, 22.1), but the predicted age of HOUSED cows was 175 d greater than their true age (95%CI: 142, 208). The direction and magnitude of systematic bias was consistent when models were restricted to 10 pre-screened predictor lipids that were mutually associated with age in both systems. Implications

Implications

Collectively, results indicated that HOUSED cows had greater metabolic-age than PASTURE cows of the same chronological age. A secondary conclusion reinforced caution regarding the extrapolation of machine-learning models: predictions may be highly precise within the training domain (e.g. Root mean square error = 24 d for HOUSED-trained models), yet substantially biased when applied to external populations. Conversely, consistent directional bias across models provided inferential evidence of biologically meaningful differences between production systems. The difference in metabolic-age may have implications for cow health and longevity across systems of dairying but has yet to be explored.

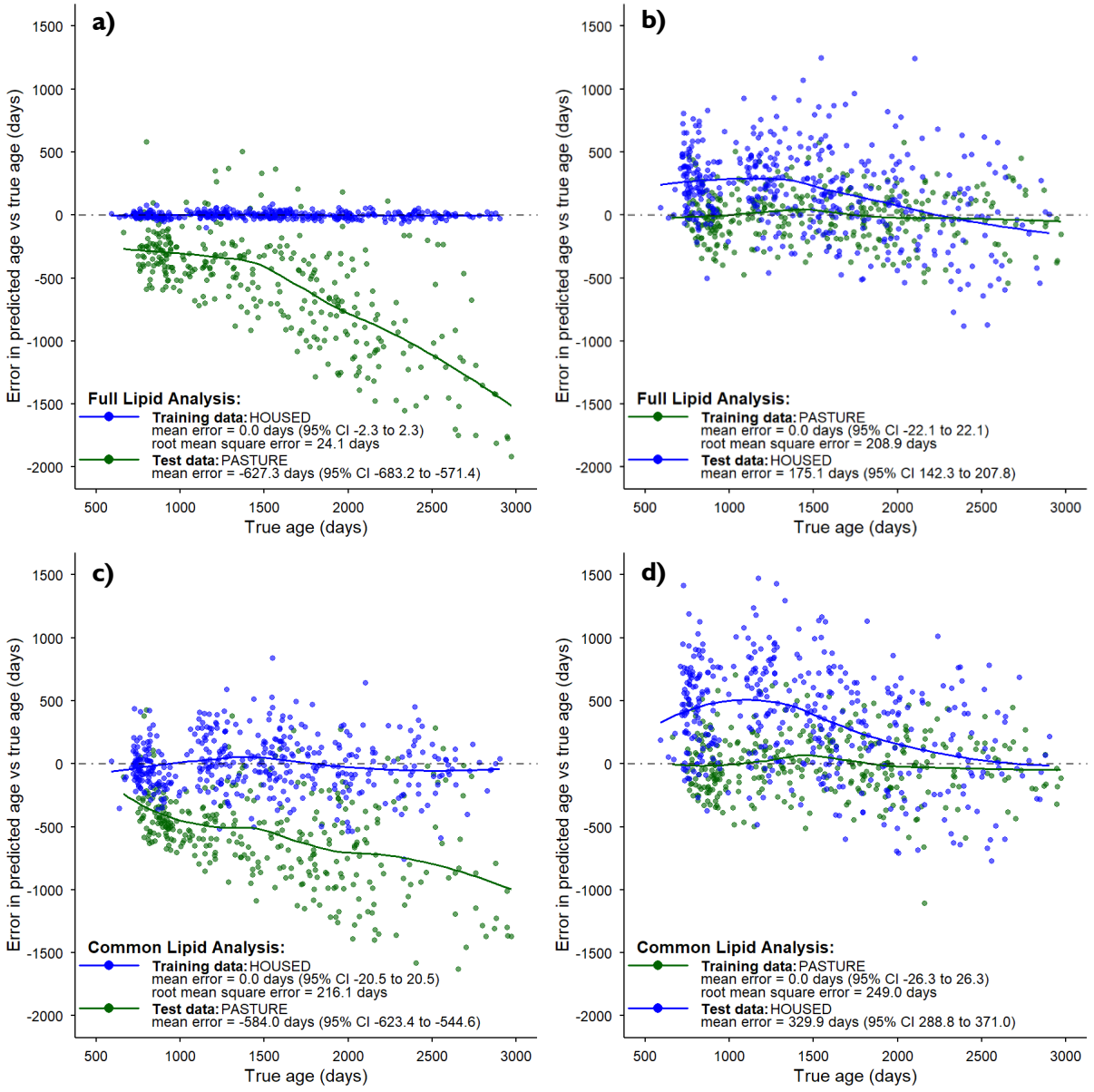


Figure 8.e: Plots of the residual error in the prediction of cow age (d) against their chronological (true) age (d) following a machine-learning metabolic clock model that utilized plasma lipid data. Models were trained exclusively on cows from either TMR, housed-based (HOUSED) or pasture-based (PASTURE) systems and tested on cows from the other system. Data were either a full lipidomic panel of 186 lipids (full lipid analysis) or models were first trained separately on cows from either system with 10 mutually important lipids selected for model training (common lipid analysis). The mean error is an indication of systematic bias in prediction.. Lines are included to indicate the average predication error at a given true age. Full lipid analysis; a) HOUSED:PASTURE, b) PASTURE:HOUSED; Common lipid analysis; c) HOUSED:PASTURE, d) PASTURE:HOUSED.

Subproject P2a Cow Longevity – v. Housing System Series

Background

An important consideration for cow longevity is the structural differences of the farming systems in which cows are managed. There are two broad dairy production housing systems: extensive, pasture-based systems and intensive, housing-based systems. The Australian dairy industry is currently undergoing a rapid transition from milk being produced almost exclusively from pasture-based farms to confinement-based systems now contributing approximately 20% of the national milk production, with expectations that 35-40% of milk will be produced from cows in confinement farms by 2030. This transition is being driven largely by motivations to expand business enterprises, an improved ability to mitigate extreme climatic events, and the decreasing availability of irrigation water. As the transition from pasture to confinement systems is recent and ongoing, there was a unique research opportunity to evaluate longevity-associated performance outcomes and biological differences between the systems of dairying in a contemporary setting.

Reproduction, mastitis and lameness in housed and pasture-based systems: associations with parity

Accepted to the Journal of Dairy Science

Authors

D.B. Sheedy, H.M. Golder, S.C. Garcia, A.K.G. Lean, I.J. Lean

Background

Dairy farming systems can be broadly classified as either housing-based dairy (**HOUSED**) or pasture-based dairy (**PASTURE**) systems. System-level differences in diet, housing, management, and milk production may influence health and reproduction of cows and the interaction of parity with these outcomes.

Methods

This multi-site prospective, observational study investigated risks of pregnancy, mastitis, and lameness for different parities and housing systems (HOUSED and PASTURE). There were 15 HOUSED and 14 PASTURE farms enrolled, with health, reproduction, and production data collected from each farm between Feb 2022 to Jan 2026. Linear regression, logistic regression, and Weibull parametric survival models were used to assess the effects of parity, production system, and season on measures of pregnancy, mastitis and lameness. Robust sandwich estimators controlled within-farm correlation.

Findings

The 100 day in-calf rate (**100DICR**, logistic regression) was not significantly different between PASTURE (34.0%; 95%CI: 28.0, 40.0) and HOUSED (32.9%; 95%CI: 28.9, 36.9) farms. The hazards of pregnancy (**HPREG**) assessed with a Weibull model were not different between PASTURE (1.04 HR, 95%CI: 0.82, 1.25) and HOUSED [1.08 hazards ratio (**HR**); 95%CI: 0.70, 1.45] farms. There was a monotonic decrease in the 100DICR and HPREG with increasing parity regardless of system. Cows breeding during Summer or Spring in PASTURE farms and Spring in HOUSED farms had reduced reproductive efficiency compared to other seasons. The effect of season was greater in PASTURE compared to HOUSED. There were 34,890 mastitis events across 129,039 lactation records. The crude incident rate of mastitis in HOUSED was 42.6 per 100 cows per 365 cow-days (95%CI: 41.9, 43.3) and 34.9 (95%CI: 33.7, 36.3) in PASTURE. The hazard of was numerically

greater in HOUSED (2.85 HR, 95%CI: 1.10, 4.61) compared to PASTURE (2.13 HR, 95%CI: 1.62, 2.64) farms. The hazard of mastitis increased monotonically with parity. There were 9,495 cases of lameness across 125,362 lactation records. The crude incident rate was 8.75 per 100 cows per 365 cow-days (95%CI: 8.43, 9.07) in HOUSED and 10.7 (95%CI: 9.97, 11.4) in PASTURE. The hazard of lameness was similar in HOUSED (1.04 HR, 95%CI: 0.24, 1.84) and PASTURE (1.19 HR, 95%CI: 0.92, 1.44) farms. The hazard of lameness were unchanged in the first three (PASTURE) and two (HOUSED) parities but increased with parity thereafter.

Implications

The association of increasing parity was far greater on health and reproductive outcomes than that of housing system. These results suggest that to support cow health and longevity we must address the impact of increased parity on health and reproduction of cows.

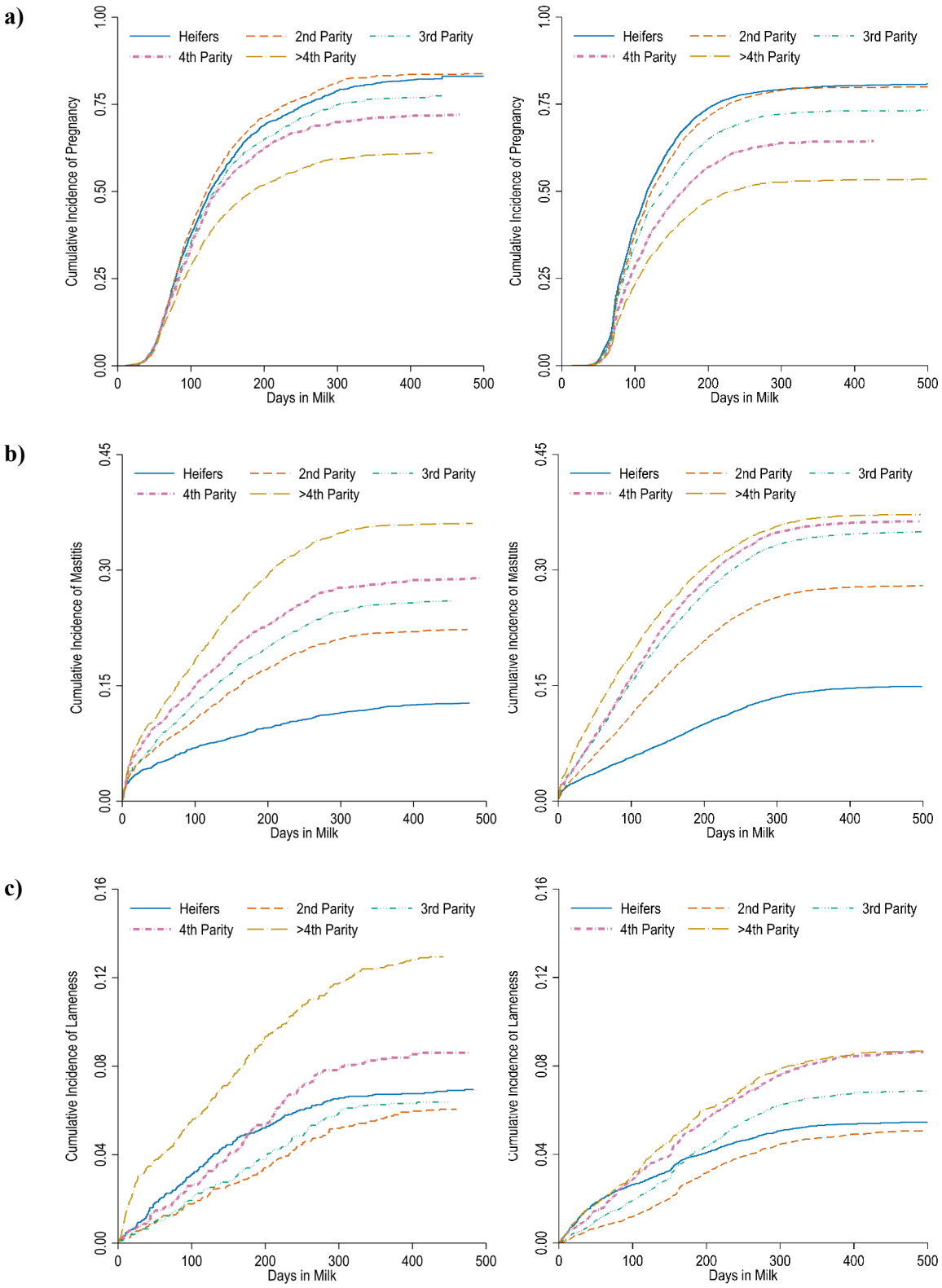


Figure 9.a: Competitive risk models showing the cumulative incidence of a) pregnancy, b) mastitis, and c) lameness by parity in pasture-based systems (left) and housing-based systems (right).

Effects of a bacterial and enzymatic product on physical and chemical properties of bedding and cow preference in compost bedded pack barns

In review

Authors

A.K.G. Lean, H.M. Golder, J.C. Quinn, I.J. Lean, D.B. Sheedy, A.J. Gunn

Background

Compost-bedded pack (**CBP**) barns are increasingly used to house cows worldwide. These are loose housing facilities where an organic bedding base is mixed daily to remove faeces and urine from the bedding surface and oxygenate the pack, allowing composting to occur. These facilities face challenges in maintaining low moisture percentages within the bedding to ensure cow comfort and good udder health. We aimed to investigate effective methods of managing CBP barns to maintain compost moisture and temperature at the recommended levels and assess the effectiveness of a bacterial and enzymatic treatment on bedding characteristics and cows' preference.

Methods

A five-week multisite replicated exposure study was conducted on 40 areas of five commercial CBP barns in Australia from late autumn to early spring in 2023 and 2024. The packs were randomly divided into alternating control (n = 20) and treatment (n = 20) areas. All areas were monitored over one week to obtain a baseline before the treatment was applied in the 20 treatment areas at the start of weeks two and four, as per manufacturer recommendations. Compost temperature (**°C**), area per cow per study area (log m^2/cow), and aerial ammonia concentrations (**ppm**) were assessed as outcome variables every 2 ± 1 day across all five farms. Three farms had additional outcome variables of compost moisture percentage and carbon to nitrogen ratio assessed weekly. Local weather conditions were measured daily, while the cultivation rate of the pack (m^2/min) was recorded as a covariable for three farms. Multilevel mixed models were used to analyse the effects of treatment, covariables, and the interaction of treatment over time.

Findings

There was no effect of treatment on bedding conditions. Aerial ammonia concentrations were only detected on six days. Slowing the cultivation rate (m^2/min) of the pack was effective at increasing compost temperature.

Implications

The product tested was not effective at improving bedding conditions nor did it change cow preference in the packs studied. Management of cultivation rate is important for maintaining bedding conditions. Minimal ammonia detection is a positive finding for human and cattle health in these barns.

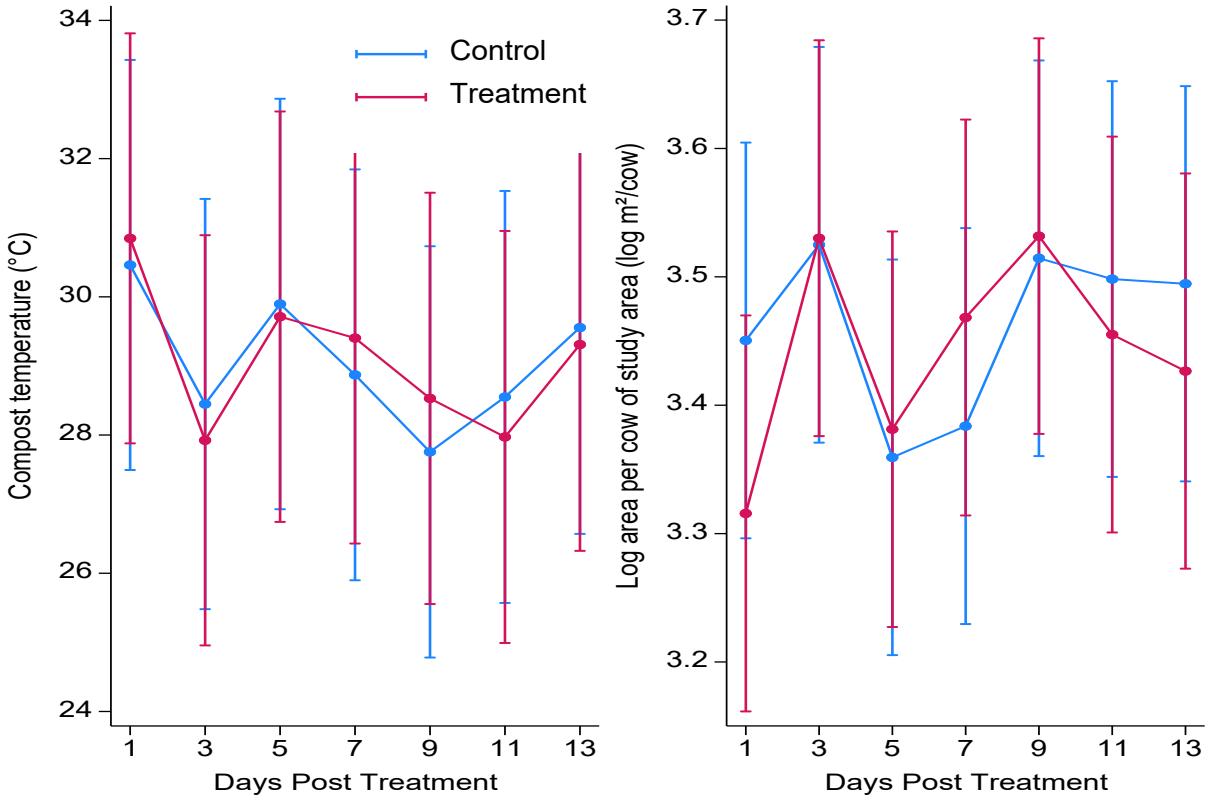


Figure 9.b: Margins plots of the 5-farm models of (A) compost temperature (°C) over time and (B) log area per cow per study area (log_{10} m^2/cow) with standard error of the mean bars

Subproject P2b – Early Alert Systems

Background

We identified a number of information streams from existing activities at Elizabeth Macarthur Agricultural Institute (**EMAI**), Department of Primary Industries and Regional Development (**DPIRD**), University of Sydney and Scibus that can be directed towards farms and their advisors in real time to ensure that diseases and risks, whether these be animal, plant or environmental can be addressed in more timely and effective manners. The use of targeted alerts, coupled with timely advice links will enable farms to more effectively reduce risks to pastures and crops, and cattle to ensure greater productivity, sustainability and health.

Outcomes

The following have been identified as targets for early alert systems; Bovine ephemeral fever, fall army worm and facial eczema. There is now a proposal for Terms of Reference for an industry panel to work on means to provide a rigorous structure to deliver early alerts (NSW DPI July 2025) and agreement from Dairy NSW committee (30/10/2024) to assist in dissemination of alerts.

