



THE GLOBAL STANDARD  
FOR LIVESTOCK DATA

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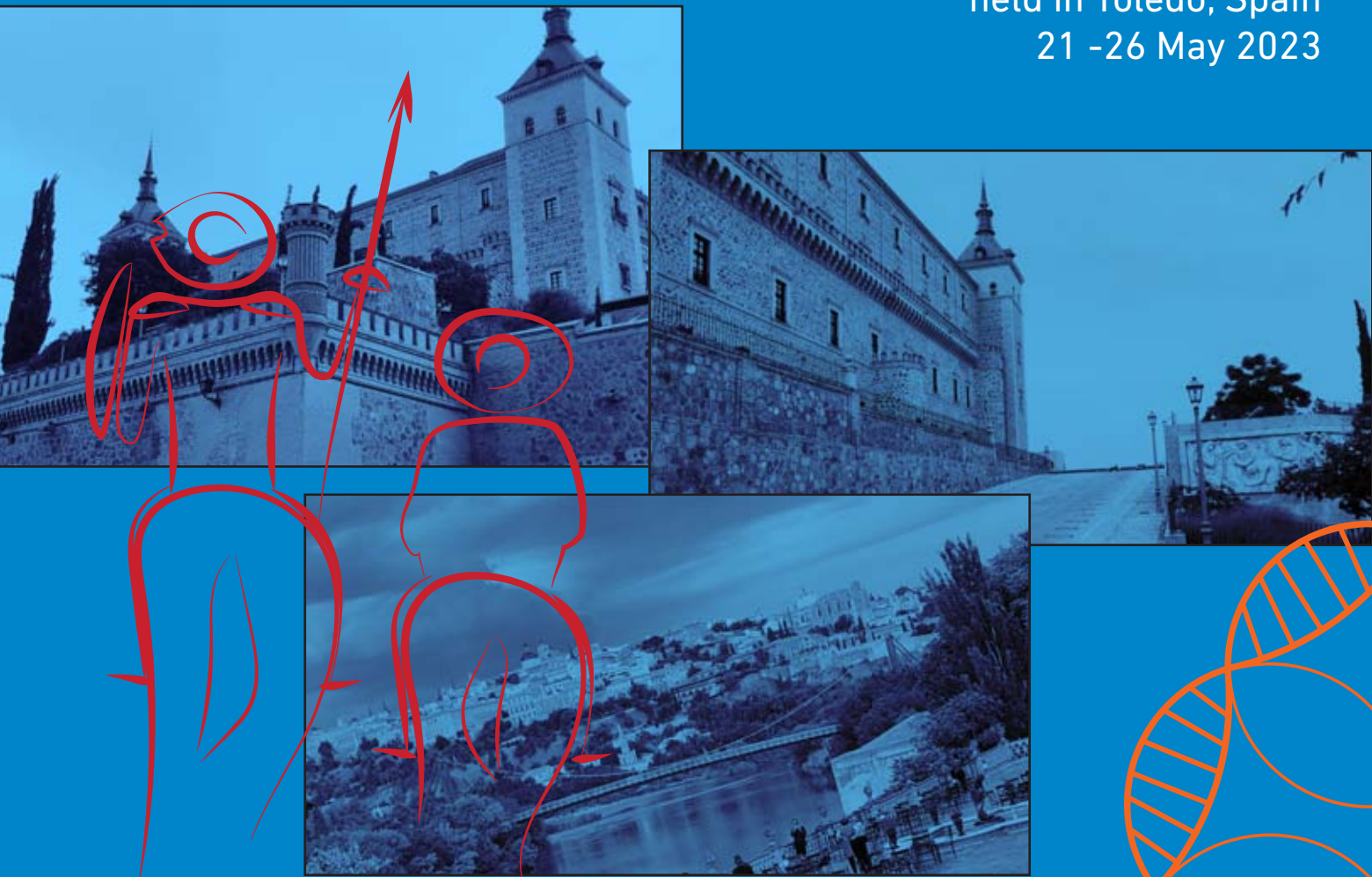


ICAR 2023  
TOLEDO SPAIN

ICAR Technical Series no. 27

# BREEDING FOR RESILIENCE: TRANSITIONING DIVERSE LIVESTOCK FARMING SYSTEMS INTO THE FUTURE

Proceedings of the 46<sup>th</sup> ICAR Annual Conference  
held in Toledo, Spain  
21 -26 May 2023



Editors: C. Diaz, I. Casasús, F. Estellés, P. Llonch, M. Luque,  
M. Burke and C. Mosconi

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November 2023



<b>Preface of the ICAR Proceedings on “Breeding for resilience: transitioning diverse livestock farming systems into the future”</b> .....	11
<b>ICAR 2023 – Summary and main conclusions of the round table on “Data ownership and data sharing”</b> .....	13
<b>Practical lessons from data hub implementation</b> .....	15
<i>J. Frandsen<sup>1</sup> and C. Murphy<sup>2</sup></i>	
<b>Development of an automated quality control pipeline to facilitate the reporting of major gene genotypes</b> .....	19
<i>K. Quigley<sup>1</sup>, T. Browne<sup>1</sup>, K. O’Connell<sup>1</sup>, P. Flynn<sup>2</sup>, R.D. Evans<sup>1</sup> and M.P. Mullen<sup>3</sup></i>	
<b>Agri-food Data Canada: A data ecosystem serving agri-food sustainability</b> .....	27
<i>L.M. Alcantara, C. Huitema and A.M. Edwards</i>	
<b>Data investment strategy - the core issue for genetics organisations in the genomics era</b> .....	33
<i>R.G. Banks<sup>1</sup>, S. Ring<sup>2</sup> and R. Evans<sup>2</sup></i>	
<b>Challenges and opportunities of milk recording methods in Irish dairy farms: A comparison of test day milk recordings and daily bulk collection samples</b> .....	41
<i>M. Thompson</i>	
<b>Identification of behavioural patterns associated with acidosis in dairy cows</b> .....	47
<i>L. Herve<sup>1</sup>, Y. Gómez<sup>2</sup>, K. Chow<sup>3</sup>, G.V. Berteselli<sup>4</sup>, E. Dalla Costa<sup>4</sup>, E. Canal<sup>4</sup>, X. Manteca<sup>2</sup> and P. Llonch<sup>2</sup></i>	
<b>A predictive model for hypocalcemia in dairy cows utilizing behavioural sensor data combined with deep learning</b> .....	53
<i>M. van Leerdam<sup>1</sup>, A. Liseune<sup>2</sup>, P.R. Hut<sup>1</sup>, E. Slavco<sup>1</sup>, J. Hulsen<sup>3</sup> and M. Hostens<sup>1,4</sup></i>	
<b>Exploration of animal-based parameters (activity level and respiratory health status) collected by sensors to monitor pig welfare on farm</b> .....	77
<i>H.-L. Ko<sup>1</sup>, D. Escribano<sup>2</sup>, M. López-Arjona<sup>1</sup>, M. Botia<sup>2</sup>, A. Ortín-Bustillo<sup>2</sup>, F. Tecles<sup>2</sup>, P. Fuentes<sup>3</sup>, J. Cerón<sup>2</sup>, X. Manteca<sup>1</sup> and P. Llonch<sup>1</sup></i>	
<b>Searching for phenotypes to improve welfare in Avileña-Negra Ibérica beef cattle breed: preliminary results</b> .....	83
<i>C. Meneses<sup>1</sup>, M.J. Carabaño<sup>1</sup>, M. González<sup>2</sup>, A. Hernández<sup>2</sup>, C. González<sup>1</sup>, F. Vicente<sup>1</sup>, M. Álvarez<sup>3</sup>, J. de la Fuente<sup>4</sup>, M. Ramón<sup>5</sup>, C. Palau<sup>6</sup>, J. González<sup>6</sup>, S. Zabala<sup>6</sup> and C. Díaz<sup>1</sup></i>	
<b>Sensor data for animal health and welfare: present perspectives and future applications</b> .....	89
<i>K. Schodl<sup>1</sup>, M. Burke<sup>2</sup>, R. van der Linde<sup>2</sup> and C. Egger-Danner<sup>1</sup></i>	
<b>Descriptive evaluation of camera-based lameness detection technology paired with artificial intelligence in dairy cattle</b> .....	97
<i>D. Swartz<sup>1</sup>, E. Shepley<sup>1</sup>, D. Norman<sup>2</sup>, J. Burchard<sup>2</sup>, Xiao Lin Wu<sup>2</sup>, A. Miles<sup>3</sup> K.P. Gaddis<sup>2</sup> and G. Cramer<sup>1</sup></i>	
<b>Assessing the impact of body condition score dynamics from dry-off to calving on the incidence of early lactation disease in Holstein cows</b> .....	103
<i>P. Pinedo<sup>1</sup>, D. Manríquez<sup>1,2</sup>, J. Azocar<sup>3</sup> and A. De Vries<sup>4</sup></i>	
<b>Towards new breeding tools in a context of climate change: Fertility results of the RUMIGEN project on new phenotypes for heat tolerance traits</b> .....	109
<i>M. Carabaño<sup>1</sup>, A. Vinet<sup>2</sup>, H. Mulder<sup>3</sup>, M. Calus<sup>3</sup>, J. Vandenplas<sup>3</sup>, M. Ramón<sup>4</sup>,</i>	

<i>C. Díaz<sup>1</sup>, B. Cuyabano<sup>2</sup>, D. Boichard<sup>2</sup>, E. Pailhoux<sup>2</sup> and S. Mattalia<sup>5</sup></i>	
<b>Genetic analysis of lactation consistency using daily milk weights in U.S. Holsteins</b> .....	115
<i>F.L. Guinan<sup>1</sup>, R.H. Fourdraine<sup>2</sup>, F. Peñagaricano<sup>1</sup> and K.A. Weigel<sup>1</sup></i>	
<b>Use of vocalizations to detect pain and discomfort in dairy cows at dry-off</b> .....	127
<i>J. Miranda<sup>1,2</sup>, C. Larrondo<sup>1,3</sup>, R. Guevara<sup>1,2</sup>, E. Vidaña-Vila<sup>4</sup>, J. Malé<sup>4</sup>, M. Freixes<sup>4</sup>, R.M. Alsina-Pagès<sup>4</sup>, L. Duboc<sup>4</sup>, E. Mainau<sup>1</sup> and P. Llonch<sup>2</sup></i>	
<b>Practical Lessons from Data Hub Implementation</b> .....	133
<i>J. Frandsen<sup>1</sup> and C. Murphy<sup>2</sup></i>	
<b>Prediction of heat stress status by infrared spectroscopy in dairy sheep</b> .....	137
<i>M. Ramon<sup>1</sup>, C. Diaz<sup>2</sup> and M. Carabaño<sup>2</sup></i>	
<b>NEU.rind - Digital farm assistant for assessing sustainability, efficiency and environmental impact on the dairy farm</b> .....	141
<i>F. Steininger<sup>1</sup>, S. Hörtenhuber<sup>2</sup>, M. Herndl<sup>3</sup>, K. Linke<sup>1</sup>, M. Stegellner<sup>4</sup>, J. Schmidt<sup>5</sup>, M. Horn<sup>6</sup>, F.J. Auer<sup>7</sup> and C. Egger-Danner<sup>1</sup></i>	
<b>The Sustainability Index: A new tool to breed for reduced greenhouse gas emissions intensity in Australian dairy cattle</b> .....	149
<i>T.T.T. Nguyen<sup>1</sup>, C.M. Richardson<sup>2</sup>, M Post<sup>3</sup>, P.R. Amer<sup>3</sup>, G.J. Nieuwhof<sup>1</sup>, P. Thurn<sup>1</sup> and M. Shaffer<sup>1</sup></i>	
<b>Farm-to-table science: dairy data mining for future resilience</b> .....	163
<i>J.I. Ohlsson, T. Klingström, D.-J. de Koning</i>	
<b>Prediction of body condition in Jersey dairy cattle from 3D images using machine learning techniques</b> .....	171
<i>R.B. Stephansen<sup>1</sup>, C.I.V. Manzanilla-Pech<sup>1</sup>, G. Gebreyesus<sup>1</sup>, G. Sahana<sup>1</sup> and J. Lassen<sup>1,2</sup></i>	
<b>How to optimize feed efficiency by automatic data exchange</b> .....	179
<i>J. Frandsen</i>	
<b>System and biological effects on quantitative milking speed phenotypes from inline milk meters</b> .....	185
<i>A.M. Miles<sup>1</sup>, R. Fourdraine<sup>2</sup>, J.L. Hutchison<sup>1</sup>, S. Sievert<sup>3</sup>, K. Gaddis<sup>4</sup>, J. Bewley<sup>5</sup>, S. Eaglen<sup>6</sup>, J. Weiker<sup>7</sup> and J. Durr<sup>4</sup></i>	
<b>Comparing peak of lactation from Automatic Milking Systems and Conventional Milking Systems</b> .....	191
<i>L. Fadul-Pacheco, G. Bisson, R. Lacroix and D.E. Santschi</i>	
<b>French experience of using ICAR approved method for predicting 24-hour fat percentage and yield from one sampled milking in Automatic Milking Systems</b> .....	195
<i>X. Bourrigan<sup>1</sup>, R. Vallée<sup>1</sup> and G. Augier<sup>2</sup></i>	
<b>Milk recording in sheep and goats: state of the art and materials used for recording and sampling</b> .....	201
<i>J.M. Astruc<sup>1</sup>, A. Carta<sup>2</sup>, M. Simčič<sup>3</sup>, M. Špehar<sup>4</sup>, E. Ugarte<sup>5</sup>, C. Mosconi<sup>6</sup> and S. Sievert<sup>7</sup></i>	
<b>Overviews of milk recording and recording devices in sheep and goats in Italy</b> .....	209
<i>M. Fioretti<sup>1</sup>, L. Pascarella<sup>1</sup>, R. Negrini<sup>1,2</sup> and G. Malvezzi<sup>3</sup></i>	
<b>Dtreo, a flexible cloud-based data-recording platform for data-driven decisions in sheep and goat industries</b> .....	219
<i>S. Savoia<sup>1</sup>, B. Santos<sup>2</sup>, S. Liebergreen<sup>2,3</sup>, E. Oo<sup>2</sup>, S. Kumar<sup>2</sup>, M. Teviotdale<sup>2</sup>, P. Amer<sup>2</sup> and T. Byrne<sup>1</sup></i>	



<b>Challenges on milk recording and sampling in the Latxa dairy sheep breeding program .....</b>	<b>227</b>
<i>M. Lasarte<sup>1</sup>, L. Minteg<sup>2</sup>, J. Bizkarralegorra<sup>3</sup>, A. Cenitagoia<sup>4</sup> and A. Díez<sup>4</sup></i>	
<b>Infrared models for the prediction of cow colostrum immunoglobulins G concentration: phenotypic variability and relationship with colostrum yield .....</b>	<b>233</b>
<i>M. De Marchi<sup>1</sup>, A. Goi<sup>1</sup>, M. Pozza<sup>1</sup>, E. Chiarin<sup>1</sup>, and A. Costa<sup>2</sup></i>	
<b>In-line real-time milk composition analysis by tunable laser spectroscopy for dairy farms .....</b>	<b>241</b>
<i>T. Bučiūnas, B. Bilinskas, I. Šimonytė, T. Žukauskas, A. Miasojedovas, V. Čerkasovas, A. Baltrėnas, A. Vizbaras, K. Vizbaras and D. Vizbaras</i>	
<b>A time-series analysis of Alpine and Saanen goat milk productivity trends in Taiwan .....</b>	<b>247</b>
<i>P.A. Tu<sup>1</sup>, J. W. Shiau<sup>1</sup>, S.T. Cher<sup>2</sup>, M.C. Wu<sup>2</sup>, J.T. Hsu<sup>3</sup>, M.K. Yang<sup>1</sup> and J.F. Huang<sup>2</sup></i>	
<b>GenoCells for dairy goats: identification of the most contributing goats to the bulk tank somatic cell count by genotyping tank milk .....</b>	<b>257</b>
<i>A. Vivier<sup>1</sup>, P. Lenormand<sup>1</sup>, T. Lallemand<sup>1</sup>, F. Perrin<sup>2</sup>, M. Calmels<sup>1</sup>, M. Foucher<sup>2</sup> and J.B. Davière<sup>1</sup></i>	
<b>Valorization of extensive breeding of the Spanish Merino Sheep through the quality wool improvement: Preliminary results of GWAS of fiber diameter from whole genome sequences.....</b>	<b>263</b>
<i>G. Anaya<sup>1</sup>, N. Laseca<sup>1</sup>, A. Domingo<sup>2</sup>, A. Granero<sup>3</sup>, F. Arrebola<sup>4</sup>, C. Ziadi<sup>1</sup> and Antonio Molina<sup>1</sup></i>	
<b>The state of the art about the development of mid-infrared based fatty acids predictions and their applications along the dairy food chain.....</b>	<b>269</b>
<i>M. Whittaker<sup>1</sup>, S. Orlandin<sup>2</sup>, C. Baumgartner<sup>2</sup>, S. Holroyd<sup>3</sup>, G. Scott<sup>3</sup>, P. Jamieson<sup>3</sup>, D. Santschi<sup>4</sup>, M. Bahadi<sup>4</sup>, B. Harris<sup>5</sup>, K. Sanders<sup>5</sup> and H. Soyeur<sup>6</sup></i>	
<b>GreenFeed and Sniffer Standard Operating Procedure (SOP) in dairy and beef cattle.....</b>	<b>281</b>
<i>L. Benzoni<sup>1</sup>, D. Berry<sup>2</sup>, E. Dressler<sup>3</sup>, R. Hegarty<sup>4</sup>, L. Koning<sup>5</sup>, C. Mc Donnell<sup>6</sup>, L. Mcnaughton<sup>7</sup>, G. Ritchie<sup>8</sup>, R. Finocchiaro<sup>1</sup>, A. Van Breukelen<sup>5</sup>, A. García-Rodríguez<sup>9</sup>, O. Gonzalez-Recio<sup>9</sup>, C. Richardson<sup>10</sup>, T.M. Villumsen<sup>11</sup> and B. Gredler-Grandl<sup>5</sup></i>	
<b>Genetic parameters and single step evaluation of sexual precocity traits in Charolais beef cattle.....</b>	<b>289</b>
<i>R. Saintilan<sup>1,2</sup>, M. Gicque<sup>3,4</sup>, A. Baur, A.<sup>1,2</sup>, H. Leclerc<sup>1,2</sup> and F. Guillaume<sup>3,4</sup></i>	
<b>Exploring opportunities to evaluate genomically MIR-predicted residual CH<sub>4</sub> exploiting correlations to MACE traits.....</b>	<b>293</b>
<i>H. Atashi<sup>1,2</sup>, A. Vanlierde<sup>3</sup>, F. Dehareng<sup>3</sup>, H. Soyeur<sup>2</sup> and N. Gengler<sup>2</sup></i>	
<b>Beef cattle breeding in Latvia.....</b>	<b>295</b>
<i>E. Skujina and I. Pilvere</i>	
<b>Application of ssGBLUP in Murrah buffaloes reared under small holder conditions in India.....</b>	<b>305</b>
<i>N. Nayee, S. Saha, A. Sudhakar, S. Gajjar, A. Mahajan and K. Trivedi</i>	
<b>Genomics improves the reliability of Breeding Value Prediction of morphological and reproductive traits in the Pura Raza Español Horse: Preliminary results .....</b>	<b>311</b>
<i>C. Ziadi<sup>1</sup>, D. Perdomo-González<sup>2</sup>, M. Valera<sup>2</sup>, N. Laseca<sup>1</sup>, A. Encina<sup>3</sup>, P. Azor<sup>3</sup>, A. Rodríguez<sup>3</sup>, S. Demyda-Peyrás<sup>1</sup> and A. Molina<sup>1</sup></i>	
<b>Validation of US genomic prediction for milk and fat yield for Holstein cattle in subtropical Taiwan .....</b>	<b>317</b>
<i>Chun Hsuan Chao, Jih-Yi Chen, Po-An Tu, Szu Han Wang and Jen-Wen Shiau</i>	
<b>Valuing the Certificate of Quality at Holstein UK .....</b>	<b>323</b>
<i>D. Todd</i>	