For ephemeral fever the following web site has been developed as a direct outcome of the Dairy UP initiative to provide information on cases in order to assist producers to make decisions around vaccination, treatments and risk of the disease. https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary/bovine-ephemeral-fever

Implications

A lack of specific budget for this project has impeded its progress to some degree. However, good will resulting in considerable in -kind contributions from NSW DPIRD and Scibus have resulted in outcomes. There is potential to develop this project further and a communications strategy would enhance the potential for delivery of benefits to animals. farmers and communities.

Subproject P2c – Milk as a Diagnostic Fluid

Background

There are a large number of diagnostic tests currently available through Government and commercial laboratories or through advanced milking platforms. The objective of the review will be to evaluate the diagnostic value of tests at an individual cow and herd level. Costs of diagnostic tools and the value of these to producers will be assessed in order to enhance existing herd recording systems or to develop novel packages for herd monitoring.

Editorial: Milk as a Diagnostic Fluid

doi.org/10.1111/avj.13299

Authors

I.J. Lean, R. Zadocks, B. Brito, H.M. Golder

Editorial

Over 40 years there have been profound changes to the Australian dairy production environment. The number of farms decreased from 21,989 in 1980 to 5055 in 2020, milk production per cow increased from 2888 L/cow per year or 1.9 million cows producing 5.49 million L per year, to 6311 L/cow per year or 8.8 million L from 1.4 million cows. Many dairy farms represent assets valued in the $10 to $100 million or more. The average herd has increased from 85 to 274 cows. Consequently, farm management has less time to engage with the individual cow. These changes influence the delivery of veterinary services as the individual cow now represents a much lower proportion of the enterprise asset value. However, herd health and productivity are critical to an enterprise and farmers are committed to stewardship of their cattle. The challenge for the veterinary profession is to deliver cost-effective services that identify, monitor, and mitigate risks to herd health and productivity. Such services must be designed to deliver better outcomes with greater labour efficiency. In this series of reviews, we evaluate the value of bulk tank milk which provides a readily available and contemporary indicator of herd status of health and production and, where appropriate, compare the value of bulk milk testing to that of individual cow testing, to determine the mastitis, viral, and metabolic status of herds. We provide quantitative and qualitative reviews of tests that may do this. We also note two coincident and valuable scoping reviews of this area, one of which includes data on the value of bulk tank milk for parasite evaluation.

Bulk milk somatic cell counts are routinely utilised by processors and veterinary advisors to assess milk quality and udder health. Because this assessment does not capture cows with clinical mastitis, diagnostics at the cow level may also be needed to manage udder health. Additional markers of inflammation or the humoral immune response are primarily available at cow level, except for antibody testing for *Mycoplasma bovis,* which can be conducted at bulk milk level to support biosecurity efforts. Elevated somatic cell counts are primarily due to intramammary infections, and its causative agents, including those with antimicrobial resistance, can be detected through culture or polymerase chain reaction (**PCR**). Specificity of PCR for contagious pathogens (*Streptococcus agalactiae, Mycoplasma bovis*) is high (0.90) but sensitivity is variable (0.15–0.99) unless repeated bulk milk testing or cow-level testing is used.

For pathogens that may be cow-derived as well as environmental (*Staphylococcus aureus, Streptococcus dysgalactiae, Streptococcus uberis*), sensitivity of bulk milk testing is low (<30%). New technologies such as matrix-assisted laser desorption/ionization time of flight (**MALDI-TOF**) or loop-

mediated isothermal amplification (**LAMP**) offer new insights into intramammary infection but are not applied at the bulk milk level yet.

Bulk tank milk was very effectively used in Australia to evaluate herd status with an enzyme-linked immunosorbent assay (**ELISA**) test to eradicate bovine leukaemia virus. Interpreting results from bulk milk tests requires careful consideration of complexities, including the test target, prior virus exposure including vaccination, duration of antibodies in milk or virus shedding, and other relevant factors. For direct detection of pathogens or the immune or metabolic responses to pathogens, increased herd size and prevalence of organisms or response has significant testing implications. Specifically, as herd size increases the probability of the assay detecting the presence decreases due to potential for dilution.

Studies identified the limits of detection for different assays and estimates for herd sensitivity (0.44–0.97) and herd specificity (0.42–1.00) for pestivirus ELISA. The use of PCR to detect persistently infected animals detected up to 1 animal in 1000, however, other studies indicated a lower sensitivity. Bulk tank milk can also be used to detect bovine alphaherpesvirus-1 (**BHV-1**) which is prevalent in Australia, while the use during a foreign animal disease outbreak is arguable.

There are valuable bulk tank milk assays that can monitor the herd nutritional status. The bulk tank milk urea and protein content provide useful indications of herd nutrition using routine milk testing from milk processors. These tests provide indicators that encourage further investigations of nutritional influences on herd fertility but are unlikely to provide strong diagnostic value. The fat to protein ratio has a high specificity, but poor sensitivity for the detection of fibre insufficiency and acidosis on an individual cow basis and is useful as an alert to evaluate other indicators of acidosis in a herd.

Integrating herd recording demographic information with Fourier transformed mid-infrared spectra (**FT-MIR**) can provide tests that are useful to identify cows with metabolic disorders and these tests will become more available in Australia. Selenium, zinc, β-carotene, and vitamin E status of the herd can be determined using bulk tank milk and could be combined to monitor herds.

Bulk tank milk is an under-utilised resource that can be used to improve the health and productivity of dairy herds. There is considerable potential to increase the availability and adoption of bulk tank milk as part of an efficient integrated farm service. Some of the tests readily available and reported on a daily basis include milk volume, somatic cell counts, fat, protein, and milk urea. More can be done with these data to evaluate herds. However, there appears to be increased scope for testing for milk minerals, vitamins, and assays for mastitis and viral pathogens. These assays can provide cost-effective rapid means of monitoring herds and have the potential to be integrated with statistical monitoring methods to automate detection of changes in herd status and equip veterinary services with new tools to assist farms.

Milk as an indicator of dietary imbalance

doi.org/10.1111/avj.13294

Authors

I.J. Lean, H.M. Golder

Background

Milk provides a readily available diagnostic fluid collected daily or more frequently on an individual animal or herd basis. Milk, as an aggregated sample in bulk tank milk (**BTM**) represents the status of a herd instead of a single animal. In this review, we examine the potential for milk to predict risks to efficient production, reproductive success, and health on the individual cow and herd level.

Findings

For many conditions related to disorders of metabolism including hyperlipdaemia and ketonaemia, improved individual cow milk testing may allow a temporally useful detection of metabolic disorder that can target intervention. However, the extension of these tests to the BTM is made more difficult by the tight temporal clustering of disorder to early lactation and the consequent mixing of cows at even moderately different stages of lactation. Integrating herd recording demographic information with Fourier-transformed mid-infrared spectra (FT-MIR) can provide tests that are useful to identify cows with metabolic disorders. The interpretation of BTM urea and protein content provides useful indications of herd nutrition. These may provide indicators that encourage further investigations of nutritional influences on herd fertility but are unlikely to provide strong diagnostic value. The fat-to-protein ratio has a high specificity, but poor sensitivity for detection of fibre insufficiency and acidosis on an individual cow basis. Selenium, zinc, β-carotene, and vitamin E status of the herd can be determined using BTM.

Implications

There appears to be increasing potential for the use of milk as a diagnostic fluid as more in-parlour tests become available for individual cows. However, the BTM appears to have under-utilised potential for herd monitoring.

Table 11.a: Details tests that are available to evaluate metabolic states including energy deficiency, and fibre sufficiency. If available, information on the diagnostic accuracy, sensitivity and specificity of the tests is provided.

Condition	Test	Index test	Test accuracy	Sensitivity	Specificity
Hyperlipidemia	Blood FFA < 0.6 mmol/L	Milk C18:1 cis-9	0.79	0.75	0.79
Hyperketonemia	Blood BHB (≥ 1.2 mmol/L)	Milk fat C18:1 cis-9-to C15:0 ratio of ≤40	0.82	0.38	0.95
Hyperlipdaemia	Blood FFA ≥ 0.95	Milk fatty acids (C17:0 to 15:0)	0.84	0.72	0.90
Hyperlipdaemia	Blood BHB mmol/L ≥ 0.7	Milk fatty acids, days in milk, lactation number, milk production	77.4	77.1	77.4
Hyperketonemia	Blood BHB (1.0 to 1.4 mmol/L)	Ketotest	0.80	0.86	0.72
Hyperketolactia	Milk acetoacetate (>0.15 mmol/L)	Fourier transformed infra-red spectrometry	0.80	0.86	0.72
Hyperketonemia	Blood BHB (≥ 1.2 mmol/L)	Fourier-transformed infra-red	77.9	84.8	77.1
Fibre sufficiency	Milk fat: Protein ratio < 1.02		0.72	0.54	0.81
Metabolic state	Balanced, unbalanced at 9 days in milk	Demographic data, milk yield and components, FT-MIR for BHB and milk fatty acids	0.85		

Milk as diagnostic fluid for udder health management

doi.org/10.1111/avj.13290

Authors

S. Rowe, J.K. House, R.N. Zadoks

Background

Mastitis is the major disease affecting milk production of dairy cattle, and milk is an obvious substrate for the detection of both the inflammation and its causative infectious agents at quarter, cow, or herd levels. In this review, we examine the use of milk to detect inflammation based on somatic cell count (**SCC**) and other biomarkers, and for the detection of mastitis pathogens through culture-based and culture-free methods.

Findings

The use of SCC at a cow or bulk milk level to guide udder health management in lactation is well-established, and SCC is increasingly used to guide selective dry cow treatment. Other markers of inflammation include electrical conductivity, which is used commercially, and markers of disease severity such as acute phase proteins but are not pathogen-specific. Some pathogen-specific markers based on humoral immune responses are available, but their value in udder health management is largely untested. Commercial pathogen detection is based on culture or polymerase chain reaction, with other tests, for example, loop-mediated isothermal amplification or 16S ribosomal ribonucleic acid microbiome analysis still at the research or development stage. Matrix-assisted laser desorption ionisation time of flight (**MALDI-TOF**) is increasingly used for the identification of cultured organisms whilst application directly to milk needs further development. Details of test sensitivity, specificity, and use of the various technologies may differ between quarter, cow, and bulk milk applications.

Implications

There is a growing array of diagnostic assays that can be used to detect markers of inflammation or infection in milk. The value of some of these methods in on-farm udder health improvement programs is yet to be demonstrated whilst methods with proven value may be underutilised.

Milk as a diagnostic fluid to monitor viral diseases in dairy cattle -

doi.org/10.1111/avj.13293

Authors

B. Brito, P. Hick

Background

Infectious viral diseases in dairy cattle have substantial implications for milk production, quality and overall animal health. Diagnostic tools providing reliable results are crucial for effective disease control at the farm and industry level. Pooled or bulk tank milk (BTM) can be used as a cost-effective aggregate sample to assess herd disease status in dairy farms.

Findings

Detection of pathogens or specific antibodies in milk can be used for monitoring endemic diseases within-farm, region or country-level disease surveillance and to make informed decisions on farm management. The suitability of assays applied to pooled milk samples relies on validation data of fit-for-purpose tests to design an optimal testing strategy. Diverse approaches and variable scope of studies determining test accuracy need to be critically appraised before sourcing the parameters to design sampling strategies and interpreting surveys. Determining if BTM or pooled milk is the best approach for a disease management programme should carefully consider several aspects that will impact the accuracy and interpretation, for example, the size of the lactating herd, the risk of infection in the lactating and non-lactating groups, the expected within-herd prevalence, the duration of infection, the duration and concentration of antibodies in milk and use of vaccination.

Implications

There are examples of tests on BTM samples providing efficient assessments of the herd disease status and supporting disease control programmes for viral diseases. However, challenges arise in pooled milk testing due to the need for accurate estimates of the imperfect sensitivity and specificity of the assays. Integration of new biotechnologies could enhance multiplexing and data interpretation for comprehensive surveillance. The development of highly sensitive assays is necessary to meet the demands of larger dairy herds and improve disease detection and assessment.

Subproject P2d Heat Stress

Effects of changes in total mixed ration nutrition and maximum temperature humidity index (THI) on dairy cow milk yield

Authors

A.K.G. Lean, A.J. Gunn, H.M. Golder, J.C. Quinn, D.B. Sheedy, I.J. Lean, P.C.Thomson

Background

There is a general understanding that changes in the nutrient concentrations of dairy cows' diet have delayed effects on production. Current nutrition models do not take these into account. There have been limited attempts to quantify these effects on milk yield. This study investigates the effects of changes in nutrition on the milk yield of housed dairy cows and the effects of changes in the weekly average maximum temperature-humidity index (**THI**). Methods

Methods

A multisite time series investigated effects of nutritional changes in TMR diets and THI on milk yield of dairy cows. Weekly diet samples were collected and tested from 8 farms for a total of 9 strings or herds for an average of 58 weeks. Nutrient components of the diet examined for effects on milk yield were neutral detergent fibre (**NDF**), lignin, crude fat, crude protein (**CP**), neutral detergent insoluble crude protein (**NDICP**), starch and non-fiber carbohydrates (**NFC**). Milk yield was measured from daily shipped milk or individual milk volumes. Weather data were measured using on-farm or nearby publicly available weather stations. The groups of cows on the diets monitored had mean milk yields between 31 L/cow and 52 L/cow. Univariable regression spline models were used to detrend the data in Stata, and residual and smooth values were generated for each variable, as well as Principal Component (**PC**)1, PC2 and PC3. Lagged variables up to -5 weeks were generated for each covariable's residual within their farm diet. A Pearson correlation matrix of the milk yield residual and the covariable residuals at each week's lag was created to determine the lag of greatest effect. These were included in univariable mixed models before a multivariable mixed model was created through backward stepping with farm diet as a random effect.

Findings

Changes in milk yield were positively associated with changes in PC1 (carbohydrates) 4 weeks before and PC3 (Crude fat/NDICP) 1 week before in univariable and multivariable analysis. In the all-diet nutrient univariable model, changes in NDF 4 weeks earlier and NDICP at the time were positively associated, NFC and starch 4 weeks earlier, and weekly average maximum THI at the time were negatively associated with milk yield changes. In a multilevel multivariable model, NFC 4 weeks earlier and weekly average maximum THI at the time were negatively associated, crude fat 1 week earlier and NDICP at the time were positively associated with milk yield changes. The diets were grouped into low-starch ($n = 3$) and high-starch diets ($n = 6$) with a cut point of 24% mean starch in the diet. The cows on high-starch diets had higher milk yield on average (high-starch = 41.8 L/cow; low-starch 34.8 L/cow). For the low-starch diets, the multivariable model showed a positive association between changes in milk yield and starch 1 week earlier and NDICP at the time. For the high-starch diets, the multivariable model found positive associations for CP 3 weeks earlier, crude fat 4 weeks earlier, while starch 2 weeks earlier and maximum average weekly THI 1 week earlier were negatively associated with milk yield changes.

Implications

This study highlighted that the effects of dietary changes take up to 4 weeks to have their greatest effect on milk yield. The carbohydrate component (PC1) and NFC had their greatest effect on milk yield 4 weeks later, while crude fat and NDICP had effects on milk yield 0-1 weeks later. The farms exceeding a mean starch of 24% likely were inducing some level of rumen dysfunction, possibly subclinical acidosis, as increased starch led to decreased milk yield. In low-starch farms, changes in starch concentration positively impacted milk yield. This study uniquely quantifies the delayed effects of diet on milk yield using time series methodology.

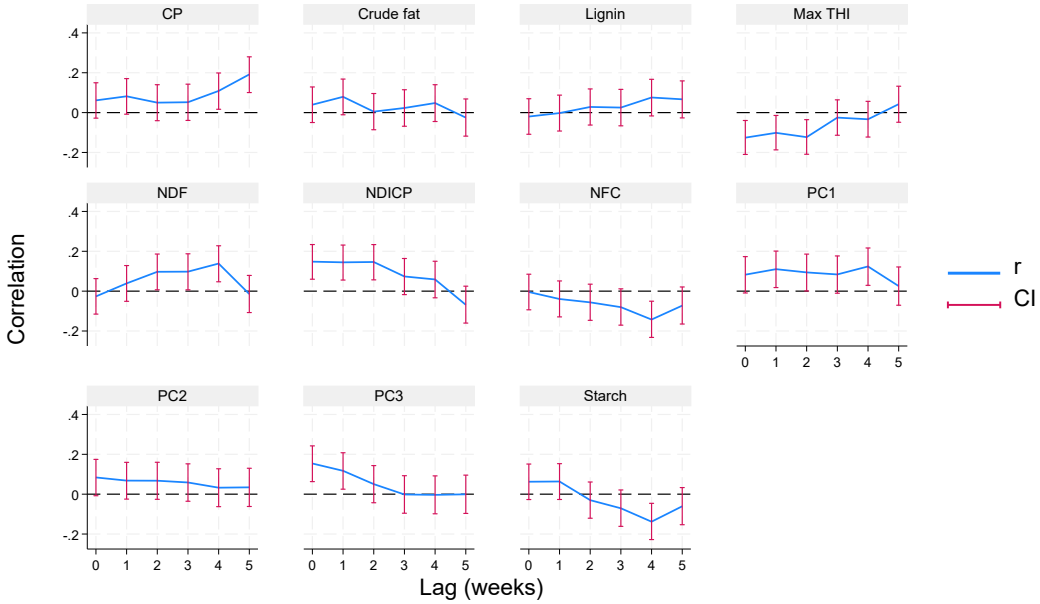


Figure 3: Pearson correlation of the milk yield residuals plotted against the different nutrition components and maximum THI residuals at lags 0 to 5 weeks. "r" is the correlation, "CI" is the 95% confidence interval.

Subproject P2e Calves/Heifers - i. Lifetime Different Diet Effects in Steers

Different lifetime dietary strategies affect carcass characteristics and rumen function in Holstein steers - doi.org/10.1071/AN25153

Authors

H.M. Golder, J. Rehberger, A.H. Smith, E. Block, R. Polkinghorne, H.E. Cuthbertson, M.A. Campbell, V. Vicic, G. Tarr, J.C. Quinn, J. Tong, S.M. Rowe, I.J. Lean

Background

The Australian dairy industry has a considerable surplus of male dairy calves for which there are limited markets and animal welfare concerns; a challenge also faced by other countries. There is a need to improve how "dairy-beef", the term for meat that originated directly or indirectly from dairy herds is integrated into red meat supply chains. Consequently, the challenge that exists with dairy-beef is how to efficiently utilise the opportunity that these calves provide. Our aim was to evaluate the effects of two diets differing primarily in starch, antimicrobial content, and milk replacer volume on (i) short- and long-term rumen adaptation; and (ii) lifetime production in Holstein steers.

Methods

Holstein males, 3-7 days old (n = 72; 36 steers/treatment; 6 per replicate) were randomised to Control (**CON**; 6 L milk replacer/calf.day; 38.2% of dry matter [**DM**] lifetime dietary starch; 50 ppm monensin, 20 ppm flavophospholipol) or Treatment (**TRT**; 4 L milk replacer/calf.day; 47.5% of DM lifetime starch diets with yeast products) strategies. Calves were fed milk replacer twice daily for 42 days, but different pre-starter (days 0-24), starter (days 25-99), and finisher diets (days 100-452). Ruminal fluid was collected at 104, 200, and 438 days old (14 days pre-slaughter) from 24 steers (2 per replicate). Fermentation, production, and carcass measures were analysed by mixed models; ruminal bacterial genera were centre log transformed and subjected to redundancy analysis.