<b>Ewes' Milk Urea Concentration methods' optimization, by difference in pH and Mid-Infrared Spectroscopy (MIRS).....</b>	<b>327</b>
<i>F. Albert<sup>1</sup>, M. El Jabri<sup>2</sup>, P. Trossat<sup>3</sup>, M. Gelé<sup>4</sup>, J.-M. Astruc<sup>1</sup>, C. Laithier<sup>6</sup> and G. Lagriffoul<sup>1</sup></i>	
<b>Development of a new equation based on MIR spectra to predict lipolysis in dairy goats .....</b>	<b>331</b>
<i>M. Gelé<sup>1</sup>, S. Meurisse<sup>1</sup>, P. Trossat<sup>3</sup>, A. Oudotte<sup>2</sup>, T. Fassier<sup>3</sup>, H. Caillat<sup>4</sup>, P. Lambertson<sup>5</sup>, A. Eymard<sup>6</sup>, H. Larroque<sup>7</sup>, L. Bernard<sup>8</sup>, C. Hurtaud<sup>6</sup> and C. Cebo<sup>9</sup></i>	
<b>Genomic selection in Spanish dairy sheep breeds.....</b>	<b>335</b>
<i>I. Granado Tajada<sup>1</sup>, E. Ugarte Sagastizabal<sup>1</sup> and M. Serrano<sup>2</sup></i>	
<b>Spanish dairy sheep breed associations were granted with the ICAR Certificate of Quality .....</b>	<b>341</b>
<i>J.M. Astruc<sup>1</sup>, F. Freire<sup>2</sup>, R. Gallego<sup>3</sup>, T. Lopez<sup>4</sup>, L. Mintegi<sup>5</sup>, E. Couto<sup>6</sup>, A. Calavia<sup>7</sup>, F. Tejerina<sup>7</sup> and C. Diaz<sup>8</sup></i>	

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## Preface

### **“Breeding for resilience: transitioning diverse livestock farming systems into the future”**

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This is the first time Spain has hosted an ICAR meeting. It has been our pleasure to have you here. We brought you to Toledo, in Castilla-La Mancha, a town with many centuries of history that provided a very nice atmosphere and which was very convenient because of its proximity to Madrid. However, our main objective was to show you the reality of the livestock production systems in a significant part of Spain, in other words, producing in harsh environments, which are increasingly affected by the climate change and where to maintain livestock activities is becoming more challenging.

Spain has very diverse livestock farming systems where all ruminant species play a crucial role in our economy including both beef and dairy cattle, dairy and meat goats and sheep. We, as in many parts of the world, are searching for new breeding objectives. RESILIENCE is one of the key words to achieve sustainable programs. Resilient production systems require resilient farmers, highly motivated toward innovations uptake, therefore understanding what drives farmers to innovations was our first proposed session. As some breeding programs have already realized, farmers are the main actors, thus we need to understand their motivations to really contribute to the development of the sector. Resilient livestock systems also require resilient producing animals, able to cope with challenging environments while maintaining their welfare (Session 2), animals that could be selected to better adapt to challenging environments or thermal stress (Session 3 and Session 8) and to limit their methane emissions and therefore mitigate climate change (Session 4). Session 5 (as well as 8) dealt with the advances in Precision Livestock Farming, covering both devices and new traits. Resilience will not be a new trait to be incorporated in breeding programs unless farmers commit themselves to innovation and to integrate devices that generate frequent records of production, behaviour or health, among other traits. These systems are well established and growing in the dairy cattle sector, but others still need to increase their adoption. However, nowadays, the free access to the data generated by these devices is subject to manufacturer's decision. This situation motivated the organization of a Round Table entitled “Data Ownership and Data accessibility”, the conclusions of which are published in these Proceedings. We also had very interesting sessions and workshops organized by ICAR working groups, where we would like to highlight the session on the Brian Wickhan Young People Exchange Program. It was

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an opportunity to honor Brian, a person who had made an enormous contribution to the livestock sector and to ICAR.

We believe that the future of the livestock sector, will benefit from the cooperation between industry and science. ICAR is the right forum where they should meet. To show you a piece of that interaction was the objective of the key speakers during the plenary session “Resilience: from science to the industry sector”. In this ICAR 2023, we have had the participation of industry, reserachers and technicians that had no previous engament with ICAR, we have also had the presence of non-traditional species for ICAR such as horses and pigs.

All this would not have been possible without the kind support of our sponsors, the Spanish National and regional authorities, the technical support of the ICAR Secretariat and the Organizing Committee, the ICAR SubComittees and working groups who helped us with the scientific organization, the Chairs of the Sessions and all those who participated in the presentations and discussions. We hope, you have enjoyed the meeting.

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## ICAR 2023 – Summary and main conclusions of the round table “On data ownership and data sharing”

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During ICAR 2023 a round table was organised to discuss data ownership and data sharing. It was moderated by Fernando Estellés and Reinhard Reents, two reputed professionals from academia and industry. The invited members of the round table included representatives from all stakeholders engaged in agricultural data including research institutions, industry, and farmers. More than 80 participants actively engaged in discussions regarding data utilization, barriers to data sharing, expectations from farmers, and considerations of standardization and interoperability. The main take-home messages from the round table are summarised.

It is important to recognise the farmer as the rightful owner of the data generated in their farms. To provide clarity on ownership, there is a collective call for formal agreements between farmers (as data originators) and the entities responsible for data collection. It is also necessary to establish a comprehensive code of conduct for the sharing of agricultural data.

In terms of data utilization, it is imperative to inform farmers about the broader potential uses of data, especially when applied to commercial farms. This broader application aims to enhance the understanding of end-user needs within the agricultural sector. The expectations from farmers concerning data usage are currently at the forefront of the discussion. Emphasis should be on the need for data to be profitable for farmers, and there is an ongoing dialogue about achieving clarity regarding the type of data (raw or processed) being referred to in these discussions. Companies emphasized the relevant economic investments made by them to transform raw data in a useful tool for farmers.

Participants identified some barriers to data sharing. Namely, there is an acknowledgement of limited awareness surrounding the existing code of conduct. Additionally, considerations are being made for national guidelines and the ownership of data insights and the progresses made by companies on data analysis and algorithm development. The importance of data security is underscored, and suggestions for implementing data hubs to ensure secure storage are and should be actively explored.