Findings

The CON had higher risk of subclinical ruminal acidosis at day 438, than TRT ($P < 0.001$). Liver abnormalities were 17.1% (CON) and 31.3% (TRT). Controls had greater fermentation with 138.4 ± 5.6 m*M* of total volatile fatty acids vs 111.6 ± 5.6 m*M* (TRT) with 8.4 m*M* higher acetate and 18.1 m*M* higher propionate, but pH was 0.31 units less ($P < 0.050$). Shannon diversity increased over time ($P < 0.001$) and was greater for the TRT at day 200, compared to CON ($P = 0.013$). Bacterial composition differed at each treatment by time comparison ($P \leq 0.01$), with variation increasing over time from 8.6 to 19.2%, suggesting the different diets lead to different microbial successions. The CON steers finished with 12 kg heavier carcasses than the TRT, with 0.8% greater dressing percentage, 1.5 mm more fat at P8, and 1.7 mm more rib fat ($P < 0.050$).

Implications

Control diets produced better carcass weights, P8 and rib fat, and had more fermentability than the TRT diets, likely reflecting better long-term adaptation. Both diets enabled integration of dairy calves into the red meat supply chain, but with differing lifetime rumen adaptations.

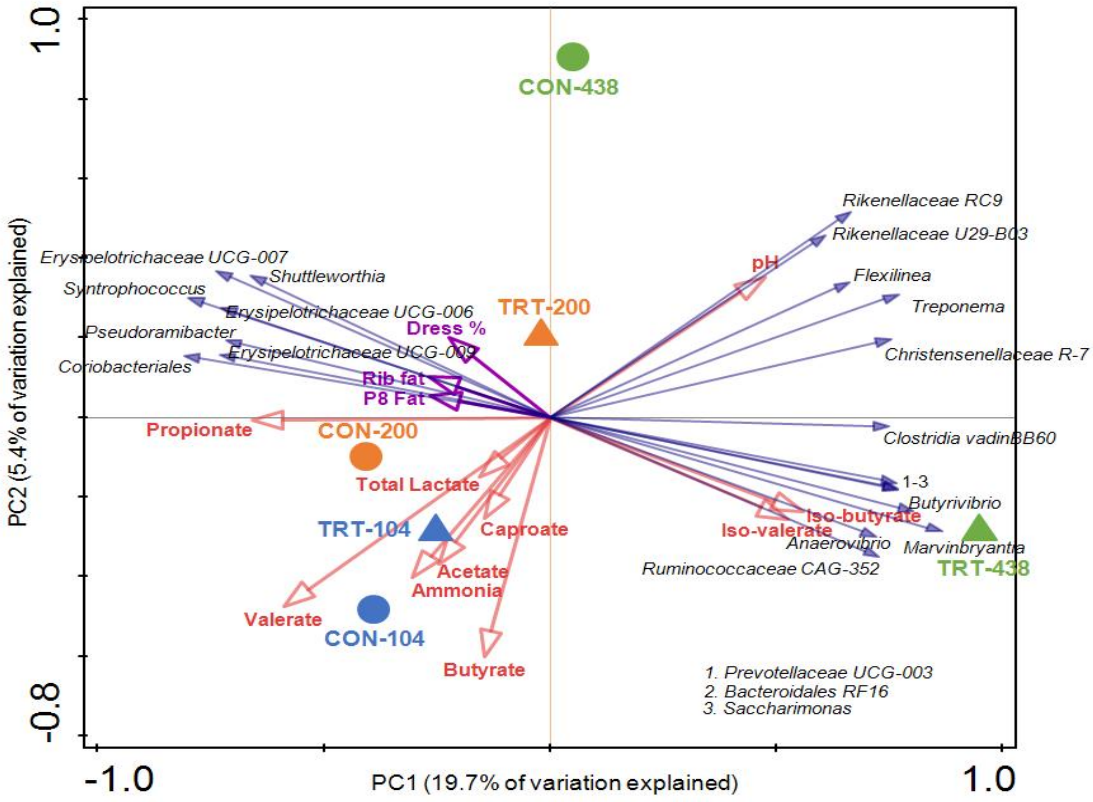


*Figure 13.a: Correlation bi-plot of the redundancy analysis of bacterial genera with respect to treatment and time combinations and ruminal fermentation explanatory variables plus carcass explanatory variables: rib fat, P8 fat, and dressing percentage (Dress %). The 20 genera with the best fit to the explanatory variables are displayed where the length of the arrows are approximate correlation coefficients between the variables and treatment-time combinations. The total variation explained by the treatment-time combinations and explanatory variables is 46.8% and the eigenvalues for the first two axes are 0.20 and 0.05. All pairwise treatment and time combinations differed ($P \leq 0.01$), with the variation in microbiota increasing over time; 8.6, 10.9, and 19.2%, for 104-, 200-, and 438-day samples respectively. Treatment and time combinations were associated with the following variations: TRT-438 = 15.6%, CON-438 = 4.5%, CON-104 = 4.5%, CON-200 = 3.9%, TRT-104 = 3.0%, TRT-200 = 2.5%. Concentrations of propionate and valerate were associated with the most variation of the ruminal fluid measures, 9.3 and 8.5%, respectively ($P = 0.002$). Less variance was associated with iso-butyrate (7.3%; $P = 0.002$) and iso-valerate concentrations (7.0%; $P = 0.002$), rumen pH (5.9%; $P = 0.002$), and concentrations of total volatile fatty acids (**VFA**; 5.8%; $P = 0.002$), ammonia (4.7%; $P = 0.004$), butyrate (3.2%; $P = 0.012$), acetate (3.0%; $P = 0.024$), total lactate (1.7%; $P = 0.204$), and caproate (1.7%; $P = 0.224$). P8 fat, rib fat, and dressing percentage were poorly associated with variance, associated with 2.8% ($P = 0.012$), 2.7% ($P = 0.020$), and 2.2% ($P = 0.072$), respectively. Categorical data is represented by a triangle or circle (blue = samples collected at a mean age of 104 days; orange = 200 days; and green = 438 days) and numerical data is represented with an arrow indicating the direction in which the variable increases (blue = bacterial genera; pink = ruminal fermentation markers, and purple = carcass measures). Blue categorical CON = Control (6 L milk replacer/calf.day for 42 days; 38.2% of DM lifetime starch diets; 50 ppm monensin, 20 ppm flavophospholipol); TRT = Treatment (4 L milk replacer/calf.day for 42 days; 47.5% lifetime starch diets with yeast products); Ruminal fluid samples were collected at a mean of 104, 200, and 438 days old; n = 12 steers/treatment; 2 steers/replicate; total n = 72 ruminal fluid samples.*

Subproject P2e Calves/Heifers - iii. Calf Performance

Milk consumption and behavior of calves in automated calf feeders as early indicators of weaning liveweight

doi.org/10.3168/jdsc.2023-0488

Authors

S.W.J. Legge, P.C. Thomson, C.E.F. Clark, S.C. Garcia

Background

Modern intensive dairy farming relies on data to aid and prioritize management decisions made on farm. Decisions made early in an animal's life can have lasting effects on welfare, productivity, longevity, and profitability. Precision technology such as automated calf feeders (**ACF**) allow the customization of feeding programs, but despite this, weaning weights (**WWT**) vary substantially between calves.

Methods

This observational study used a 3-yr dataset comprising 1,440 female Holstein Friesian calves at a single intensive commercial dairy farm (Dairy Australia feeding system 5; indoor, total mixed ration) using ACF to (1) determine the variability in WWT (as a proxy of animal performance) of calves within this system; (2) identify the contributing factors responsible for the variation in WWT; and (3) identify potential early management intervention points that could be indicative of the performance of calves at weaning within the system. Calves entered the ACF at 10 d of age with 12 calves per ACF; calves were weighed at birth and weaning using weigh scales.

Findings

We discovered a large range of calf WWT (41–118 kg/head) at ~60 d of age despite the application of strict uniform management protocols. Our results from modeling showed that WWT was significantly and positively associated with birth weight (**BWT**), with low BWT calves (<36 kg) achieving an average of 70 kg weight at weaning. In contrast, heavier BWT (>36 kg) calves achieved an average of 82 kg at weaning. Based on calf feeder data, cumulative milk consumption and cumulative unrewarded visits to the feeder, as well as BWT, were identified as indicators of greater WWT as all these were highly significant terms in the model for WWT. Results suggest that quantifying consumption and number of visitations to the ACF at d 5 may allow farmers to identify, with time to intervene, calves underperforming within the feeder or system, therefore increasing their potential for growth.

Implications

this demonstrated that greater milk consumption (>30 kg) and interaction with the feeder up to d 5 in the feeder is more likely to yield a WWT >75 kg, identifying a potential point for management intervention for calves below consumption and interaction thresholds (e.g., by developing alarm systems based on consumption or visitation number).

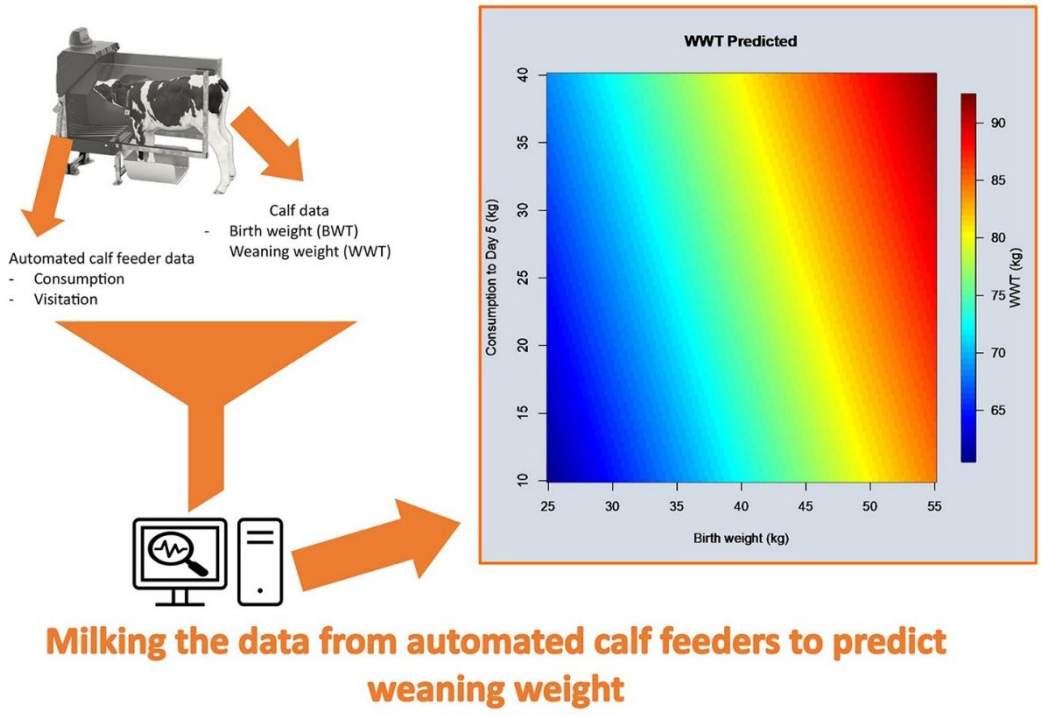


Figure 14.a: Predicted response surface showing the model-based mean weaning weight (WWT) for varying birth weights and cumulative consumption up to day 5. A scale is indicated on the right side of the plot.

Subproject P2e Calves/Heifers - iv. Feedlot Adaptation

Feedlot adaptation: characterising rumen fermentation profiles that determine success or failure

Authors

M. Zhang, I.J. Lean, R. Polkinghorne, H.E. Cuthbertson, M.A. Campbell, G. Tarr, J.C. Quinn, S.M. Rowe, H.M. Golder

Background

Ruminal acidosis is a major digestive disorder of feedlot cattle that leads to significant reductions in cattle health, welfare, and performance, and accounts for 3.7% of mortalities related to digestive disorders in feedlot cattle. We propose that identifying the rumen fermentation characteristics of cattle that adapt well to feedlot induction based on bodyweight gain over the induction period or those that fail to adapt well to feedlot induction may provide vital clues for future interventions to control acidosis, and further knowledge on the aetiology of acidosis. The objective of this study was to evaluate changes in the gastrointestinal tract function of steers introduced to feedlot diets and identify differences between steers with high-weight gain (performing) and low-weight gain (non-performing) during the feedlot induction period (first 3 wk in the feedlot).

Methods

A total of 94 steers of Holstein, British, and European origin were enrolled in the study at entry to the feedlot in 2 batches. After 3 wk, 10 steers from each breed were allocated to 1 of 6 treatment groups based on weight gain between feedlot entry and the end of feedlot induction: Holstein low-bodyweight gain (**HLG**), Holstein high-bodyweight gain (**HHG**), British low-bodyweight gain (**BLG**), British high-bodyweight gain (**BHG**), European low-bodyweight gain (**ELG**), and European high-bodyweight gain (**EHG**). Performance indicators included bodyweight which was measured at feedlot arrival, 3 wk post-feedlot entry (sampling 1; S1), monthly, and 2 wk before slaughter (sampling 2; S2), and average daily gain (**ADG**) which was calculated between each weighing session. Rumen samples were obtained using a stomach tube at S1 and S2 and analysed for pH, volatile fatty acid (**VFA**), ammonia, and lactic acid concentrations. These rumen fermentation measures were used in a validated model based on discriminant analysis and K-means clustering to determine acidosis risk. Following slaughter, carcases, organs and the rumen were inspected for abnormalities.

Findings

No differences in acidosis risk between gain categories (**GC**) or breed were observed ($P = 0.125$ and 0.494, respectively), although HHG steers had a higher risk of acidosis compared to HLG steers ($P = 0.024$). A lack of significant differences in rumen fermentation measures were observed between GC and treatment groups, with the exceptions being isovalerate ($P = 0.001$), acetate to propionate (A:P; $P = 0.010$), isobutyrate ($P = 0.001$), and isovalerate ($P = 0.036$). Steers in the high GC had greater monthly bodyweights ($P < 0.001$) and ADG ($P = 0.003$), final feedlot ADG ($P = 0.003$), hot carcase weight (**HCW**; $P = 0.008$), P8 fat depth ($P = 0.045$), and eye muscle area ($P = 0.039$) compared to low GC steers. There was an overall low incidence of carcase, organ, and rumen abnormalities and no rumen lesions were observed.

Implications

It was concluded that changes in rumen fermentation measures did not influence weight gain, pathology, or meat quality, but rather that the effects of high performance during feedlot induction may translate to more desirable carcase traits at slaughter. The carcass traits demonstrated by Holstein breeds also provide support for the emergence of a dairy beef industry in Australia to reduce male bobby calf wastage.

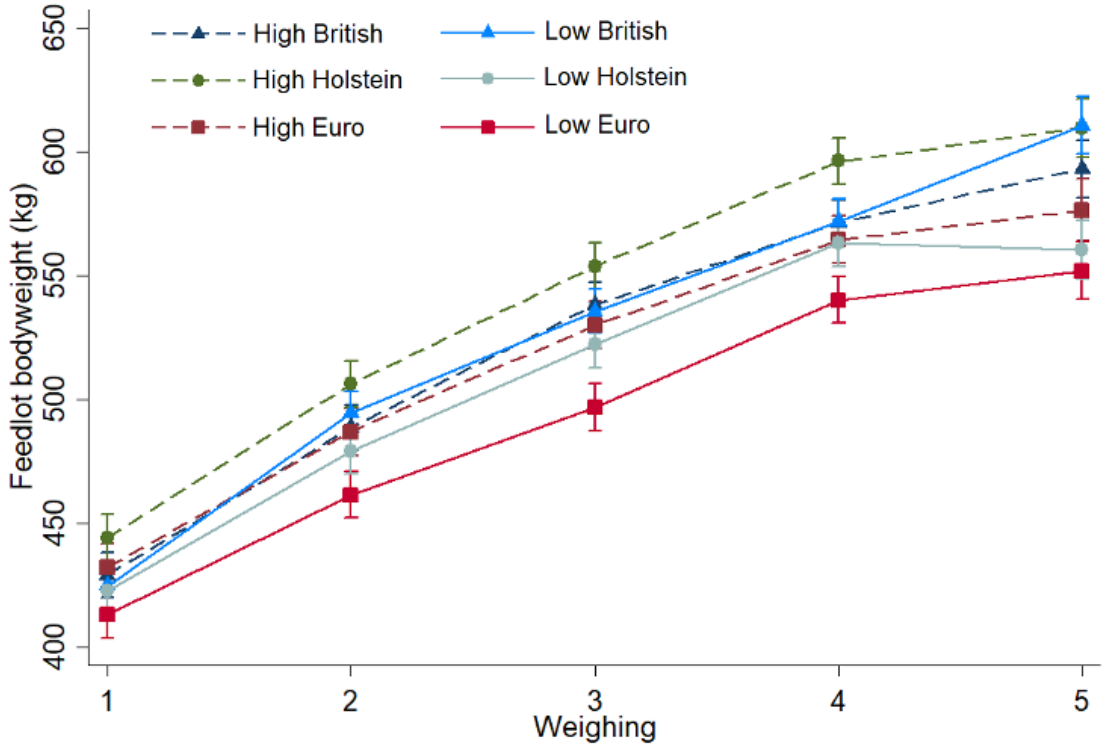


Figure 15.a: Average feedlot bodyweight of Holstein, British and European steers in high and low gain categories at each monthly weighing. High = high gain category; Low = low gain category.

Subproject P2f The Infectome

Background

Infectious diseases are the main cause of disease and mortality in calves. The knowledge of the prevalence and diversity of infectious disease-causing agents in NSW dairy cattle has not been comprehensively assessed. Thus, the immediate goal of this project was to redress this knowledge gap using untargeted microbial genomic sequencing to characterise and identify known and emerging enteric and respiratory pathogens in dairy calves. We determined the occurrence and distribution of their microbial species across all NSW dairy regions. This enables the Australian dairy industry to improve animal health and productivity, and diagnostic capacity, which will allow farmers to make informed management decisions about disease control strategies.

The main objectives of the project were:

To identify the respiratory and enteric infectome diversity in NSW dairy farms: we determined the calves' respiratory and enteric viral, bacterial and eukaryotic species taxonomic diversity in farms across NSW using meta-transcriptomics.

To describe the occurrence and spread of selected ribonucleic acid (**RNA**) viruses and different genotypes through shotgun sequencing and phylogenomics analyses.

To obtain representative isolates and samples from *E. coli* across dairy farms in NSW and characterise the whole genome by the study of *E. coli*'s molecular epidemiology and the study of antimicrobial resistance profile and mechanisms of antimicrobial resistance spread.

Next-generation detection in bovine respiratory and enteric diseases: metagenomic and amplicon sequencing insights into microbial diversity

doi.org/10.3389/fvets.2026.1788101

Authors

Z.U. Abedien, I.J. Lean, S.P. Djordjevic, P.M. Hick, M.E. Westman, J. Mckay-Demeler, J. Webster, B.P. Brito

Summary

Respiratory and enteric diseases are major contributors to morbidity, mortality, and economic loss in cattle production, with significant implications for animal welfare, particularly in calves. Traditional diagnostic approaches have laid the foundation for pathogen detection in cattle, providing essential tools for disease surveillance and control. However, their targeted nature limits the capacity to identify unexpected, novel, or polymicrobial infections that often underlie complex respiratory and enteric syndromes. Recent advances in molecular technologies, particularly amplicon sequencing (metataxonomics), metagenomics, and metatranscriptomics, enable untargeted, high-resolution profiling of microbial communities directly from clinical samples, offering transformative potential for research and diagnostics. This review synthesises current applications of these approaches in bovine respiratory and enteric disease research, highlighting key findings across virology, bacteriology, and parasitology. Collectively, these studies have expanded the catalogue of the microbial diversity, yet their interpretation remains challenged by the still-evolving understanding of microbial contributions to pathogenesis. Progress toward clinical integration is further hindered by the need for methodological standardisation, validation, and improved interpretive frameworks. Looking ahead, advancing these technologies will require harmonised protocols, integration of multi-omics datasets,

and robust experimental and epidemiological studies to establish causal links between microbial signatures and disease outcomes. By bridging discovery and application, these approaches hold the potential to enhance diagnostic accuracy, strengthen surveillance, and support sustainable cattle production systems. As these technologies continue to evolve, they are likely to play an increasingly central role in bovine disease research and diagnostics.