Several queries arose from the audience inquiring about the use and control of data generated in farms. Clarifications from experts were provided, affirming that farmers must give consent for any use of their data. Challenges related to data flow are currently being addressed, with an emphasis on establishing reciprocal benefits and fostering a win-win situation for both companies and farmers. Suggestions for data anonymization and considerations about farmers' understanding of agreements are actively being discussed. There is an ongoing exploration of the possibility of allowing farmers to opt-out of sharing data for algorithm improvement.

For effective data sharing, standardization and interoperability were identified as a crucial aspect. Participants favour collaboration over mandatory standardization and efforts should be made towards data interoperability to facilitate seamless collaboration among various stakeholders.

Steps to enhance data sharing are currently under consideration, including the formation of data unions or cooperatives. Participants are emphasizing the importance of data integration and standardization processes to facilitate collaboration effectively.

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A paradigm shift is evident in the perception of data, transitioning from capital to a public good. Suggestions are being considered for agreements between companies, farmers, farmer's organizations and researchers to address societal needs such as animal welfare, environmental impact, and food security.

The meeting evidenced the need for follow-up discussions involving relevant stakeholders to progress on the issues addressed. This follow-up aims to build upon the current dialogue and address the multifaceted issues raised during the meeting, fostering continued collaboration in the dynamic landscape of agricultural data.

In conclusion, stakeholders are exploring collaborative approaches to enhance data sharing while acknowledging the challenges and complexities inherent in the agricultural sector. This round table sought to foster a shared understanding among diverse stakeholders and lay the foundation for continued collaboration in agricultural data management.

## Practical lessons from data hub implementation

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The International Dairy Data Exchange Network (iDDEN) was developed to optimise data exchange between dairy herds, dairy data organisations, farm service providers, dairy equipment manufacturers and on-farm software organisations. The implementation of iDDEN's data exchange hub by dairy data organisations and equipment manufacturers provides practical lessons on solving the drivers, barriers and challenges of innovation uptake and change in this area.

The drivers of more streamlined data exchange are from organisations seeking operational efficiencies and lower operating costs (including reduced manual data entry and transfer), standardisation of interfaces and animal data, access to data that is currently inaccessible or difficult to retrieve, and a consistent data transfer mechanism for the increasing number of devices and sensors on-farm.

Barriers and challenges to optimising data exchange and uptake that iDDEN has addressed are a combination of technical, regulatory, and organisational elements.

A standardised approach and the use of open standards means that technical difficulties and obstacles to implementation are relatively minor compared to these other factors.

Regulatory barriers are usually due to confusion about data use regulations and oversight and having to translate and understand legal jargon, especially across different countries. These hurdles can be overcome via good communication and the use of standardised, simple data use agreements.

Organisational barriers include a desire to 'control' data, a lack of a data management strategy, or an unclear business case on the value of data sharing. iDDEN has worked with both technical teams and senior management to ensure there is not a disconnect in the organisation about the importance of data exchange and it is seen as a business imperative.

*Keywords: data exchange, data transfer, innovation, standardisation.*

The International Dairy Data Exchange Network (iDDEN) was developed to optimise data exchange between dairy herds, dairy data organisations, farm service providers, dairy equipment manufacturers and on-farm software organisations.

iDDEN is the largest international dairy data partnership, bringing together farmer-owned organizations and national databases across thirteen countries representing approximately 200,000 dairy herds, 20 million dairy cows in total and 13 million milk recorded dairy cows.

### Abstract

### Introduction

iDDEN is owned and governed by a consortium of farmer-controlled member organizations from different countries providing dairy data services in Australia, Austria, Belgium, Canada, Denmark, Germany, Iceland, Finland, Luxembourg, Norway, Sweden, The Netherlands, and the United States. The seven iDDEN foundation shareholders are CRV, DataGene, Lactanet, National Dairy Herd Information Association (NDHIA), NCDX (Nordic countries), RDV, and vit.

The iDDEN hub enables two-way data exchange between farm management system software located on-farm and cloud-based farm management system solutions with milk recording and other industry organisations databases located around the world.

The drivers of more streamlined data exchange are from organisations seeking operational efficiencies and lower operating costs (including reduced manual data entry and transfer), standardisation of interfaces and animal data, access to data that is currently inaccessible or difficult to retrieve, and a consistent data transfer mechanism for the increasing number of devices and sensors on-farm.

The implementation of iDDEN's data exchange hub by dairy data organisations and equipment manufacturers provides practical lessons on solving the drivers, barriers and challenges of innovation uptake and change in this area.

## The barriers and challenges to data exchange

The barriers and challenges to optimising data exchange and uptake that iDDEN has addressed are a combination of technical, regulatory, and organisational elements.

### Technical

#### Technical barriers and challenges

Data hub implementations involve integrating data from various sources and systems. This can result in complexity and challenges associated with data integration, such as data format differences, data quality issues, and data synchronization problems. It underscores the need for robust and standardised data integration strategies and tools.

Data hub implementations often involve an iterative process of development and refinement. Many organizations realize the importance of adopting an agile approach, enabling them to iterate and test the data integration before going into the full 'production' environment. Flexibility, adaptability, and continuous 'real world' testing become essential for addressing technical barriers and challenges with implementing a data exchange.

A data hub implementation highlights the importance of establishing centralized data governance processes and frameworks. It is evident that consistent data definitions, standards, and policies are crucial for ensuring data quality, integrity, and security across the organization and its data exchange partners.

Specific technical barriers and challenges to data exchange encountered to date include dairy data organisations, farm service providers, dairy equipment manufacturers and on-farm software organisations operating across different countries, languages, and data providers (using different data definitions). The key technical barrier however is a lack of people or resources allocated to the data hub implementation and integration.

A standardised approach and the use of open standards means that technical difficulties and obstacles to implementation are relatively minor compared to these other factors. Key approaches have been:



- Standardize as much as possible – for example, using Open Standards and International Committee for Animal Recording (ICAR) Animal Data Exchange (ADE) data definitions. iDDEN has implemented the ICAR ADE data message standards, and these common standards and guidelines make data interchange easier and more effective.
- Provide a high level of technical support, including an information pack for new users, biweekly coordination meetings during the integration phase, and technical discussions via a dedicated Slack channel.
- Provide support tools such as a Translation Tool to support companies for different languages and markets and an Admin Tool to monitor the day-to-day operation of the data exchange.
- Engage a professional service partner; in iDDEN's case, Mtech (Finland), an experienced agricultural software service provider.
- Ensure a critical size of the organisation to finance the necessary technical infrastructure and to influence or set data standards.

Solutions and practical lessons learned.

Data hub implementations necessitate handling data from multiple sources. Organizations appreciate the criticality of robust technical and organisational measures to protect data security and ensure compliance with regulations. Specific regulatory or legal barriers and challenges encountered to date with some of iDDEN's partners include confusion about data use regulations in different countries or jurisdictions, disconnection between technical teams, senior management, and legal representatives (especially around interpreting and understanding legal jargon). However, the key concern raised during discussions on data hub implementation is whether data is stored or not.

Regulatory / legal

Regulatory and legal barriers and challenges

The iDDEN solution is designed to ensure that no data is stored within the system other than temporarily to deal with technical interruptions and the use of log files to help customers monitor their own data exchange.

Solutions and practical lessons learned

iDDEN uses standardised, simple data use agreements and authentication approaches to ensure that farmers retain control of their data. iDDEN also has an international approach in dealing with regulatory and legal matters, especially as many current and potential partners most are global or operate across several countries.

Implementing a data hub often requires a cultural shift within the organization towards a data-driven mindset. iDDEN's experience shows that organizations with successful data exchange implementations have senior executive sponsorship, technical buy-in, and a "data sharing" culture at all levels. Collaboration and communication become vital to achieving data-driven objectives.

Organisational

Organisational barriers and challenges

Companies that are unclear on the value of a data exchange usually have no data management strategy or want to 'control' data (both their 'own' and even that from other sources). Often, in these cases, data exchange is seen as a "technical function" instead of a business priority and there is a disconnect between the technical teams

and senior management (the “decision makers”). As a result, there is limited focus and budget allocated to data exchange.

### Solutions and practical lessons learned

A key lesson from data hub implementations is the focus on deriving tangible business value. Successful organizations align data initiatives with strategic objectives and prioritize use cases that deliver significant outcomes. The key lessons in this area have been the importance of ongoing engagement and communication at multiple levels with partner organisations. Other key factors to overcome organisational barriers include simplifying the data exchange process as much as possible, having ownership and governance of iDDEN by a consortium of farmer-controlled member organisations from around the world, and implementing an Advisory Committee comprising iDDEN shareholders and strategic partners.

### Conclusions

Overall, implementing a data hub has provided practical insights into various aspects of data management, governance, integration, security, and culture. These lessons have guided iDDEN and its partner organizations in building robust data ecosystems that support informed decision-making, innovation, and competitive advantage.

A summary of practical lessons from iDDEN’s data hub implementation:

- Farmers want their service organizations to help them make better use of data.
- The benefits to data exchange are clear – but not all companies or organisations have seen the value (yet).
- Overcoming technical barriers to data exchange is relatively easy, especially when a standardised approach and common data standards are used.
- Legal or regulatory barriers can be overcome by only exchanging (not storing data), authentication, and standard agreements.
- Any organisational barriers are addressed by frequent communication, simplifying data exchange, and building trust.

## Development of an automated quality control pipeline to facilitate the reporting of major gene genotypes

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The Irish Cattle Breeding Federation (ICBF) national cattle database stores in excess of 3.1 million genotypes, from both dairy and beef herds consisting of both purebred and crossbred animals. The reporting of genomic mutations with large effect termed major genes, is of benefit to breeders and industry, providing valuable information on both desirable and undesirable major genes segregating within herds. Pre genomics, the major gene status of an animal, for example for some genetic diseases, was only discovered following the birth of an affected calf. Genotyping allows the identification and management of animals and their major gene status before the birth of any progeny. Recently, the ICBF have developed an automated pipeline to facilitate the largescale reporting of a panel of major gene genotypes. The pipeline consists of a series of additional quality control steps to increase reliability in the final genotype call for automated reporting. Current quality control steps in the pipeline include a manifest call rate of  $\geq 97\%$ , custom confidence scores (variant and genotype specific), SNP classification categories (plate specific), custom clustering separation (variant and genotype specific), minimum X and Y signal intensities, heterozygosity threshold check, and sire/dam/trio (where available) Mendelian checks. The development of this major gene pipeline will provide additional information to industry to aid breeding decisions and an opportunity to develop mating strategies where useful.

### Abstract

The Irish Cattle Breeding Federation (ICBF) national cattle database formed in 1998, stores in excess of 3.1 million genotypes, from both dairy and beef herds consisting of both purebred and crossbred animals. Services such as genetic/genomic evaluations, parentage verification, gender verification, breed composition and more recently major gene status, are provided to farmers and can be used to support breeding decisions on farm.

### Introduction

Such services are enabled by using the ICBF custom genotyping platform, termed The International Dairy and Beef SNP Chip (Mullen *et al.*, 2013), of which there are five iterations to date. The latest iteration of this platform (IDBv5), consists of 51,421 SNPs including the International Society of Animals Genetics (ISAG) recommended 200 SNPs for parentage verification, the International Committee for Animal Recording (ICAR) 554 SNPs for parentage discovery, an updated ICBF 800 parentage SNP panel (McClure *et al.*, 2018), a large number of genome wide polymorphisms for genomic evaluation and research purposes, and approximately 190 major genes which can be further categorised based on their effect; Beneficial, Meat, Milk, Colour, Unwanted and Lethal. These major genes underly a broad range of genetic conditions observed in many cattle breeds such as conditions that are favourable to breeders, conditions underlying pigmentation, conditions affecting milk and meat productivity, conditions

that affect the animal before it can make an economic return, and conditions that result in embryonic lethality (McClure & McClure, 2016). Leveraging genomic data will help to monitor and manage genetic conditions known to be segregating within Irish cattle populations, including the identification of carrier animals.

The major gene status of animals is of interest to cattle breeders due to both the desirable and undesirable effects of genetic conditions on production and performance, ultimately impacting the profitability of farming enterprises (Cole *et al.*, 2016). Prior to the genomics era, a major gene carrier was only identified after an affected calf was produced, due to the phenotypic resemblance between both carrier and normal animals for many genetic conditions (Cieploch *et al.*, 2017). Since the advent of genotyping, major gene status can be determined before the animal reaches sexual maturity, allowing breeders to make more informed breeding decisions, for example, to develop mating strategies where carriers have been identified and reduce the risk of producing affected offspring or conversely to increase the frequency of a desirable major gene in their herd (McClure *et al.*, 2013).

The current process in place for the reporting of major genes in Ireland is handled by a commercial service provider (Weatherby's Ireland), where any breeder, artificial insemination (AI) company or herdbook can make a request. For the service provider, this is a manual process whereby the genotype is analysed on output from the genotyping process. There is also a cost for the breeder, irrespective of the royalty status of the major gene requested. With this in mind, the ICBF aimed to develop an automated quality control pipeline which would facilitate the largescale routine reporting of major gene genotypes. The metrics applied as part of the pipeline are described herein..

## Methods

### Major Gene Reporting

The International Dairy and Beef (IDB) is the ICBF custom genotyping platform, of which there are five iterations to date. The current iteration termed IDBv5, is a ThermoFisher Applied Biosystems™ Axiom™ Genotyping array. The major gene pipeline consists of a series of quality control metrics associated with each SNP, genotype and genotyping plate (n=384 samples). The pipeline is initially focused on genotypes derived from the IDBv5 platform. The aim of the pipeline is to improve the reliability of the final genotype call for automated reporting. At the time of submission, there were a total of 3,124,175 genotypes in the database, of which 1,114,739 are IDBv5 genotypes (CR>=0.97) and are eligible for the major gene pipeline.

Current quality control metrics included in the major gene pipeline are described in Table 1. Generic quality control thresholds applied to genotypes include; an animal call rate of >=0.97 and a Mendelian check (Parents and Trio). Three additional metric thresholds which are outputs of the Thermofisher genotyping process are also applied to each SNP and genotype; Clustering separation X contrast values, Confidence Score and minimum X and Y signal intensity values. Thresholds applied are specific to each SNP and genotype. One quality control metric (SNP classification) is applied to each genotyping plate.

## Results and discussion

Genotypes from the major gene pipeline, released since November 2022, include major genes of immediate interest to breeders and industry, namely Myostatin and Polled status. With regards to Myostatin, there are nine variants routinely reported including *L64P*, (Dierks *et al.*, 2014) *F94L*, *nt419*, *Q204X*, *E226X*, *C313Y* (Grobet *et al.*, 1998), *nt821del11* (Grobet *et al.*, 1997) *S105C* and *D182N* (Dunner *et al.*, 2003) (Table 2).

Table 1 Description of QC metrics included in the major gene pipeline\*.

Metric	Specific to	Description
Animal Call Rate (ACR)	Genotype	ACR threshold of $\geq 97\%$ . The call rate is defined as the proportion of SNPs with a genotype call for each individual i.e. the number of called SNPs/ the total number of SNPs.
Mendelian Check	Genotype	To detect scenarios where the genotype of the individual is not consistent with the transmission pattern expected according to Mendel's law of inheritance using comparisons to both parents individually and as a trio where available.
Cluster Separation	SNP and Genotype	Thresholds applied to contrast values based on the clustering resolution of each genotype class. Clusters should be well separated and distinct from each other, be well-formed and have no visible cluster abnormalities.
Confidence Score	SNP and Genotype	The confidence score is described as 1 minus the posterior probability of the genotype belonging to the assigned genotype cluster. It can range between zero and one, with lower confidence scores indicating more confident genotype calls.
SNP Classification	Plate	Each genotyping plate is classified into one of the six SNP classification categories – PolyHighResolution, MonoHighResolution, NoMinorHom, CallRateBelowThreshold, Off Target Variant and Other. This metric analyses the performance of the AA, AB and BB clusters, and their relationship to each other.  <b>PolyHighResolution</b> - SNPs with well separated, distinct genotyping clusters and $>2$ occurrences of the minor allele. <b>MonoHighResolution</b> - SNPs with one distinct and well-formed genotyping cluster - all genotyped samples are monomorphic/homozygous. <b>NoMinorHom</b> - SNPs with well separated, distinct genotyping clusters with no minor homozygous genotypes i.e. One cluster is homozygous and one is heterozygous (for biallelic SNPs). <b>OffTargetVariant (OTV)</b> - SNP sites whose sequences are significantly different from the sequences of the hybridisation probes. <b>CallRateBelowThreshold</b> - SNP call rate is below the threshold, but other QC metrics are acceptable. <b>Other</b> – At least one QC metric is not meeting the required threshold.
Signal Intensity	SNP and genotype	Minimum thresholds applied to X and Y intensity values to identify and exclude low intensity genotypes.

\*More details can be found in Thermo Fisher Scientific Inc (2020).

Some variants are positioned in exonic regions of *MSTN*, located on BTA2 (Positions based on assembly ARS UCD 1.2 of the *Bos taurus* genome build). Additionally of interest is the Polled Celtic variant, a complex rearrangement positioned between IFNAR2 and OLIG1 (Aldersey *et al.*, 2020; Allais-Bonnet *et al.*, 2013; Medugorac *et al.*, 2012) (Table 3).

Myostatin has been the subject of interest to cattle breeders for some time, primarily due to its effect on carcass performance but also due to its negative impact on calving difficulty (Purfield *et al.*, 2019; Bellinge *et al.*, 2005; Casa *et al.*, 1999). Additionally, Polledness in cattle resulting in the absence of horns, is a favourable trait for many breeders, alleviating the cost associated with dehorning and averting associated injuries, safety and welfare concerns (Aldersey *et al.*, 2020; Allais-Bonnet *et al.*, 2013; Medugorac *et al.*, 2012).