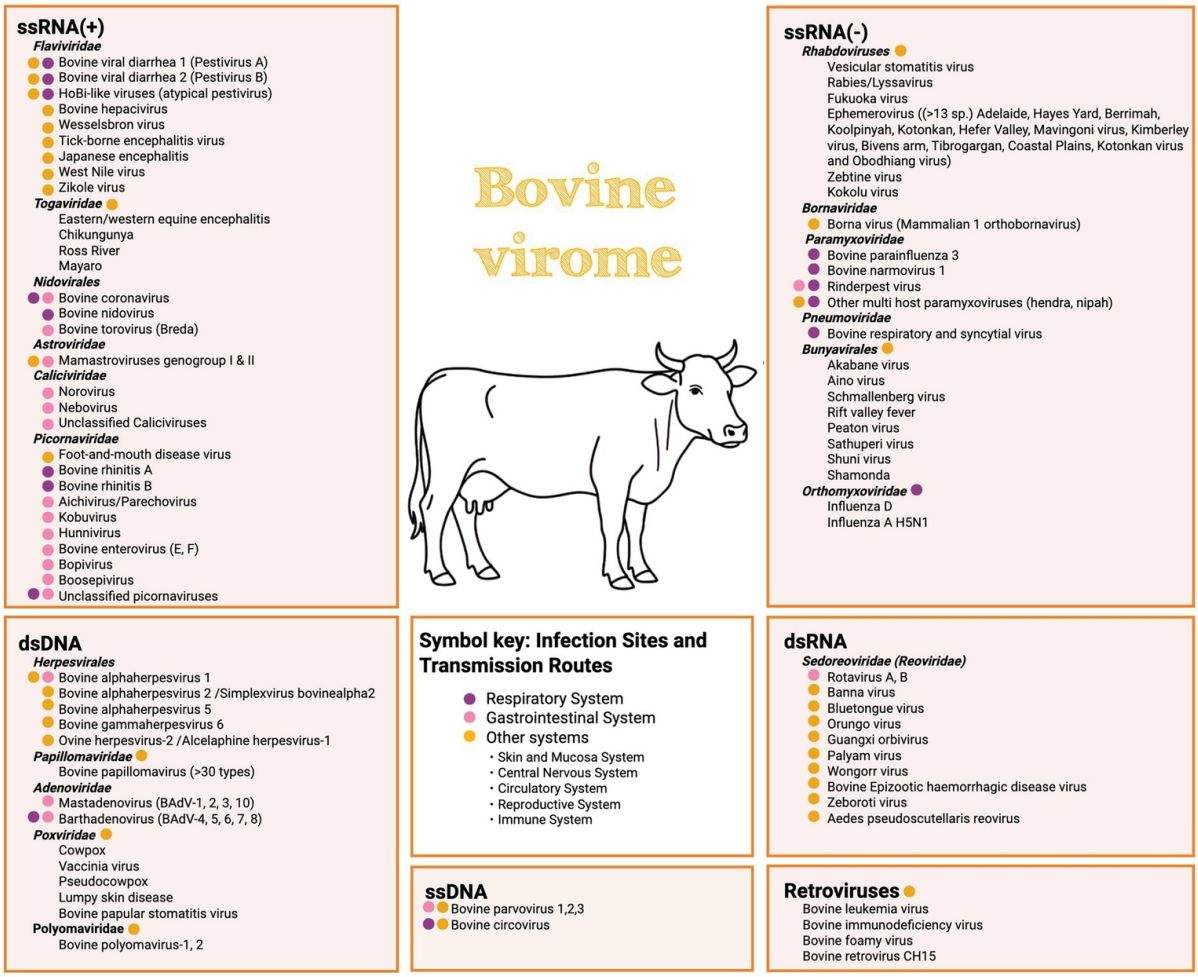


Figure 16.a:Summarises viruses detected in cattle, organised by genome type and taxonomic group. Coloured dots indicate the primary infection sites or transmission routes. This overview highlights both viruses known to cause infection in cattle and those more recently detected in cattle, whose pathogenic potential or effects remain to be explored.

Integrated Metatranscriptomic Profiling of Enteric Pathogens in Bovine Neonatal Diarrhea

Authors

B. Brito, Z.U. Abedien, J. Webster, S. Djordjevic, E.C. Holmes, I.J. Lean

Project Background

Diarrhea in newborn calves is a significant health challenges. It is a leading cause of death and illness in pre-weaned calves, and the costs to producers extend well beyond animal loss, encompassing veterinary treatment, labour, and the long-term impact on growth and milk production later in life. Despite decades of research, the precise combination of infectious agents responsible for diarrhea in calves under real farm conditions has not been investigated using untargeted detection methods. Standard diagnostic tests are limited in scope, typically checking for only a handful of known pathogens at a time, while missing many others that may be contributing to disease.

This project set out to address that gap by applying metatranscriptomics, a cutting-edge genomic sequencing technology, to characterise the full range of actively infectious agents present in calves across New South Wales dairy farms. Unlike conventional diagnostics, metatranscriptomics captures the genetic expression of bacteria and can simultaneously, detect viruses and parasites, providing an unprecedented window into what is actually happening inside a sick or healthy calf's gut. Importantly, it detects organisms that are actively replicating or expressing virulence traits, rather than simply present. This project represents the most comprehensive field-scale investigation of calf enteric disease in Australia to date, spanning 72 farms across all major dairy regions of NSW.

Methods

Between November 2022 and December 2023, the research team visited dairy farms across all major dairy-producing regions of New South Wales, including the Far North Coast, Central West, Riverina, Hunter, Mid Coast, South Coast, Far South Coast. Farms were selected using stratified random sampling to ensure the study was representative of the NSW dairy industry. In total, rectal swabs were collected from 918 pre-weaned calves (under seven weeks of age) from 72 farms, with each farm contributing samples from both healthy and diarrheic calves wherever possible. Calves were grouped into four age brackets to allow comparisons at different life stages.

Swabs were preserved immediately using a nucleic acid stabilisation medium and stored at -80°C at the Elizabeth Macarthur Agricultural Institute (**EMAI**). In the laboratory, 593 samples were selected for metatranscriptomic sequencing. The RNA was extracted and sequenced using the Illumina NovaSeq X platform, generating an average of over 60 million read pairs per sample. Bioinformatic analysis involved removal of host and background sequences, followed by detection and quantification of viral, parasitic, and bacterial virulence gene transcripts against curated reference databases. Multiple statistical frameworks were applied to identify associations between specific pathogens and diarrheal disease, including machine-learning models, mixed-effects regression, and differential abundance analysis, all accounting for farm-level differences and calf age.

Region	Healthy	Respiratory signs	Respiratory + diarrhea	Diarrhea	Unspecific signs	Total
Central West	51	5	1	8	5	70
Far South Coast	121	8	2	34	5	170
Far North Coast	51	4	0	18	3	76
Hunter	49	1	0	13	0	63
Mid Coast	165	13	5	29	7	219
Riverina	68	31	3	26	12	140
South Coast	74	14	1	42	8	139
Wagga & Inland	31	6	0	4	0	41
Total	610	82	12	174	30	918

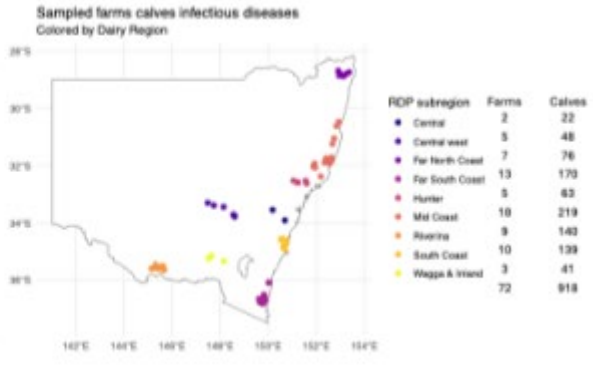


Figure 17-b Geographic distribution and health status of 918 pre-weaned calves sampled across major dairy regions in New South Wales, Australia. The table shows calf counts by clinical status and region, while the map visualizes farm locations coloured by dairy region.

Key Findings

Viruses: Astrovirus was the most abundant viral transcript detected across all samples, followed by Rotavirus A and Kobuvirus. Rotavirus A, long recognised as a primary cause of calf diarrhea, was confirmed as a significant early-life pathogen. Bovine Kobuvirus emerged as a particularly strong predictor of diarrhea in calves under three weeks of age, with disease risk linked to the amount of virus present rather than its mere detection. Strikingly, many viruses, including astroviruses and picornaviruses, were detected at high levels in clinically healthy calves, demonstrating that viral presence alone does not equal disease.

Parasites: Cryptosporidium was detected on 83% of farms and was the only parasite significantly associated with diarrhea. Giardia, although widespread, showed a negative association with diarrhea, potentially reflecting disruption of its intestinal niche during disease rather than a protective effect. Substantial shedding of all parasites was observed in healthy calves, underscoring that farm-wide exposure is the norm rather than the exception.

Bacteria: Rather than simply identifying which bacteria were present, this study measured the expression of bacterial virulence genes, the tools bacteria use to cause disease. Genes putatively associated with *Escherichia coli* adhesion, invasion, and iron acquisition were significantly enriched in diarrheic calves during the first weeks of life. Campylobacter-associated motility and colonisation genes were also strongly linked to diarrhea, particularly their abundance in positive samples. Farm of origin was the strongest determinant of bacterial virulence profiles, explaining over 45% of variation, highlighting how farm management, hygiene, and environment shape the bacterial disease landscape far more than any single pathogen.

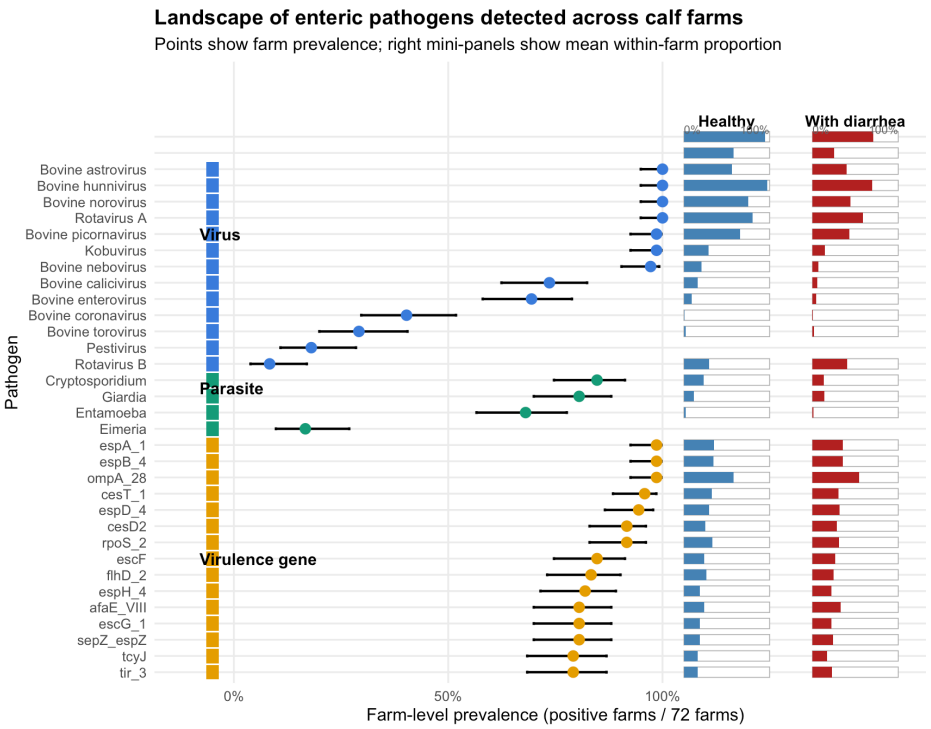


Figure 17-c: Landscape of enteric pathogens detected across calf farms. Points represent the farm level prevalence (n = 72 farms) with 95% Wilson confidence intervals. Pathogens are grouped by category (viruses, parasites, and bacterial virulence genes), indicated by the coloured sidebar. Right mini-panels show the mean within-farm proportion of positive calves in healthy animals (blue) and calves with diarrhea (red), averaged across farms.

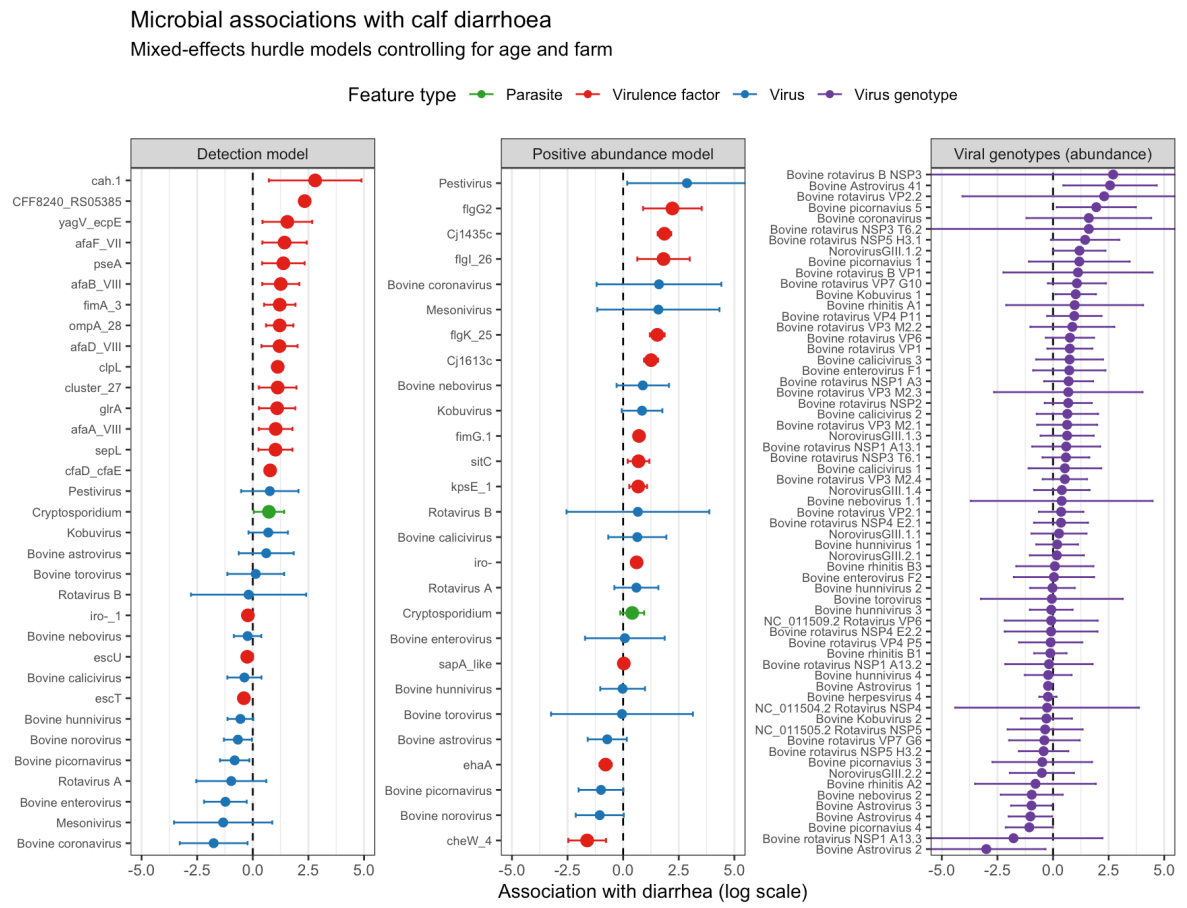


Figure 17-d: Microbial features (taxa) associated with calf diarrhoea identified using mixed-effects hurdle models. Detection models (left) show associations with the probability of presence (log-odds), while positive abundance models (middle) show associations with abundance among positive samples (log fold change). The right panel shows genotype-level viral associations based on the abundance component of the model. Points represent model coefficients and horizontal bars indicate 95% confidence intervals. Positive values indicate higher detection probability or abundance in diarrheic calves, whereas negative values indicate higher detection or abundance in healthy calves. Colours denote microbial feature type: parasites (green), virulence factors (red), viruses (blue), and viral genotypes (purple). All models include farm as a random effect and adjust for calf age.

Implications for the Dairy Industry

These findings have practical implications for diagnostics of enteric disease in dairy calves. The study demonstrates that calf diarrhea is rarely caused by a single pathogen, and the microbial variation is shaped mostly by the farm environment. Current diagnostic tests, which typically screen for only four or five pathogens, likely miss a significant proportion of the microbial complexity driving disease. This study identifies bovine Kobuvirus as a potential emerging relevant pathogen of particular concern for young calves that is not currently included in standard diagnostic panels or commercial vaccines in Australia.

A major practical takeaway is the understanding of the high viral or bacterial load carried by some healthy calves can frequently shed high levels of pathogens without becoming ill, meaning a positive test result alone provides limited guidance for treatment or intervention. Future diagnostic frameworks should incorporate pathogen load and age-specific context, and potentially host biomarkers to better guide producer and veterinary decision-making. The strong farm-level effect on bacterial virulence profiles also points to the central role of on-farm management practices, namely colostrum delivery, hygiene, stocking density, and antibiotic stewardship, in determining disease risk, reinforcing the value of farm-specific advisory services.

From a biosecurity and public health perspective, the identification of *Campylobacter* virulence activity in calves with diarrhea has further implications. Understanding the conditions under which virulence genes are expressed in calves can inform strategies to reduce contamination risk at the farm level. More broadly, this project has generated a rich, publicly available dataset, including 593 metatranscriptomes deposited in the National Center for Biotechnology Information (**NCBI**) Sequence Read Archive, that will serve as a foundational resource for future research into bovine enteric disease, antimicrobial resistance, and emerging infectious agents relevant to Australian dairy production.

Bovine Respiratory Disease in NSW Dairy Calves- Understanding Respiratory Viruses, Bacteria, and Calf Immune Responses Across NSW Dairy Farms

Authors

B. Brito, Z.U. Abedien, S. Djordjevic, E.C. Holmes, I.J. Lean

Project Background

Bovine respiratory disease (**BRD**), commonly one of the costliest and welfare-significant infectious disease affecting cattle worldwide. In pre-weaned calves, it is a significant cause of illness, death, and long-term production losses, contributing to reduced growth, impaired future milk yields, and increased antibiotic use. Despite its economic and animal welfare importance, several questions remain at a fundamental level: we know that multiple viruses and bacteria are involved, but we have had a limited ability to determine which specific pathogens matter most, how they interact with each other, and how the calf's own immune system responds to infection. In New South Wales (NSW), there was no systematic, state-wide data on which respiratory viruses and bacteria are circulating in dairy calf populations, or how prevalent they are. This information gap has hampered the design of effective prevention strategies and has left the industry relying on diagnostic tools and vaccines originally developed for overseas or feedlot systems, contexts that differ substantially from dairy farming. This project was designed to fill these gaps. Using a cutting-edge genomic technique called metatranscriptomics, which allows us to simultaneously detect all active viruses, bacteria, and the calf's own immune gene activity from a single nasal swab, we conducted the first comprehensive, state-wide survey of respiratory pathogens in NSW dairy calves. The study enrolled 72 farms and 908 pre-weaned calves across all major dairy regions of NSW, generating what is now the largest dataset of its kind for Australian dairy cattle, and one of the most comprehensive globally.

Methods

Between November 2022 and December 2023, the research team visited dairy farms across NSW and collected nasal swabs from calves under 60 days of age. Farms were selected randomly from a state registry to ensure results were representative of the NSW dairy industry. Each calf was assessed clinically and classified as healthy, showing non-specific signs, having respiratory disease, having diarrhoea, or a combination.

The nasal swabs were processed using a technique called metatranscriptomics, a form of genetic sequencing that reads all the active biological "messages" present in a sample at the time of collection. This captures a snapshot of everything that is actively happening in the calf's nasal cavity: which viruses are replicating, which bacteria are active, and which of the calf's own defence genes are switched on or off. This approach is far more comprehensive than conventional diagnostic tests such as polymerase chain reaction (PCR) panels, which only detect a pre-selected list of known targets and can miss unexpected or newly recognised pathogens.

Each swab generated approximately 75 million genetic sequence reads. These were processed through a computational pipeline that separated calf RNA from microbial RNA, then matched sequences to databases of known viruses, bacteria, and calf immune genes. Statistical analyses were used to determine which pathogens were most prevalent across NSW farms, which were most strongly associated with disease, and how the calf's immune system responded differently depending on which pathogen was present. The farm of origin was also factored into all analyses to account for the natural variation between properties.

Table 17-a: Number of nasal swabs metatranscriptomes successfully sequenced per health status, and age category.

Age strata	Healthy	Respiratory	Respiratory & diarrhea	Diarrhea	Unspecific	Total
<25	104	23	3	33	12	175
≥25	102	42	4	15	13	176
Unknown	31	10	4	6	4	55
Total	237	75	11	54	29	406

Findings

The study generated several important discoveries. First, and most strikingly, the farm a calf was born on was the single biggest factor shaping which viruses and bacteria were present in its nasal secretion, and how its immune system was responding, far outweighing the effect of whether the calf was sick or healthy. This tells us that respiratory disease in dairy calves is fundamentally a farm-environment problem, driven by the microbial world each herd harbours.

Second, the pathogens most commonly found in NSW dairy calves were different to the ones currently targeted by most commercial vaccines and diagnostic tests. Bovine rhinitis viruses A and B (never routinely tested in Australia), bovine torovirus, and bovine adenovirus 3 were the most prevalent respiratory viruses detected, found on 35–52% of farms. In contrast, viruses such as bovine respiratory syncytial virus and infectious bovine rhinotracheitis virus were detected rarely or not at all in this age group.

Third, the bacterial genera most consistently associated with disease were Moraxella, Mannheimia, and Pasteurella, supporting their role as key drivers of clinical pneumonia. Critically, when these bacteria were abundant, the calf's immune system showed signs of tissue damage and inflammation.

Finally, the calf's immune response followed a clear pattern: early in infection, antiviral defence genes were activated; in calves with established disease, the response shifted toward damaging inflammation. This transition appears to be a key feature of disease progression.

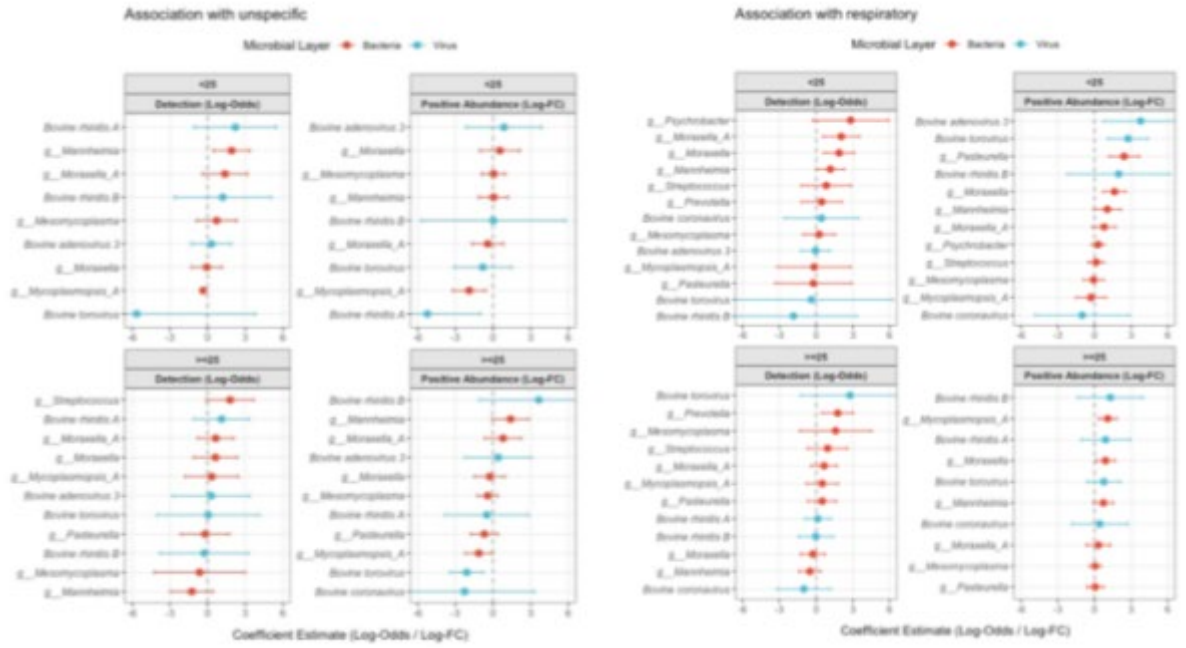


Figure 17-e: Microbial associations with animals showing respiratory or unspecific signs across age groups. Associations between microbial features (viruses and bacteria) and clinical status were assessed using mixed-effects hurdle models, separating detection (presence/absence; log-odds) and positive abundance (log fold change). Results are shown for two comparisons: unspecific vs. healthy (left) and respiratory vs. healthy (right). Within each comparison, analyses were stratified by age (<25 days and ≥25 days), and results are presented separately for detection and positive abundance components. Points represent model estimates, and horizontal lines indicate 95% confidence intervals. Positive estimates indicate higher prevalence or abundance in diseased animals relative to healthy ones, while negative estimates indicate association with healthy status. Models included clinical status as a fixed effect and farm as a random intercept to account for clustering. Only features meeting predefined quality control criteria (minimum sample size and sufficient positive observations per group) were included in each model component; therefore, not all features are present in all panels. Colours indicate microbial layer (viruses in blue; bacteria in red).

Implications

These findings have direct and significant implications for how BRD is managed in dairy calves. The dominance of the farm environment as a driver of disease risk means that the most effective strategies for reducing calf respiratory disease lie in improving management at the herd level. This supports a shift from reactive to preventive farm health planning, and highlights that consistent implementation of existing best practices could substantially reduce disease burden across the NSW industry. The identification of rhinitis viruses and bovine torovirus as the most prevalent respiratory viruses in Australian dairy calves is a pivotal finding for the vaccine and diagnostics industry. While with this investigation we identified the microbial populations present in the upper respiratory tract of the calves and its association with disease, the next step is to determine their association with lower respiratory tract infection and pneumonia. Despite this limitation, this study helps guide the development of relevant diagnostic panels for respiratory disease investigations. Internationally, these findings add to a growing body of evidence that BRD vaccine portfolios need updating to reflect the actual pathogens circulating in dairy calf populations. The host immune data provide a roadmap for future therapeutic development. By identifying the specific immune pathways that are protective

versus damaging, researchers and industry can begin to explore targeted interventions, such as immune modulators or mucosal vaccines, that strengthen early defence without triggering the excessive inflammation that causes lung damage. Together, these findings provide a science-based foundation for rethinking calf respiratory health strategy at the farm, national, and global level.

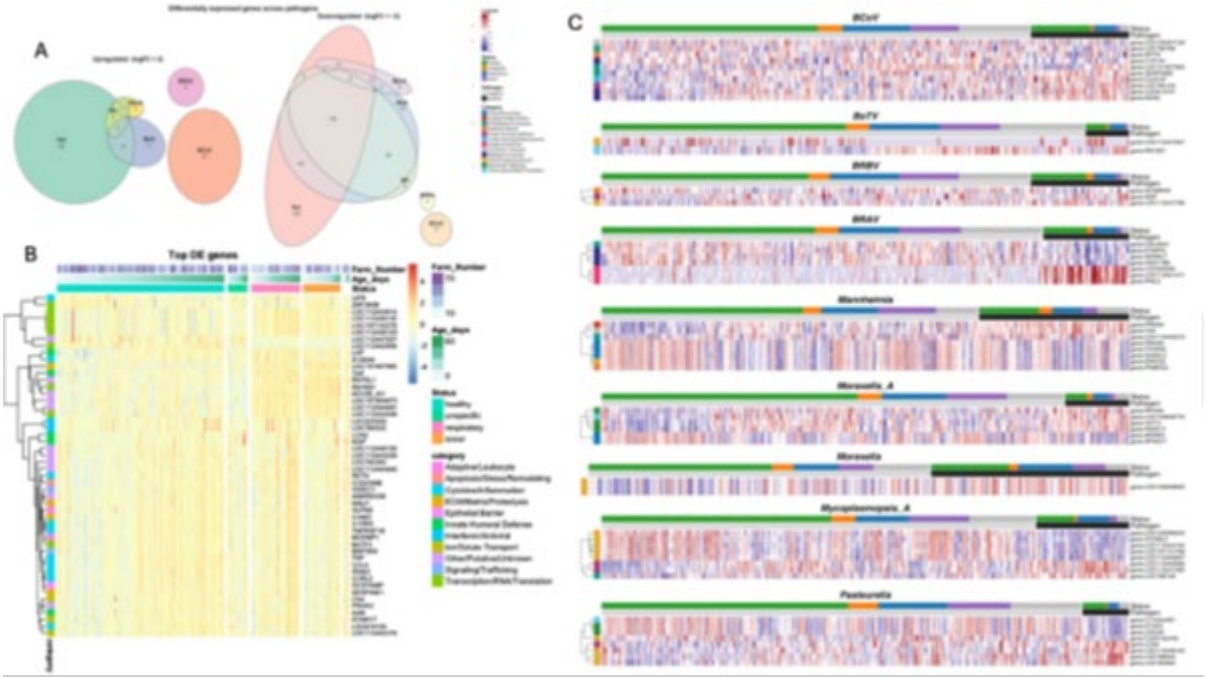


Figure 17-f: A: Euler diagram of differentially expressed genes in animals with unspecific symptoms or respiratory symptoms compared healthy animals based on pathogen positivity. B: Global host transcriptional response in bovine nasal swab metatranscriptomes stratified by clinical status. Heatmap of z-score-scaled expression values for the top differentially expressed host genes across clinical status.

The molecular epidemiology of rotaviruses in NSW dairy calves, Australia

Authors

Y. Kida, Z.U. Abedien, C.M. Donato, S. Djordjevic, M.J. Frost, A. Read, P. Hick, E.C. Holmes, I.J. Lean, B. Brito

Project overview

Neonatal calf diarrhea is a leading cause of mortality in young calves worldwide, with bovine rotavirus A (BoRVA) recognized as a major etiological agent. Genomic variation among circulating strains can influence antigenicity, virulence, transmissibility, and potentially vaccine effectiveness. To comprehensively characterize BoRVA diversity and associated host responses in NSW, Australia, we performed a large-scale metatranscriptomic study of pre-weaned calves, analyzing 593 rectal and 408 nasal transcriptomes.

Rotavirus genomes were frequently detected in both diarrheic and clinically healthy calves, with substantial viral loads observed in asymptomatic animals, indicating widespread subclinical shedding. Genotyping revealed that dominant bovine genotypes: G6 or G10 in combination with P[5] or P[11], were consistently distributed across NSW. Rare genotypes, including G24 and G8, as well rotavirus B, were also identified.

Genotype-specific age patterns were evident. Rotavirus G10P[11] genotypes were detected across a broader age range and maintained high abundance over an extended window compared to G6P[5], which exhibited a narrower peak in early life.

Host transcriptome analysis revealed that high-burden infections were associated with coordinated downregulation of epithelial barrier, transport, and metabolic pathways, alongside upregulation of epithelial stress and remodeling signatures, consistent with functional mucosal disruption rather than isolated inflammatory amplification.

Together, these findings highlight the local genetic diversity, the extensive subclinical BoRVA circulation, genotype-specific age dynamics, and distinct host transcriptional responses, underscoring the importance of genomic surveillance to inform vaccine and control strategies.

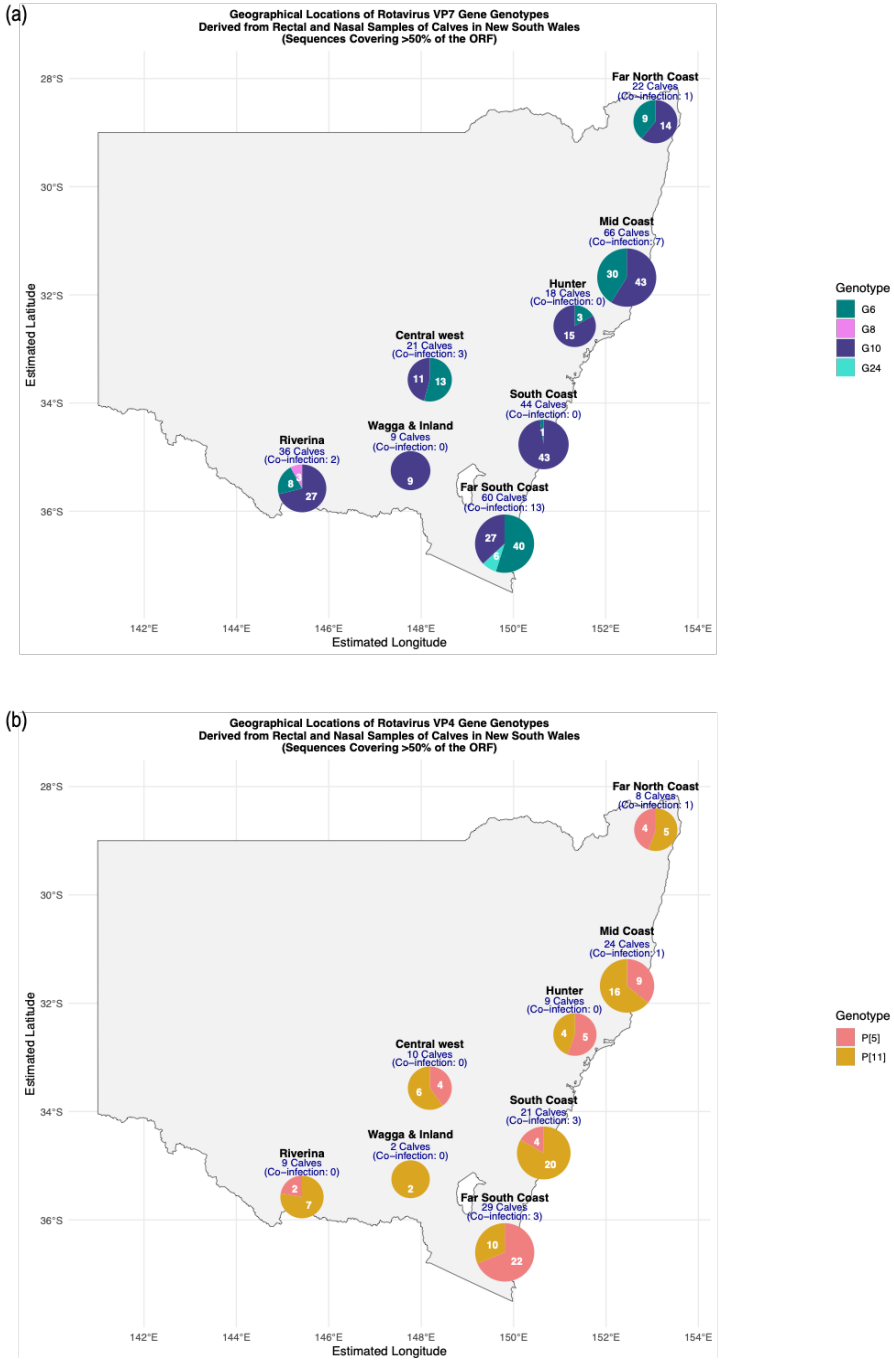


*Figure 17-g: Geographical distribution of rotavirus genotypes in calves across NSW. (a) Geographical locations of each genotype of viral protein (**VP**)7 of rotavirus A (**RVA**). Pie charts show the proportion of VP7 genotypes detected in all rectal and nasal samples combined for each area. The size of each pie reflects the total number of calves in which rotavirus was detected. Numbers inside pies indicate the number of calves carrying each genotype. Areas with co-infected calves are highlighted. Colours represent genotypes: G6 (teal), G8 (violet), G10 (dark slate blue), and G24 (turquoise). (b) Geographical locations of each genotype of VP4 of the detected RVA. Pie charts represent the proportion of VP4 genotypes detected in rectal (R) or nasal (N) samples for each geographic area. The size of each pie chart is proportional to the number of unique calves sampled. Numbers inside each pie indicate the number of calves*

carrying each genotype. Areas with co-infected calves are indicated separately. Colours correspond to genotypes as follows: P[5] (light coral), and P[11] (golden).

Bovine Respiratory Disease Study – Mesonivirus Findings

Authors

B. Brito, E. Holmes, I.J. Lean.

Project background

Respiratory disease is a leading cause of illness in young dairy calves, yet many cases have no identified cause even after extensive testing. This project used advanced genetic sequencing to characterize all microorganisms present in nasal samples from calves across New South Wales farms, with the goal of uncovering previously undetected pathogens.

Methods

Nasal swabs were collected from 406 pre-weaned calves, both healthy and showing signs of respiratory illness, across 72 dairy farms. Samples were analyzed using broad-spectrum RNA sequencing capable of detecting any actively replicating microorganism, without needing to know in advance what to look for.

Findings

The objective of this study was to investigate an unexpected finding consisting of the detection of Alphamesonivirus-1 in calves from 12 farms. This virus belongs to the family Mesoniviridae within the order Nidovirales and has previously been considered insect-specific, with reported associations limited primarily to mosquitoes. Notably, the five calves with the highest viral abundances were all affected by respiratory disease.

Importantly, no mosquito-derived genetic material was detected in these samples, reducing the likelihood that the viral sequences originated from an accidentally sequenced mosquito. Instead, these findings support the possibility that Alphamesonivirus-1 was present within the upper respiratory tract of the calves themselves. While the biological significance and host range of this virus remain unclear, its repeated detection across multiple farms and association with respiratory disease warrant further investigation to determine whether it may represent a previously unrecognised bovine-associated virus or an opportunistic respiratory agent.