*Table 2. RS IDs (where available), coordinates and OMIA references for Myostatin variants routinely released since November 2022 as part of the ICBF major gene pipeline (Positions based on assembly ARS UCD 1.2 of the Bos taurus genome build).*

Variant	Rs ID	Coordinates	Amino Acid Change	OMIA
L64P	rs449270213	2:6279187	p.Leu64Pro	000683-9913
F94L	rs110065568	2:6279278	p.Phe94Leu	000683-9913
S105C		2:6279310	p.Ser105Cys	000683-9913
nt419		2:6281243	-	-
D182N		2:6281368	p.Asn182Asp	000683-9913
Q204X	rs110344317	2:6281434	p.Gln204X	000683-9913
E226X		2:6281500	p.Glu226X	000683-9913
nt821del11	rs382669990	2:6283674	p.Glu275ArgfsX14	000683-9913
C313Y		2:6283794	p.Cyc313Try	000683-9913

*Table 3. RS IDs (where available), coordinates and OMIA references for the Polled Celtic variant routinely released as part of the ICBF major gene pipeline - (Positions based on assembly ARS UCD 1.2 of the Bos taurus genome build).*

Variant	Rs ID	Coordinates	Amino Acid Change	OMIA
Polled Celtic	-	1:2429327_2429336del- 2429109_2429320dupins	-	000483-9913

Since the implementation of the pipeline in November 2022, 1,107,481 genotypes have been released for the nine Myostatin polymorphisms and the Polled Celtic variant. Of this number, the sample pass rate ranges from 91.5 % for S105C to 99.1 % for C313Y, with an average sample pass rate of 95.6 % (Table 4)

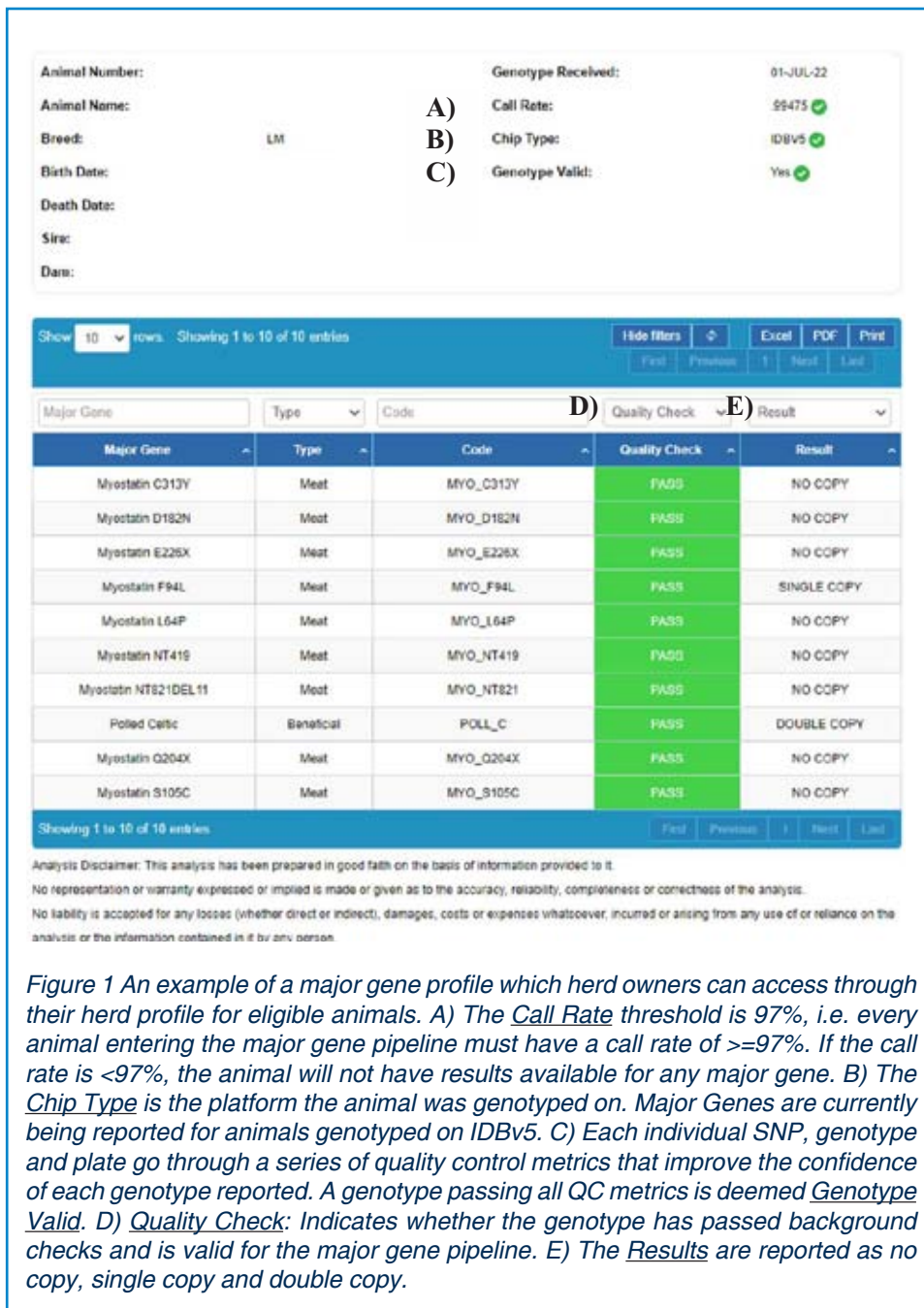
Communication of results to farmers, herd-books and AI companies is through herd profiles on the ICBF website, where herd owners can access major gene reports for each animal that meets the criteria (Figure 1). Additionally, results may be reported on EU Zootechnical certificates where results are stated in a section dedicated to genetic defects and genetic peculiarities.

This pipeline provides valuable information on the major gene status of animals which herdowners may incorporate to aid breeding decisions. Moreover, the reporting of results to cattle herd-books ensures the monitoring and management of major genes

*Table 4. Animal Pass rates for the 10 major genes routinely released as part of the ICBF major gene pipeline since November 2022.*

Locus	MG variant	Total passed*	Pass rate (%)
MSTN	L64P	1,059,810	95.7
MSTN	F94L	1,038,833	93.8
MSTN	nt419	1,037,607	93.7
MSTN	S105C	1,012,891	91.5
MSTN	D182N	1,083,609	97.8
MSTN	Q204X	1,091,075	98.5
MSTN	E226X	1,044,293	94.3
MSTN	nt821del11	1,091,882	98.6
MSTN	C313Y	1,097,347	99.1
POLLED	Polled Celtic	1,046,889	94.5

\*Total passed samples of total samples through the pipeline (n=1,107,481).



segregating within cattle breeds. Ongoing and future work includes expanding the major genes incorporated into the pipeline for routine release, including Polled Friesian, lethals and colour related major genes.

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## Agri-food Data Canada: A data ecosystem serving agri-food sustainability

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Agri-food Data Canada (ADC) is creating a data ecosystem serving agri-food sustainability. Through investments in technology, infrastructure, and culture, we are helping researchers and the research community get more value from the data researchers are already collecting. Agri-food Data Canada's approach is guided by the FAIR data principles (that data should be Findable, Accessible, Interoperable and Reusable). To improve data FAIRness ADC is

1. Creating a semantic engine that will help researchers create and use better machine-actionable, reusable, and accessible descriptions and governance for their data, projects, algorithms, tools, workflows, and other digital research outputs;
2. Collaborating on projects supporting the federation of data silos, to ensure that data, metadata, and access rights can travel with the data from source to destination within the ADC federation;
3. Developing tools to help researchers with data provenance and traceability; and
4. Creating a culture of FAIR data by developing knowledge-sharing resources such as webinars, training, and teaching materials.

ADC works with partners to align our approaches and contribute to the global research community, with the goal to ensure research data is FAIR. One collection of tools that are under development at ADC is the Semantic Engine. While there are many approaches to harmonizing data through the creation of data platforms, ADC sees the value in adding value to heterogeneous data through the creation of tools that improve data without the necessity of data platform infrastructure. Researchers can improve their data documentation workflows by adding context to their data through the creation of machine-actionable data schemas. At the heart of the Semantic Engine is the Overlays Capture Architecture (OCA), an international open standard created by the non-profit organization Human Colossus Foundation. OCA's layered architecture is machine-actionable and easy to generate. OCA schemas allow multiple contributors to improve a schema independently and permits the bundling of schemas with appropriate task-specific schema overlays. Schemas can be internationalized through the creation of language-independent overlays, and their additions do not change the underlying structure of the schema which ensures interoperability and allows schemas to be continually improved throughout the dataset's lifecycle. OCA also permits the use of downstream data validation rules carried by schemas and enables the incorporation of ontological terms. For example, ontologies, terms, and data standards endorsed by ICAR can be added to schemas to improve data interoperability and harmonization, which are essential for advancing the international agri-food sector. Agri-food Data Canada is developing a powerful collection of tools and creating a data ecosystem that will reduce barriers to data documentation, ease data sharing, and support the international agri-food sector's data needs.