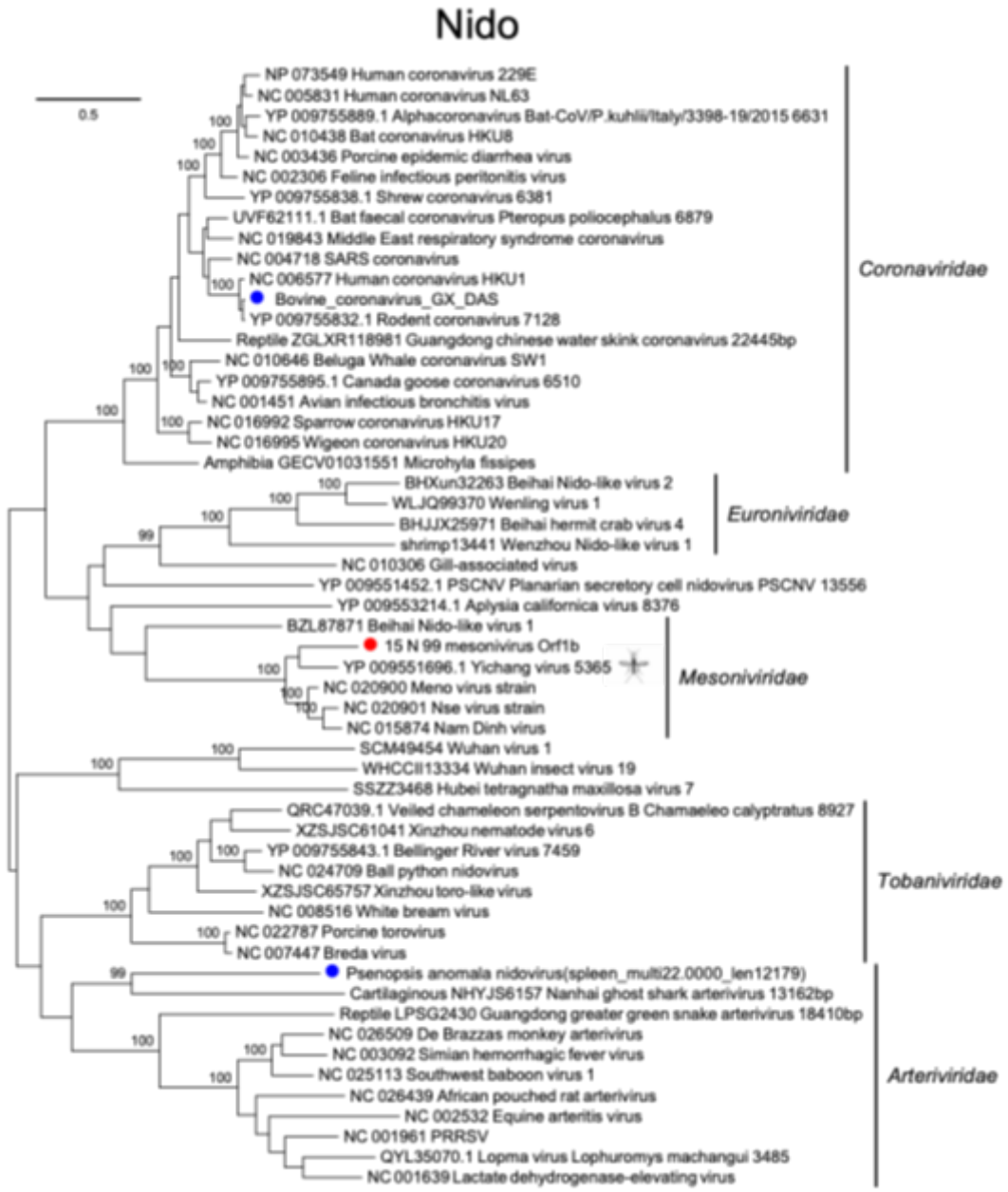


Figure 17-h: Phylogenetic analysis of the amino acid sequence of ORF1b from Nidovirales representative species. The virus found in nasal swabs in this study is highlighted in red (15 N 99 mesonivirus Orf1b).

Implications

This is one the first reports of this virus in cattle and only the second time it has been detected in any land animal (previous detection was from a respiratory case in a horse). While it remains unclear whether this virus causes disease, the finding suggests it may have a broader host range than previously recognized and warrants further investigation as a potential contributor to unexplained respiratory illness in cattle.

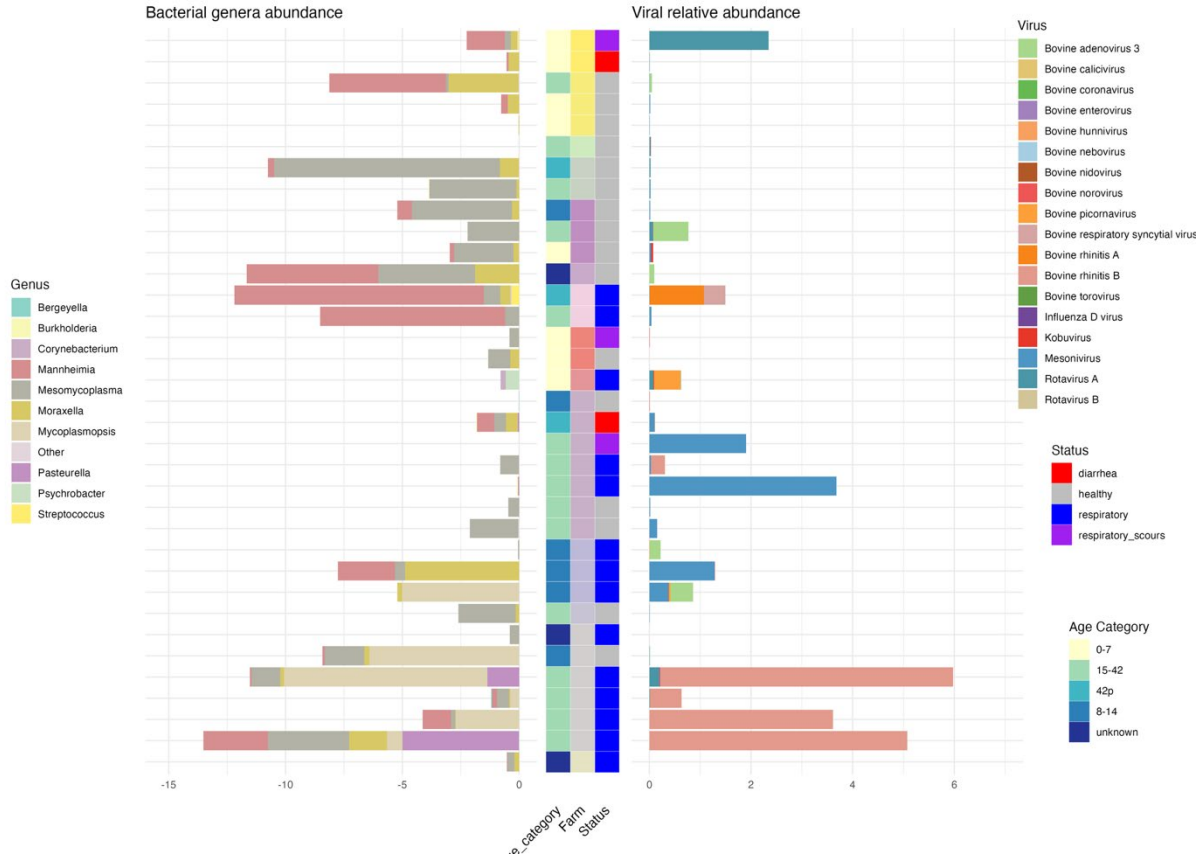


Figure 17-i: A diverse range of bacteria and viruses was present in nasal samples where mesonivirus was detected. Among the Mesonivirus-positive animals, 14 animals with respiratory disease (as indicated by blue annotation tiles) showed low Mesonivirus abundance and instead had high levels of Bovine rhinitis A and B viruses, along with Mannheimia, Mycoplasma, and Pasteurella, all common respiratory bacteria.

Whole-genome surveillance of Escherichia coli from healthy and scouring dairy calves across New South Wales: a matched case-control study

Authors

A.M. Stanczak, V.M. Jarocki, E.R. Wyrsch, I.J. Lean, B. Brito, S. Djordjevic

Background

Neonatal calf diarrhoea ("scours") is the leading cause of morbidity and mortality in pre-weaned dairy calves worldwide, imposing substantial economic and welfare costs on the dairy industry and driving early-life antimicrobial use. E. coli, particularly enterotoxigenic (ETEC) and other diarrhoeagenic pathotypes, is a key contributor, yet the population structure, virulence repertoire and resistance gene burden of E. coli circulating in healthy versus scouring calves on Australian dairy farms remain poorly characterised, limiting evidence-based diagnostics, intervention design and antimicrobial stewardship.

Methods

A matched case-control study was conducted across 40 commercial dairy farms spanning eight major dairy regions of New South Wales. Faecal samples were collected from 126 calves (63 scour cases, 63 clinically healthy controls) stratified by age (0–7, 8–14, 15–42 and >42 days). Multiple colonies per animal were recovered on chromogenic (ECC) and ESBL-selective media to capture within-host diversity and ESBL-selection-context strains, then subjected to whole-genome sequencing on the Illumina short-read platform. Following quality control, 451 high-quality E. coli genomes (352 ECC, 99 ESBL-selection) were retained for downstream genomic analysis.

Results

In silico multilocus sequence typing resolved 91 distinct STs across the case-control cohort, with the assemblage dominated by globally distributed lineages, ST10 (n=74), ST58 (n=49), ST88 (n=34) and ST362 (n=22), alongside 20 isolates with novel STs. Twenty-eight STs were shared between healthy and scouring calves, while 37 were recovered exclusively from healthy controls and 26 exclusively from scour cases; most exclusive STs were singletons from single farms, but several were supported by multiple animals and farms. ST10 and ST69 were over-represented among scouring calves, and ST602, ST56, ST711 and ST75 were recovered exclusively from scour cases across multiple animals and farms, marking them as priority candidates for pathotype characterisation. Conversely, ST3268, ST3018, ST38 and ST1613 were recovered exclusively from healthy controls, alongside lineages such as ST117, ST1434 and ST3268 that were strongly skewed to healthy carriage. Comprehensive downstream analyses will include in silico pathotyping and virulence-gene screening (Enterotoxigenic E. coli/Enteropathogenic E. coli/Shiga toxin-producing E. coli determinants, fimbrial adhesins, toxins, iron-acquisition systems), antimicrobial resistance gene and plasmid replicon profiling, mobile genetic element (insertion sequence, integron, transposon, prophage) characterisation, and pangenome analysis to identify accessory gene content and lineage-specific signatures associated with disease status.

Implications

This dataset provides one of the most comprehensive genomic baselines of E. coli from Australian dairy calves to date and establishes a framework for disentangling commensal carriage from disease-associated lineages. Identification of ST- and gene-level markers linked to scours will inform targeted on-farm diagnostics, evidence-based antimicrobial use, and One Health surveillance of high-risk E. coli lineages with zoonotic and food-chain relevance.

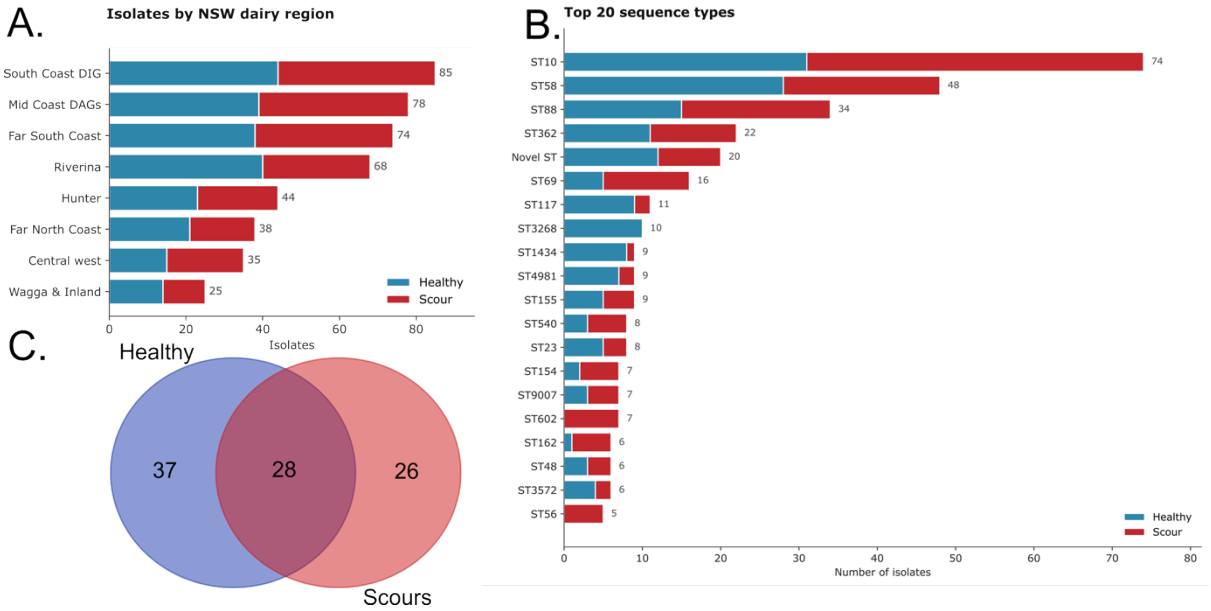


Figure 17-j: E. coli diversity in healthy and scouring dairy calves A) cohort overview B) Stacked bars showing healthy/scour split for each of the top 20 most abundant lineages C) Venn diagram illustrating ST overlap between E. coli derived from healthy and scouring calves.

Detection of blaCTX-M genes in Escherichia coli in dairy calves in Australia.

Authors

A.M. Stanczak, V.M. Jarocki, E.R. Wyrsch, I.J. Lean, B. Brito, S. Djordjevic

Background

Escherichia coli is a leading cause of antibiotic-resistant urinary tract (UTI) and blood stream (BSI) infections, ventilator-associated pneumonia, meningitis and wound infections. E. coli is also a major pathogen across intensive animal production systems causing gastrointestinal and extraintestinal disease. From a genomic perspective, E. coli has been intensively studied as a human pathogen but remains understudied in livestock, fresh produce, retail meats, waste streams, and wildlife, hindering One Health considerations of antimicrobial resistance (AMR) and pathogen evolution.

Methods

A comprehensive E. coli sampling regime devised to examine phylogeny, AMR and virulence gene carriage across different dairy production systems generated 1300 rectal samples from 67 farms across NSW. E. coli was isolated from calves <7 weeks old in 67 farms using both non-selective (Chrome-Agar) and selective (Chrome-agar with an extended spectrum β-lactam (ESBL) antibiotic) approaches.

Results

Phenotypic resistance to ESBLs was observed on 59 farms (88%) with ESBL non-susceptible E. coli isolates collected from 52 farms. After quality control analysis of whole genome sequencing data, 117 isolates representing 49 farms comprised the final collection. Among these, 23 sequence types (ST) were identified with ST10 (n=22), ST88 (n=15), ST9007 (n=10), ST155 (n=10) and ST117 (n=9) dominant. All E. coli phylogroups except B2 were represented with A (n=48), B1 (n=31) and C (n=15) common. Phenotypically resistant isolates had blaCTX-M-14 (n=59), blaCTX-M-15 (n=45), blaCTX-M-27 (n=1), blaCTX-M-1 (n=1), blaDHA-1 (n=1), and blaCMY-2 (n=4) present; we could account for ESBL resistance in six isolates. Genes encoding resistance to aminoglycosides, sulphonamides, tetracyclines, β-lactams, trimethoprim, and macrolides were also prevalent on a herd basis and most isolates (n=75; 64%) carried the class 1 integrase gene intI1. Virulence gene carriage analysis of the genome sequences indicated that ColV (n=36; 31%) plasmids and the Yersinia High Pathogenicity Island (n=42; 36%), both high priority ExPEC virulence factors found in E. coli that cause UTI and BSI, were frequently detected in these E. coli genomes.

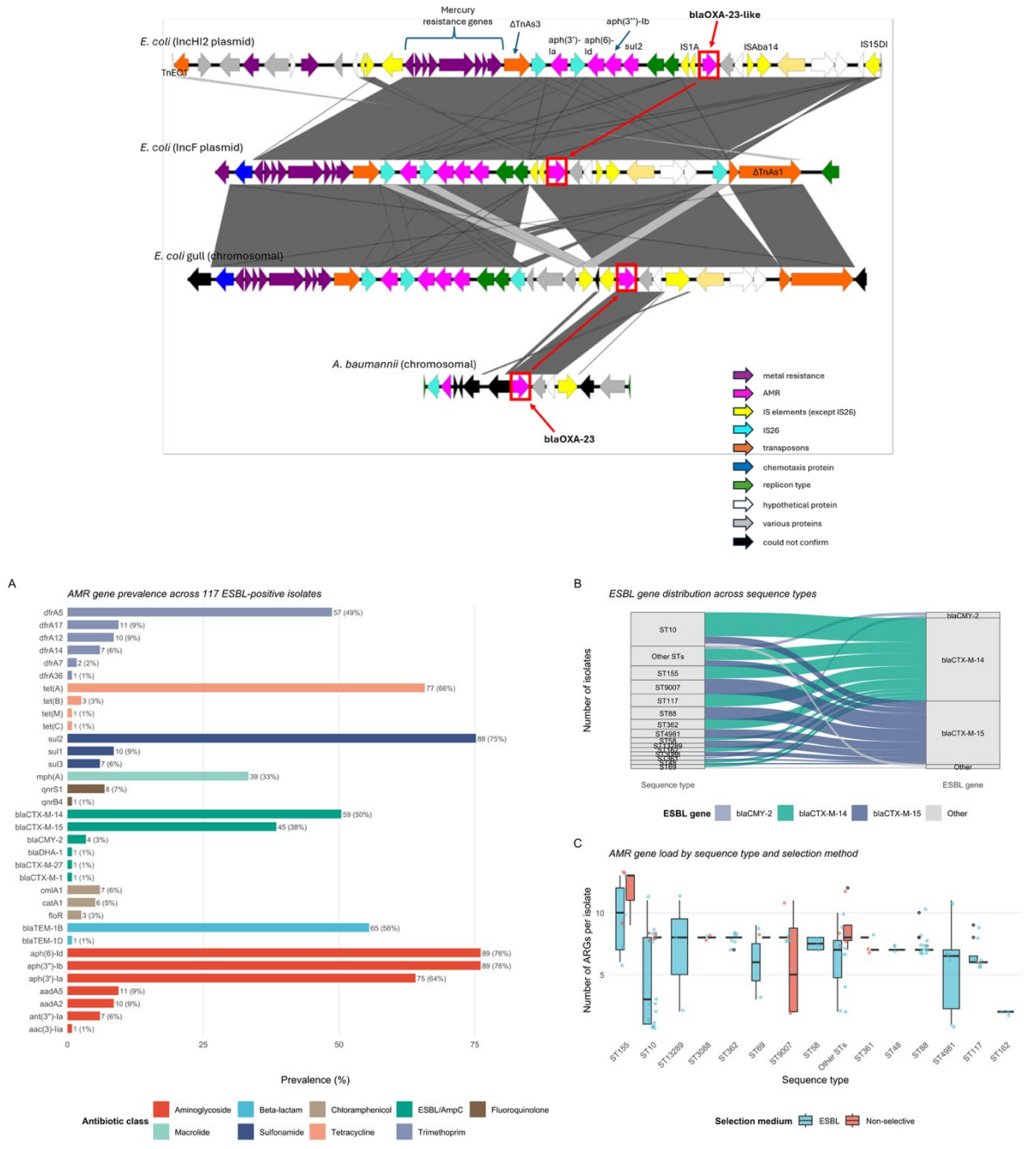


Figure 17-k: Top panel: Genetic context of blaOXA-23 gene in an E. coli plasmid and the comparison with the genetic context of a related gene found in gull. Lower panels: A. The resistome found in rectal swabs from NSW healthy calves. B. ESBL gene distribution across different E. coli ST.

Conclusions/implications:

This study represents the most comprehensive whole-genome sequencing analysis of third-generation cephalosporin–resistant E. coli subpopulations in calves from Australian dairy operations. Although a high prevalence of ESBL-producing E. coli was observed on a herd basis in this early-life cohort, previous studies indicate that such colonization is typically transient in young calves.

Our findings provide important genomic context for interpreting early-life AMR prevalence. Collectively, these data establish a critical genomic baseline for ongoing AMR surveillance and support sustainable antimicrobial stewardship within the dairy sector.

Subproject P2g Heifers Early Calving

Background

We identified in P2a marked declines in health and reproductive performance with increased parity. Further, we found marked differences in metabolite and lipids with increased parity and changes in BCS and BW. In this series of studies we are investigating whether reducing the age at calving can reduce the risks of removal and reproductive failure while maintaining milk production. We also identified that there was considerable potential to reduce the age at calving in some pasture-based herds as these had a much older age for heifers at calving.

Meta-analysis of the effects of age at first calving on production outcomes, calving difficulty, and reproduction in dairy heifers

doi.org/10.3168/jds.2025-27004

Authors

A.K.G. Lean, A.J. Gunn, J.C. Quinn, I.J. Lean, K. Breinhild, H.M. Golder

Background

This study aims to determine the effects of feeding, management, and reproductive strategies used to reduce age at first calving (**AFC**) on production and reproductive performance through meta-analytic methods.

Methods

A literature search using three search engines was conducted to identify prospective studies that evaluated AFC for data extraction. Inclusion criteria included specific dairy breeds, a treatment group with an AFC below 26 mo of age, and prospective enrolment of heifers. There were few prospective studies suitable for meta-analysis, with 16 studies containing 35 appropriate experiments. There was substantial heterogeneity likely due to differences in study design and interventions used to induce an AFC difference. Outcomes were assessed using classical multilevel random effects meta-analytic models with standard mean difference, effect sizes, or risk ratios being evaluated where appropriate. Meta-regression was performed using the difference in age at first calving.

Findings

There was a loss of production for first lactation milk (-2.06 L/d of earlier calving), fat (-0.12 kg/d of earlier calving), and protein (-0.08 of kg/d earlier calving) yield for heifers calving at a younger age than their peers. There were no differences in second and third lactation milk yield, calving difficulty, heifer reproductive performance, survival to calving, and survival to the end of first lactation.

Implications

While the production in first lactation is decreased, this may be offset by fewer days to first calving without compromising the heifers' ability to calve, become pregnant for the first time, or survive to the end of first lactation. Given the lack of prospective studies, more randomized controlled experiments over multiple lactations, including health, production, and reproductive data, are needed to evaluate this topic further.

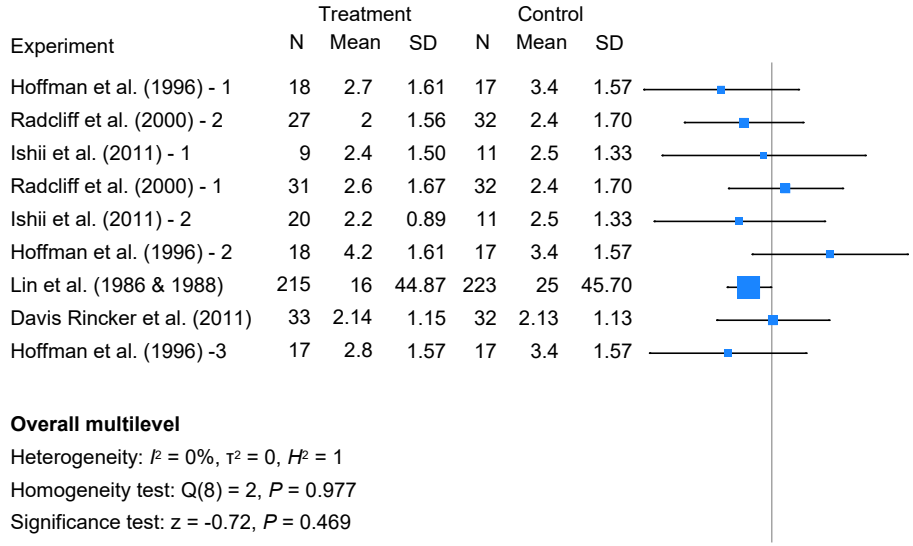


Figure 17.a: A forest plot representing the effect size of calving difficulty. The solid vertical line represents no effect or zero. Points to the left of the line indicate a reduction in calving difficulty, while points to the right indicate increased calving difficulty. Each square on the graph represents the mean effect size for comparison at the univariate level, with the lines extending from the square being the 95% CI of that experiment. The size of the square indicates the weighting of the experiment at a univariate level, the value of which is recorded in the right-hand column. The diamond at the base represents the results from the overall multilevel model, which was non-significant (P = 0.469). The forest plot is ordered by ascending age at first calving (AFC) of the treatment group from the top. There is little heterogeneity between the experiments, as can be seen by the low values of I2 and τ2 and high H2

Age at first breeding in Holstein dairy cattle: Effects on age at first calving, production, dystocia, health and reproduction in primiparous heifers

In Review

Authors

A.K.G. Lean, H.M. Golder, J.C. Quinn, I.J. Lean, A.J. Gunn

Background

Although lowering the age at first calving (**AFC**) for dairy heifers has been investigated over the last 60 years, recent prospective studies are lacking (Lean et al., 2026). Age at first breeding (**AFB**), a driver of AFC, has been less well investigated as a mechanism for production gain. The objective of this study was to investigate AFB and AFC in well-grown modern Holstein dairy heifers and their effects on production, reproduction, and health, with heifers bred from the age of > 10 mo.

Methods

Age at first breeding was investigated by breeding heifers from 5 commercial farms at > 330 kg at younger or older than 13 months of age. Heifers were weighed, and body condition scored at recruitment. Where the farmer elected to breed heifers < 330kg at the same sites, these were included in the study population based on their AFB. A total of 489 heifers were recruited, with heifers entering the normal reproductive programs of the farms; of these, 477 were bred. A second weight was recorded at the transition or periparturient period. Heifers were monitored for reproductive performance, abortions, and survival to calving (< 13 months, N = 249, or > 13 months, N = 228) and AFC group (< 22 months, N = 131, 22-24 months, N = 180 or > 24 months, N = 128). In primiparous lactation, all outcomes were investigated for the effects of AFB, including 305 d milk production per lactating heifer and enrolled heifer, milk fat and protein percentages, days of medical treatment, time to first mating and pregnancy, dystocia, stillbirths, and survival in primiparous lactation and over the whole trial. All outcomes were investigated using multilevel, multivariable models either in the nulliparous or primiparous year with farm location as a random effect. Mixed linear, logistic regression, Poisson regression, and Weibull survival models were used.

Findings

Heifers with an AFB > 13 months had a higher BW at enrolment and increased odds of abortion in their nulliparous year compared with the heifers with an AFB < 13 months. In primiparous lactation, heifers with an AFB > 13 months an increased hazard of exit from the herd, a decreased risk for medical treatment and produced 814 L less milk over 305 d per enrolled heifer when compared with the heifers with an AFB < 13 months. Each kg of extra BW at enrollment was associated with 13.0 L of milk over a 305 d lactation. There was no difference in milk production per lactating heifer between the AFB groups.

When the data were analyzed for AFC groups, there was no difference between groups for milk production per lactating or enrolled heifer. There was a decrease in fat percentage in older AFC heifers when compared to the < 22 mo AFC group. The 22-24 mo AFC group had a decrease of 0.18 % milk fat, while the > 24 mo AFC group had a decrease of 0.28 % fat in the milk. There was a decreased risk of medical treatment for heifers having a female calf and an AFC of > 24 mo when compared to those with an AFC of < 22 mo. Odds of stillbirth were decreased with an AFC of > 24 mo compared to < 22 mo.

Implications

Lowering the recommended AFB for well-grown (> 330kg) heifers to 10-13 months of age may therefore be of benefit as a management strategy, though risks of increased medical treatment and stillbirth must be considered.

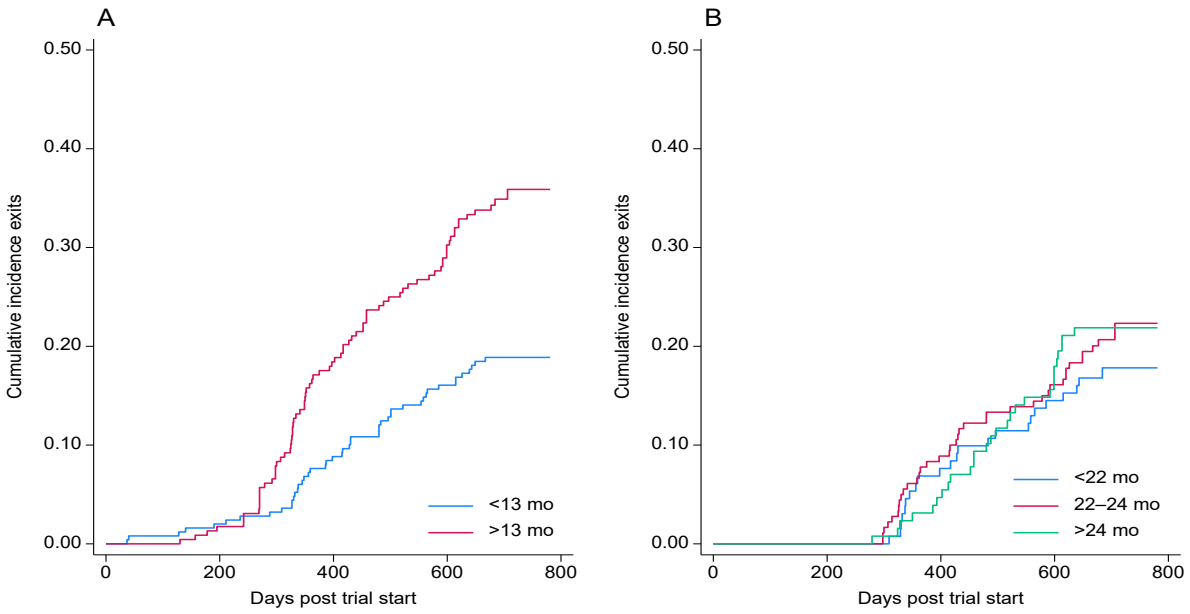


Figure 17.b: Kaplan Meier failure graphs illustrating the cumulative incidence of exits by age at first breeding (Figure A) and age at first calving (Figure B). As heifers exit the proportion of heifers exiting increases illustrating exits over time. Heifers can only have an age at first calving after reaching calving, resulting in fewer heifers and a later start time in Figure B. In Figure A the groups are age at first breeding < 13 mo and > 13 mo. In Figure B the groups are age at first calving < 22 mo, 22-24 mo, and > 24 mo.

Age at first breeding in Holstein dairy cattle: Associations of hormone, biochemical and protein measures - *In Review*

Authors

A.K.G. Lean, H.M. Golder, J.C. Quinn, I.J. Lean, A.J. Gunn

Background

Age at first breeding (**AFB**) has traditionally been targeted to 15 mo of age to allow for calving before the spring flush in seasonal calving systems. Despite this being an industry norm for many decades, few studies have evaluated metabolism and AFB and insights are needed to help optimize management strategies. The objective of this study is to investigate heifer reproductive and growth performance by assessing the BW, BCS, presence of a corpus luteum (**CL**), time to pregnancy and survival to first lactation in relation to hormonal, biochemical, and protein metabolism as well as AFB. Understanding these interactions may enable the selection of heifers for mating at earlier ages based on metabolite status and BW, markers that could be readily tracked for performance traits in dairy systems.

Methods

Holstein dairy heifers (N = 411) sourced from 4 commercial farms were aged from 11 to 18 mo of age at first breeding. Heifers were sourced from 4 commercial farms that fed total mixed rations. Most utilized pasture feeding for the heifers at times. Heifers were weighed, BCS scored and enrolled for immediate breeding if > 330 kg in BW and assigned to mating groups of < 13 mo or > 13 mo at first breeding. Of 411 heifers enrolled into the trial, a subset of 149 heifers were systematically blood sampled at the time of weighing. A metabolic profile was tested consisting of total protein, albumin, globulin, urea, creatinine, glucose, Ca, Mg, P, Cu, Zn, vitamin B12, glutathione peroxidase, leptin, insulin growth factor (**IGF**)-1, IGF binding protein 6 (**IGFBP-6**), insulin, undercarboxylated osteocalcin, and osteocalcin. A further subset of 76 heifers from this group were tested for the presence of a corpus luteum. Heifers were monitored for time to pregnancy, age at first calving and survival. The effects of AFB and the above metabolites were investigated for associations with the BW, BCS, presence of a corpus luteum, reproductive performance and survival at first calving. Analysis applied multilevel mixed linear, logistic regression and Weibull survival models with farm as a random effect.

Findings

No difference was found between the AFB groups for the BCS, presence of a corpus luteum, time to pregnancy or survival. Older heifers had a heavier BW. Albumin and Mg were positively associated with heifer BW, whereas vitamin B12 and log insulin were negatively associated. There were positive associations for urea, BW, log IGF-1 and P with BCS. Non-esterified fatty acid, Mg and vitamin B12 were associated negatively with Cu, and vitamin B12 were positively associated with the presence of a corpus luteum. Globulin and glutathione peroxidase were negatively associated with time to pregnancy. There were no metabolic associations with survival.

Implications

The negative association of NEFA with the presence of a CL highlights the importance of effective nutrition for growth in heifers so they are ready for mating. Blood Cu may have importance in the onset of puberty and needs further investigation. These results give insights into the metabolic influences on Holstein dairy heifers at first breeding.

Table 17.a: Univariable results from analysis of time to pregnancy, survival, presence of the corpus luteum, BW, and BCS with a random effect of farm. Body weight and blood test results have been centered by farm for the analysis.

Variable	**Time to pregnancy**			**Survival at calving**			**Corpus luteum**		
Statistical model	**Weibull Survival**			**Weibull Survival**			**Logistic regression**		
	Hazard ratio	**SE**	***P*-value**	**Hazard ratio**	**SE**	***P*-value**	**Odds ratio**	**SE**	***P*-value**
Age at first breeding (d)	1.004	1.004	0.038	1.003	0.006	0.631	1.002	0.008	0.820
Breeding group (< 13 mo[ref] or > 13 mo of age)	1.211	0.244	0.342	2.733	1.784	0.123	0.623	0.05	0.982
BW at blood test (kg)	1.003	0.002	0.118	0.995	0.009	0.598	0.993	0.008	0.374
Total protein (g/L)	0.986	0.012	0.259	0.997	0.044	0.950	1.011	0.042	0.801
Albumin (g/L)	0.990	0.038	0.801	0.838	0.133	0.185	0.936	0.132	0.614
Globulin (g/L)	0.981	0.015	0.198	1.024	0.053	0.652	1.025	0.050	0.623
Urea (mmol/L)	0.971	0.111	0.794	0.717	0.323	0.303	1.300	0.317	0.408
Glucose (mmol/L)	1.032	0.153	0.839	1.018	0.569	0.975	0.847	0.590	0.778
Creatinine (μmol/L)	0.996	0.008	0.558	0.970	0.024	0.203	0.969	0.026	0.218
NEFA (mmol/L)	2.738	0.465	0.030	0.036	2.671	0.213	0.001	2.666	0.012
Leptin (ng/ml)	1.020	0.013	0.143	1.038	0.049	0.439	0.985	0.072	0.827
Undercarboxylated Osteocalcin (ng/ml)	1.242	0.114	0.056	1.139	0.384	0.734	0.943	0.310	0.850
Osteocalcin (ng/ml)	1.007	0.004	0.051	1.003	0.016	0.826	1.003	0.019	0.882
Ca (mmol/L)	0.782	0.659	0.709	2.032	1.974	0.719	0.429	2.003	0.672
Mg (mmol/L)	17.712	1.084	0.008	0.253	3.524	0.696	0.001	3.544	0.043
Ca to Mg ratio	0.459	0.328	0.017	1.724	1.003	0.587	4.749	1.016	0.125
P (mmol/L)	1.096	0.305	0.764	0.938	1.076	0.953	1.626	1.024	0.635
Glutathione peroxidase (U/gHB)	0.996	0.002	0.066	0.993	0.007	0.283	0.976	0.012	0.035
Cu (μmol/L)	1.002	0.044	0.956	1.064	0.146	0.669	1.204	0.134	0.166
Zn (μmol/L)	0.968	0.039	0.410	1.004	0.126	0.974	1.277	0.124	0.048
Vitamin B12 (pmol/L)	0.997	0.002	0.098	1.007	0.006	0.204	0.990	0.006	0.080
Log Insulin (μmol/L)	0.944	0.088	0.511	1.370	0.337	0.351	0.945	0.274	0.838
Log IGF1(ng/L)	1.009	0.086	0.918	0.998	0.302	0.995	1.121	0.260	0.661
Log IGFBP-6 (ng/ml)	0.913	0.089	0.305	0.842	0.335	0.608	0.804	0.387	0.574

Project-wide Dissemination

The project findings have been disseminated through multiple formal research channels, including peer-reviewed publications, conference oral and poster presentations, and PhD theses. Two PhDs have been completed by Sarah Legge and David Sheedy, with Andrew Lean in the final stages of completion. Academic and industry conferences where findings have been presented include the American Dairy Science Association Annual Meeting, the Dairy Research Foundation Symposium, the Australian Cattle Veterinarians Conference, the ANZCVS Vet Science Week, the and International Society for Veterinary Epidemiology and Economics Symposium.

Findings have also been communicated directly to industry through Dairy UP workshops, Dairy UP project update meetings, field-based activities, producer and advisor engagement, and industry events including Raising the Roof, Appetite for Success and Herd '25. International visibility was strengthened through Dairy UP's special symposium, The DairyUp Project: Attacking Major Challenges for Dairy in a Multidisciplinary Project, at the American Dairy Science Association Annual Meeting in 2025, helping showcase Australian dairy research to a global audience.

Table: Peer-reviewed publications.