### Abstract

*Keywords: FAIR data, metadata, data governance*

## Introduction

Agri-food Data Canada (ADC) is creating a data ecosystem serving agri-food sustainability. Through investments in technology, infrastructure, and culture, we are helping researchers and the research community get more value from the data researchers are already collecting. Agri-food Data Canada's approach is directed by the FAIR Guiding Principles. According to Wilkinson *et al.* (2016), the FAIR principles provide a framework for making data Findable, Accessible, Interoperable, and Reusable.

Findability emphasizes the need for data to be assigned globally unique and persistent identifiers, described with rich metadata that includes identifiers, and registered or indexed in searchable resources. Accessibility focuses on ensuring data can be easily retrieved using standardized protocols that are open, free, and universally implementable, while also maintaining access to metadata even when the data itself is no longer available. Interoperability emphasizes the use of formal and broadly applicable languages for knowledge representation, along with vocabularies that align with FAIR principles, and the inclusion of qualified references to other data. Reusability stresses the importance of richly describing metadata with accurate attributes, associating data with clear and accessible usage licenses, and meeting domain-relevant community standards. By adhering to the FAIR principles, data becomes discoverable, accessible, compatible, and usable, enabling broader and more effective data sharing and integration across disciplines and communities.

To enhance the FAIRness of data, ADC is implementing several initiatives. These include:

- **Creating the Semantic Engine:** A tool designed to assist researchers in generating and utilizing machine-actionable, reusable, and accessible descriptions and governance for their data, projects, algorithms, tools, workflows, and other digital research outputs.
- **Collaborating on federated data projects:** ADC actively engages in collaborative efforts aimed at supporting the federation of data silos. This ensures that data, metadata, and access rights can seamlessly travel with the data from its source to its destination within the ADC federation.
- **Developing data provenance and traceability tools:** ADC is committed to building tools that aid researchers in capturing and tracking data provenance, promoting transparency and reproducibility in research.
- **Cultivating a culture of FAIR data:** ADC fosters a culture that promotes FAIR data principles by creating and sharing knowledge-sharing resources, such as webinars, training sessions, and teaching materials. These resources aim to educate and empower researchers to adopt and implement FAIR data practices.

Agri-food Data Canada is actively contributing to improve the FAIRness of data and facilitate the adoption of FAIR data principles within the agri-food research community. Unlike other methods of harmonizing data through data platforms, ADC recognizes the significance of enhancing heterogeneous data directly without relying on complex data infrastructure. One promising suite of tools that are under development at ADC is the Semantic Engine, which will empower researchers by enabling them to enhance their data documentation workflows effortlessly for effective uptake of data share and reuse.

## Semantic Engine

The Semantic Engine is a suite of tools being developed by ADC to help researchers write rich contextual data documentation based on machine-actionable data schemas, thereby improving its overall quality, portability, standardization, and reuse. These tools

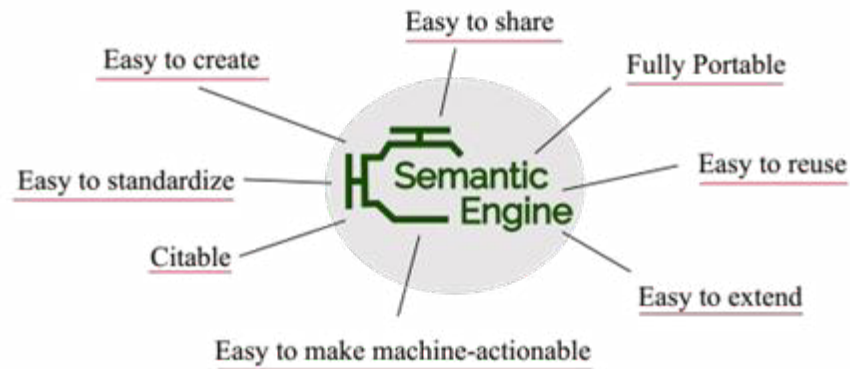


Figure 1. Semantic Engine is a suite of tools that will help researchers write rich contextual data documentation.

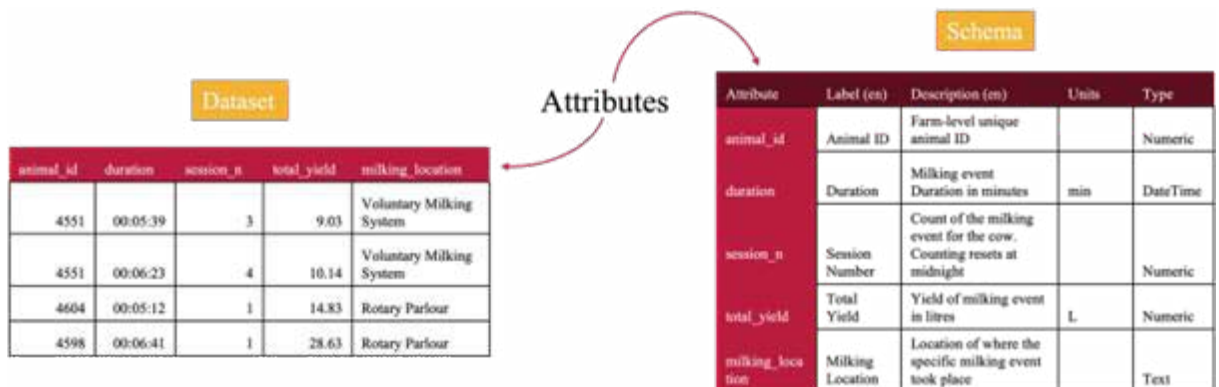


Figure 2. Data must be structured to be understood and a schema describes the structure of the dataset.

will offer a practical solution for researchers looking to maximize the value of their data without the burden of implementing extensive data platform frameworks.

In practical terms, the Semantic Engine offer numerous benefits that cater to various stakeholders and collaborators, including the researchers themselves. These tools will avoid ‘mystery’ data by providing improved data descriptions, allowing for a clearer understanding of the data’s content and context. Additionally, they allow researchers to adjust the level of detail based on specific needs, ensuring that the data is comprehensive yet focused. By using these tools, researchers can deposit high-quality data with less effort, saving time and resources while enhancing the overall value and usefulness of the data.

Another significant advantage of the Semantic Engine is its ability to aid others in utilizing research data effectively. By providing better data descriptions and context, researchers can reduce the time spent supporting individuals who are working with their data, enabling them to navigate and interpret it more efficiently (Figure 2). This is particularly valuable in cross-disciplinary research where data from different domains may need to be integrated. These tools empower researchers to communicate the necessary information clearly, improving data understanding and facilitating collaboration across diverse research fields.

There is an additional benefit, as it also caters to machine consumption of data. Using machine-readable schemas, data can be easily discovered and utilized by automated systems. Publishing these schemas promotes better collaboration and interoperability, allowing researchers to integrate data from various sources seamlessly. The ability to assign a separate DOI to the schema ensures that it can be cited and used by others, contributing to a more robust and efficient scientific ecosystem. By improving data accessibility and usability for machines, these tools pave the way for better science outcomes derived from high-quality and well-documented data.

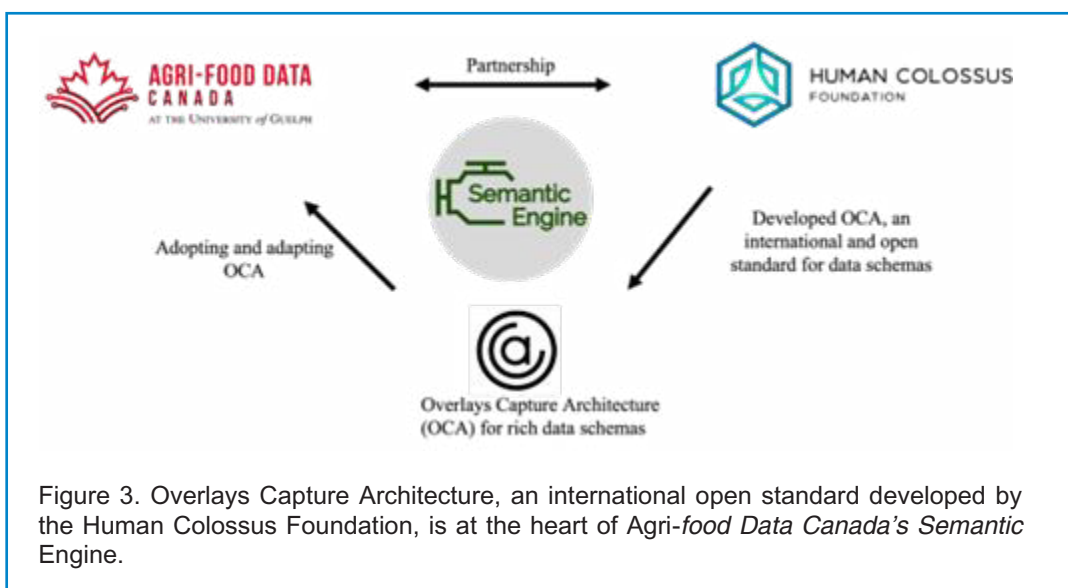


Figure 3. Overlays Capture Architecture, an international open standard developed by the Human Colossus Foundation, is at the heart of Agri-food Data Canada’s Semantic Engine.