Authors	Title	Journal	Year Published	Citations (to April 26)
I.J. Lean, H.M. Golder, S.J. LeBlanc, T.F. Duffield, and J.E.P. Santos	Increased parity is negatively associated with survival and reproduction in different production systems	Journal of Dairy Science	2022	35
Lean, I.J., D.B. Sheedy, S.J. LeBlanc, T. Duffield, J.E.P. Santos, and H.M. Golder	Holstein dairy cows lose body condition score and gain body weight with increasing parity in both pasture-based and total mixed ration herds	Journal of Dairy Science Communications	2022	12
Lean, I.J., S.J. LeBlanc, D.B. Sheedy, T. Duffield, J.E.P. Santos, and H.M. Golder	Associations of parity with health disorders and blood metabolite concentrations in Holstein cows in different production systems	Journal of Dairy Science	2023	96
Brito, B., and P. Hick	Milk as a diagnostic fluid to monitor viral diseases in dairy cattle	Australian Veterinary Journal	2024	14

Lean, I.J., and H.M. Golder	Milk as an indicator of dietary imbalance	Australian Veterinary Journal	2024	6
Rowe, S., J.K. House, and R.N. Zadoks	Milk as diagnostic fluid for udder health management	Australian Veterinary Journal	2024	4
Legge, S.W.J., P.C. Thomson, C.E.F. Clark, and S.C. García	Milk consumption and behavior of calves in automated calf feeders as early indicators of weaning liveweight	Journal of Dairy Science Communications	2024	1
Lean, I., R. Zadoks, B. Brito, and H. Golder	Milk as a diagnostic fluid	Australian Veterinary Journal	2024	-
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Moate, P. Reddy, S.J. Rochfort, J.E. Pryce, and I.J. Lean	A large multisite lipidomic investigation of parity and aging in dairy cows	Journal of Dairy Science	2025	6
Sheedy, D.B., H.M. Golder, S.C. Garcia, P. Reddy, J.E. Hemsworth, D.E. Vincent, S.J. Rochfort, J.E. Pryce, and I.J. Lean	Associations among body condition score, body weight, and serum biochemistry in dairy cows	Journal of Dairy Science	2025	3
Golder, H.M., J. Rehberger, A.H. Smith, E. Block, R. Polkinghorne, H.E. Cuthbertson, M.A. Campbell, V. Vicic, G. Tarr, J.C. Quinn, J.K. Tong, S.M. Rowe, and I.J. Lean	Different lifetime dietary strategies affect carcass characteristics and rumen function in Holstein steers	Animal Production Science	2025	-

Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Hemsworth, D.E. Vincent, J.E. Pryce, and I.J. Lean	Confinement and pasture-based dairy herds differ in plasma lipid profiles	Journal of Dairy Science	2025	-
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Hemsworth, D.E. Vincent, J.E. Pryce, and I.J. Lean	A large, multisite investigation into the lipidomics of survival in dairy cows	Journal of Dairy Science	2026	I
Sheedy, D.B., H.M. Golder, S.C. Garcia, and I.J. Lean	Glycerophospholipids in dairy cow health and longevity: a review	Journal of Dairy Research	2026	-
Lean, A.K.G., A.J. Gunn, J.C. Quinn, I.J. Lean, K. Breinhild, and H.M. Golder	Meta-analysis of the effects of age at first calving on production outcomes, calving difficulty, and reproduction in dairy heifers	Journal of Dairy Science	2026	-
Abedien, Z.U., I.J. Lean, S.P. Djordjevic, P.M. Hick, M.E. Westman, J. Mckay-Demeler, J. Webster, and B.P. Brito	Next-generation detection in bovine respiratory and enteric diseases: metagenomic and amplicon sequencing insights into microbial diversity	Frontiers in Veterinary Science	2026	-

Annexes

Table: Conference presentations, abstracts and other meetings

Authors	Title	Presentation Type	Conference/ Event	Location	Year
B. Brito, Z.U. Abedien, Y. Kida, J. Webster, S. Djordjevic, E. Holmes, and I.J. Lean	Epidemiology 2.0: Metagenomic Pathogen Detection in NSW Dairy Calves - High-Resolution Mapping of Respiratory and Enteric Microbial Populations	Oral, conference	Australia and New Zealand College of Veterinary Scientist Vet Science Week	Gold Coast, QLD	2026
Sheedy, D.B., H.M. Golder, S.C. Garcia, A.K.G. Lean, and I.J. Lean	Reproduction, mastitis, and lameness in confinement and pasture-based systems: Associations with parity	Poster, conference	American Dairy Science Association Annual Meeting	Milwaukee, USA	2026
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Hemsworth, D.E. Vincent, J.E. Pryce, and I.J. Lean	Challenges and consequences of dairy cow longevity: Genetics, management, health and assessing the economics	Oral, conference	Australian Cattle Veterinarian Conference	Brisbane, QLD	2026
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Pryce, and I.J. Lean	System-level bias in lipidomic biological clock models imply greater metabolic age in confinement vs pasture-based cows	Poster, conference	American Dairy Science Association Annual Meeting	Milwaukee, USA	2026
Stanczak, A., B. Brito, S. Hem, I.J. Lean, V. Jarocki, and S. Djordjevic	Detection of blaCTX-M genes in Escherichia coli in dairy calves in Australia	Oral, conference	Environmental Dimensions of Antimicrobial Resistance Conference	Brisbane, QLD	2026
Abedien, Z.U.	Cracking the Code of Calf Health: Insights from Enteric Microbes	Oral, conference	Dairy Research Foundation Symposium	Wollongong, NSW	2025
Abedien, Z.U., S. Djordjevic, I.J. Lean, J. Webster, and B. Brito	Microbial diversity and gene expression using meta-transcriptomics study, an insight to untargeted approach in dairy calves	Poster, conference	American Dairy Science Association Annual Meeting	Louisville, USA	2025

Abedien, Z.U., S. Djordjevic, I.J. Lean, J. Webster, and B. Brito	Meta-transcriptomic insight into resistome, virulome, and microbial diversity in calves	Oral, conference	Australia and New Zealand College of Veterinary Scientist Vet Science Week	Gold Coast, QLD	2025
Brito, B.	When bugs go bad- who is attacking your calves?	Oral, conference	Dairy Research Foundation Symposium	Wollongong, NSW	2025
Brito, B.	NSW dairy calves microbial genomics study- preliminary findings	Oral, conference	Elizabeth Macarthur Agricultural Institute Lunch and Learn Seminar	Menangle, NSW	2025
Brito, B., Z.U. Abedien, and I.J. Lean	The enteric and respiratory viral diversity of calves in health and disease—A state-wide metatranscriptomic study.	Oral, conference	American Dairy Science Association Annual Meeting	Louisville, USA	2025
Golder, H.M., and I.J. Lean	Investigating genotype and environment effects on the rumen microbiome	Oral, conference	American Dairy Science Association Annual Meeting	Louisville, USA	2025
Kida, Y., Z.U. Abedien, I.J. Lean, and B. Brito	Molecular epidemiology of Rotavirus in New South Wales dairy calves	Poster, conference	American Dairy Science Association Annual Meeting	Louisville, USA	2025
Kida, Y., Z.U. Abedien, I.J. Lean, and B. Brito	Comprehensive analysis of genetic diversity in bovine rotavirus	Poster, conference	Australia and New Zealand College of Veterinary Scientist Vet Science Week	Gold Coast, QLD	2025
Kida, Y., Z.U. Abedien, I.J. Lean, and B. Brito	Comprehensive analysis of genetic diversity in bovine rotavirus	Oral, conference	Australia and New Zealand College of Veterinary Scientist Vet Science Week	Gold Coast, QLD	2025
Lean, A.K.G, H.M. Golder, J. Quinn, I.J. Lean, and A. Gunn	Meta-analysis of the effects of age at first calving on production outcomes, calving difficulty, and reproduction in dairy heifers	Oral, conference	Australia and New Zealand College of Veterinary Scientist Vet Science Week	Gold Coast, QLD	2025

Lean, A.K.G, H.M. Golder, J. Quinn, I.J. Lean, D.B. Sheedy, and A. Gunn	How early should you calve your heifers?	Oral, conference	Dairy Research Foundation Symposium	Wollongong, NSW	2025
Lean, A.K.G, H.M. Golder, J. Quinn, I.J. Lean, D.B. Sheedy, and A. Gunn	Evaluation of the effects of an enzymatic and biological additive on bedding in compost bedded pack barns in Australia	Oral, conference	American Dairy Science Association Annual Meeting	Louisville, USA	2025
Lean, I.J.	Unlocking the potential of the cow; adaptation to achieve profit	Oral, work shop	Appetite for Success	Sale, Inverloch and Warrnambool, VIC	2025
Lean, I.J.	Calcium metabolism and practical transition management	Oral, conference	New Zealand Veterinary Association	Wellington	2025
Lean, I.J., H.M. Golder, A.K.G. Lean, and D. Sheedy	Harvesting the power of the cow	Oral, conference	Dairy Research Foundation Symposium	Wollongong, NSW	2025
Lean, I.J., H.M. Golder, B. Brito, N. Moss, D. Sheedy, A.K.G. Lean, S. Garcia, C. Clark, A. Gunn, and J. Quinn	You don't eat the coat! How does beef on dairy stack up?	Oral, conference	Herd '25	Bendigo, VIC	2025
Lean, I.J., H.M. Golder, D.B. Sheedy, C. Old, and A.K.G. Lean	Case definition and metabolic disorders: More accurate phenotypes	Oral, conference	American Dairy Science Association Annual Meeting	Louisville, USA	2025
Legge, S.W.J., P.C. Thomson, C.E.F. Clark, and S.C. García	The positive associations between calf and lactation performances in dairy cattle	Oral, conference	American Dairy Science Association Annual Meeting	Louisville, USA	2025
Legge, S.W.J., P.C. Thomson, C.E.F. Clark, and S.C. García	Identifying key factors affecting dairy cow longevity	Oral, conference	Dairy Research Foundation Symposium	Wollongong, NSW	2025
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort,	Do cows in total mixed ration systems have old lipid profiles compared with pasture-based cows?	Oral, conference	American Dairy Science Association Annual Meeting	Louisville, USA	2025

J.E. Hemsworth, D.E. Vincent, J.E. Pryce, and I.J. Lean					
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Hemsworth, D.E. Vincent, J.E. Pryce, and I.J. Lean	Life and death in pasture and TMR farms - the fat?	Oral, conference	Dairy Research Foundation Symposium	Wollongong, NSW	2025
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Pryce, and I.J. Lean	Variable stabilization in adaptive lasso cox frailty models to explore lipidomic associations with survival of dairy cows.	Oral, conference	Animal Science Modellers Special Interest Group	Louisville, USA	2025
Stanczak, A., B. Brito, S. Hem, I.J. Lean, V. Jarocki, and S. Djordjevic	Molecular Characterisation of blaOXA-23 genes identified during a genomic survey of Escherichia coli in Australian dairy cattle.	Oral, conference	MedVetPathogens - FEMS	Prato, Italy	2025
Sheedy, D.B.	P2a Project Updates	Oral, meeting	Dairy UP Teams Meeting	On-line	2023 2024 2025
Brito, B.	P2f Project Updates	Oral, meeting	Dairy UP Teams Meeting	On-line	2022 2023 3x 2025
A.K.G, H.M. Golder, J. Quinn, I.J. Lean, D.B. Sheedy, and A. Gunn	Age at first calving	Oral, conference	Australia and New Zealand College of Veterinary Scientist Vet Science Week	Gold Coast, QLD	2024
Golder, H.M.	P2c Project Updates	Oral, meeting	Dairy UP Teams Meeting	On-line	2024
Lean, A.K.G, H.M. Golder, J. Quinn, I.J. Lean, and A. Gunn	Assessment and management of dairy compost bedded pack barns	Oral, conference	Australia and New Zealand College of Veterinary Scientist Vet Science Week	Gold Coast, QLD	2024

Lean, A.K.G, H.M. Golder, J. Quinn, I.J. Lean, and A. Gunn	Understanding and assessing compost bedded pack dairy barns	Oral, conference	Australian Cattle Veterinarian Conference	Wodonga, VIC	2024
Lean, A.K.G, H.M. Golder, J. Quinn, I.J. Lean, and A. Gunn	Managing and improving loose housing facilities with a compost bedded pack	Oral, conference	Raising the Roof	Cessnock, NSW	2024
Lean, I.J., H.M. Golder, B. Brito, N. Moss, D. Sheedy, A.K.G. Lean, S. Garcia, C. Clark, A. Gunn, and J. Quinn	Unlocking the potential of the cow	Oral, conference	Raising the Roof	Cessnock, NSW	2024
Sheedy, D.B., H.M. Golder, S.C. Garcia, and I.J. Lean	Metabolic investigation into dairy cow longevity	Oral, conference	School of Life & Environmental Sciences Higher Degree Research Showcase	Sydney, NSW	2024
Sheedy, D.B., H.M. Golder, S.C. Garcia, and I.J. Lean	Survival and longevity	Oral, conference	Raising the Roof	Cessnock, NSW	2024
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Pryce, and I.J. Lean	Associations among body condition score, body weight and serum biochemistry in dairy cows	Poster, conference	American Dairy Science Association Annual Meeting	West Palm Beach, USA	2024
Sheedy, D.B., H.M. Golder, S.C. Garcia, Z. Liu, P. Reddy, S.J. Rochfort, J.E. Pryce, and I.J. Lean	Associations among body condition score, body weight and serum biochemistry in dairy cows	Poster, conference	International Society for Veterinary Epidemiology and Economics	Sydney, NSW	2024
Brito, B.	The bugs of calves- a state-wide study of endemic infectious diseases	Oral, work shop	Dairy UP workshops	Bega, Bodalla, Casino	2023
Brito, B., H.M. Golder, and I.J. Lean	The respiratory infectome of dairy calves characterized by a total RNA sequencing approach.	Oral, conference	American Dairy Science Association Annual Meeting	Ottawa, CAN	2023

Brito, B., H.M. Golder, E. Wrysch, S. Djordjevic, and I.J. Lean	Expression of virulence factors and antimicrobial resistant genes in total RNA sequenced from rectal swabs from diarrheic calves	Poster, conference	American Dairy Science Association Annual Meeting	Ottawa, CAN	2023
Brito, B., H.M. Golder, E. Wrysch, S. Djordjevic, J. Rothwell, and I.J. Lean	Untargeted metatranscriptomic methods to characterize the enteric infectome of calves with and without diarrhea	Poster, conference	American Dairy Science Association Annual Meeting	Ottawa, CAN	2023
Lean, A.K.G, H.M. Golder, J. Quinn, I.J. Lean, D.B. Sheedy, D. Lewis, and A. Gunn	Assessment and microbial enhancement of dairy compost-bedded pack barns	Oral, conference	Charles Sturt University Faculty of Science and Health Higher Degree by Research and Honours Symposium	Wagga Wagga, NSW	2023
Lean, I.J, A.K.G. Lean, and D. Sheedy	Cow longevity and metabolomics	Oral, work shop	Dairy UP workshops	Bega, Bodalla, Casino	2023
Sheedy, D.B., H.M. Golder, S.C. Garcia, and I.J. Lean	Insights into exit reasons between intensively housed and pasture-based dairy systems	Poster, conference	School of Life & Environmental Sciences Higher Degree Research Showcase	Sydney, NSW	2023
Sheedy, D.B., H.M. Golder, S.C. Garcia, and I.J. Lean	The metabolomics of dairy cow longevity	Oral, conference	Dairy Research Foundation Symposium	Camden, NSW	2023
Brito, B.	Infectious diseases calves, kikuyu poisoning	Oral, conference	District Veterinarians Conference	Camden, NSW	2022
Sheedy, D.B., H.M. Golder, S.C. Garcia, and I.J. Lean	A metabolomic investigation into Australian dairy cow longevity.	Oral, conference	School of Life & Environmental Sciences Higher Degree Research Showcase	Sydney, NSW	2022

Table: Technical reports, pamphlets, published material and other media engagements

Authors	Title	Place Published	Year
	P2f fact sheets https://dairyup.com.au/project/p2f-factsheets/		

Conclusion and Recommendations

Dairy UP Project 2 has delivered a substantial and integrated body of work addressing dairy cow health, productivity, welfare and longevity across multiple life stages and production systems. The project has generated knowledge through a broad program of database development, retrospective and prospective studies, lipidomics, milk diagnostics, heat stress research, molecular infectious disease investigations, calf and heifer studies, and housing system studies. Collectively, these outputs demonstrate both the breadth and depth of the project and its value to the Australian dairy industry.

The project has achieved strong research and industry dissemination, with outcomes communicated through peer-reviewed publications, industry presentations, conference abstracts, field days and direct engagement with producers and advisors. The national and international relevance of the work is reflected in the breadth of publications and presentations, including Dairy UP's presence as a special symposium, The DairyUp Project: Attacking Major Challenges for Dairy in a Multidisciplinary Project, at the America Dairy Science Association annual meeting in 2025. This visibility has helped place Australian dairy research on the global stage.

Specific outcomes for the projects include;

- A clear understanding of the increased risks of disease and metabolic disorder with increased parity of cows. This will provide farmers and advisors with clarity around the increased risks with increased parity of cows.
- Identifying, that there are marked differences in lipid profiles with age of cows and that cows in housed facilities have older profiles than those at pasture.
- These differences suggest means by which the problems of aging might be modified with lipid supplementation.
- Cows lose body condition but gain weight as they age, indicating a pathway to reducing risks of problems in older cows.
- We identified differences in herd removals between housed and pastured herds which will help farmers and advisors understand the benefits and risks from different systems.
- We identified methods to improve bedding management in compost bedded pack barns.
- An early alert system for bovine ephemeral fever was established.
- Milk has great value as a diagnostic fluid and is under-utilised by the Australian industry, Milk urea nitrogen will be very valuable to routinely report.
- An important finding was to quantify the lagged influence of diet on milk production highlighting that nutritional responses to diet occur over days to weeks.
- Diets differing in formulation provided the potential to integrate dairy calves into the beef supply chain but with different rumen lifetime adaptations.
- Greater milk consumption in early life (up to day 5) had substantial effects on weaning weight of calves.
- Holstein cattle achieved excellent feedlot performance compared to beef breeds. High performing cattle at feedlot induction were higher performing overall compared to those with lesser early performance.

- Metagenomic and amplicon sequencing methods will change understandings and improve accuracy of veterinary diagnostic testing.
- Calf diarrhoea is rarely caused by a single pathogen and is substantially explained by the farm environment. This reinforces the importance of good husbandry for farmers and advisors.
- Kobuvirus was identified as an emerging pathogen associated with diarrhoea.
- Pestivirus, while of moderate prevalence, was a substantial pathogen. This can be controlled with vaccination.
- Insights were gained on host responses to potential pathogens providing avenues for future control of disease without using antimicrobial treatments.
- Similarly respiratory disease risks were dominated by farm environment. Rhinitis-viruses and Torovirus were identified as pathogens. Again, disease occurs as result of interactions among potential pathogens.
- Host responses to pathogens were an important part of the pathogenesis of the disease.
- Reducing the age at first mating of heifers weighing >330 kg to 10 to 13 months as opposed to mating at >13 months resulted in > 800 L greater milk production in the first lactation as a result of more heifers entering and remaining in the herd. This has implications for costs of rearing and green-house gas footprint.
- Heifers with higher blood free fatty acids had a lesser risk of being actively cycling indicating the importance of a positive nutritional plane near mating.

Further investment is recommended to build on the momentum created by Project 2. Support is needed for extension activities that translate findings into practical farm decision-making, and for early- and mid-career researchers who have developed expertise through the program. Continued support would help retain research capacity, strengthen industry relationships and ensure that the knowledge generated through Dairy UP is converted into measurable benefits for farmers, veterinarians, nutritionists and the wider dairy sector.

A key priority for future funding should be to move from exploratory findings to targeted implementation studies. Several projects have identified promising areas for intervention, including feeding strategies, omega fatty acid biology, heat stress mitigation, heifer development, housing design and early warning systems. The risk of inaction is that the collaborations, data infrastructure, research capacity and industry momentum developed through Dairy UP will not be fully realised. Continued investment would allow the program to progress from discovery to practical application, supporting a more productive, resilient and socially sustainable Australian dairy industry.