### Overlays Capture Architecture

To create the Semantic Engine, ADC is partnering with the Human Colossus Foundation to adopt its work on Overlays Capture Architecture (OCA) as the underlying schema standard and adapt it to the agri-food research area (Figure 3).

Overlays Capture Architecture is an extensible, flexible, international, open, and machine-accessible standard for data schemas (Knowles, 2022). From a table representation of a schema, an OCA schema splits each feature into a separate layer and each layer is a separate file (written in a machine-readable format) that recognizes the capture base, or the foundation of the schema describing the data set (Figure 4). Layers are added to the schema to provide more detail, making it easier to understand and use data collected and structured according to the associated schema.

Overlays Capture Architecture schemas offer a flexible and collaborative approach to schema development by allowing multiple contributors to enhance a schema individually. This decentralized approach enables each contributor to work on specific aspects or components of the schema without disrupting the underlying structure. Furthermore, the ability to add new elements or modify existing ones in overlays ensures that schemas can be continuously improved and refined throughout the lifecycle of a dataset, promoting long-term data quality and adaptability to evolving requirements.

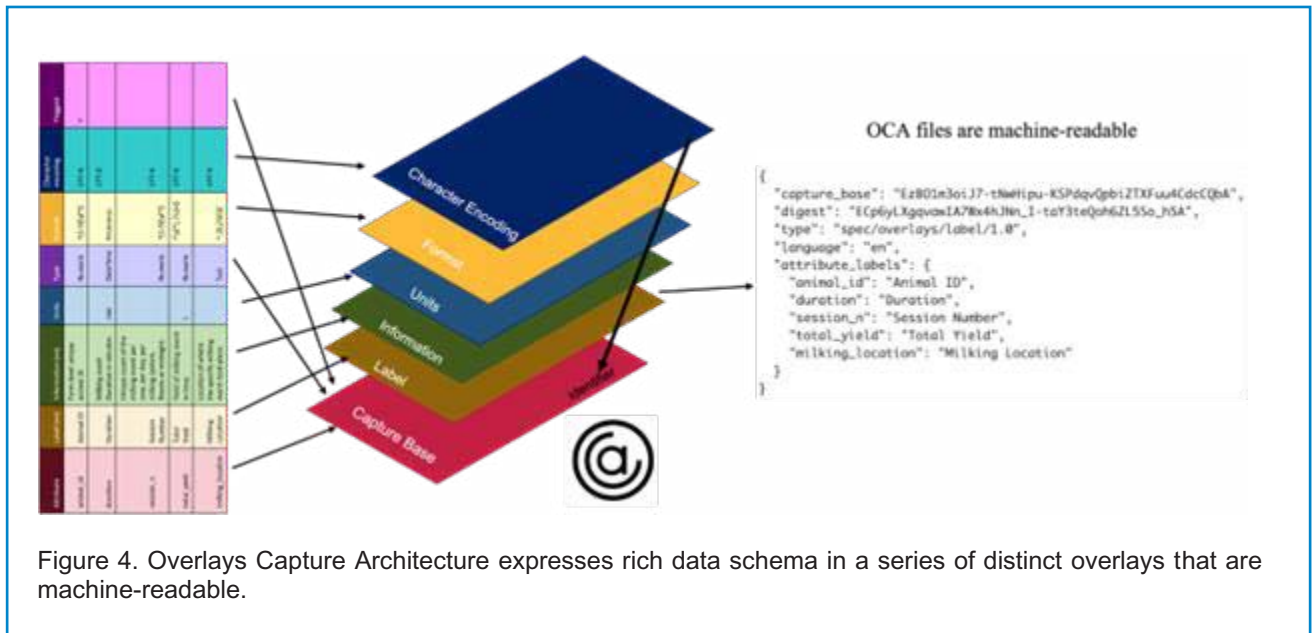


Figure 4. Overlays Capture Architecture expresses rich data schema in a series of distinct overlays that are machine-readable.

Additionally, OCA schemas support internationalization through the creation of language-independent overlays, which enables the translation of schema elements into different languages without altering the schema's core structure. This feature enhances interoperability across different linguistic contexts and enables the seamless exchange of data between diverse systems.

The benefits offered by ADC through the Semantic Engine and OCA are applicable for researchers in the agri-food sector and directly extendable to members of the International Committee for Animal Recording (ICAR) in numerous ways. Firstly, OCA allows for the inclusion of downstream data validation rules through schemas, ensuring data quality and consistency. This feature is particularly valuable for ICAR members as it enables the incorporation of ontological terms and data standards endorsed by the committee, facilitating data interoperability and harmonization. By leveraging OCA and its integration capabilities, ICAR members can enhance the international agri-food sector by promoting standardized and consistent data management practices.

ADC's development of a comprehensive collection of tools and a robust data ecosystem further benefits ICAR members. These initiatives address the challenges faced by the international agri-food sector, such as barriers to data documentation and data sharing. ADC's tools simplify the process of data documentation, making it more accessible and efficient for researchers to capture and share valuable data.

Agri-food Data Canada is developing a data ecosystem to serve agri-food sustainability through a powerful collection of tools that will reduce barriers to data documentation, ease data sharing, and support the international agri-food sector's data needs. This data ecosystem will foster collaboration and cooperation among stakeholders, enabling seamless data integration and enhancing the overall data needs of the agri-food sector.

### Applications for the agri-food sector



## References

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## Data investment strategy - the core issue for genetics organisations in the genomics era

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Genetic evaluation and improvement in the genomics era is underpinned by genomic reference populations. Traditionally, evaluation systems servicing multi-stakeholder situations (such as national or breed evaluation systems) have acted as recipients of data – essentially dealing with what is submitted by breeders and others, usually with some protocols around data collection and encouragement in relation to traits seen as most important. Typically, this stance results in a preponderance of data relating to easy-to-measure traits, such as milk volume or early life weight records. This may be despite such traits only comprising a moderate proportion of the value defined by the breeding objective(s), and accordingly, genetic improvement must be limited both in direction (alignment with the breeding objective) and speed (rate of progress for the objective). This limitation can become worse under genomic selection, as differences in accuracies among traits with differing numbers of records will be increased.

Addressing this potential problem requires an active strategy for phenotypes – either via direct investment and/or by contracting to obtain more desirable (economically valuable).

The extent of potential problems is here explored via a case study using ICBF data for several beef and dairy breeds, and elements of an active phenotype investment strategy are then discussed.

Failure to implement such strategies will increase risks of fragmentation, leading to possible reductions in rate and value of genetic improvement. Data investment strategy also interacts with progeny testing and approaches to finding new elite animals, and reflect the importance for evaluation organisations of clarifying their role(s)

### Abstract

Genomic prediction is now central to all major livestock genetic improvement applications, including evaluation systems at both the breed and national level.

Fundamental to useful genomic prediction is the genomic reference population (or information, or library), which consists of animals with pedigree information of some form, along with phenotype and genotype records.

The size, sampling and trait coverage determines the accuracy of breeding values estimated for animals without phenotypes, and can make a significant contribution to the accuracy of breeding values for animals with phenotypes. In broad terms, the reference population thereby determines the attraction to submit animals for evaluation,

### Introduction

whether they are potential candidates for selection or simply to assess their value for commercial production.

To date, genetic evaluation systems have in the main conducted analyses for the traits that are present ie that have been recorded at some reasonable scale, in the population of interest. This pragmatic approach may be despite the breeding objective(s) developed for the population: for example, fertility may be an objective trait (ie one with an economic weight) but if recording for the trait is limited or absent, then any genetic change in fertility will result almost solely from correlated responses to selection for well-recorded traits.

Extending this point, where a novel trait is developed via research and introduced, volumes of phenotypes for that trait may be limited for some time, particularly if it is costly or otherwise difficult to record. In this situation, the accuracy of genomic BVs for that trait will likely remain low despite its economic importance.

This situation generates risk for an evaluation system in two ways:

- The risk that selection for the trait will be limited, generating an opportunity cost
- The risk that alternative providers of genetic information may offer BVs for that trait, with whatever level of technical rigour, and attract participants away

This perspective points to a view of evaluation systems, whether at breed or national level, as platforms – infrastructure which can support a range of information services. As a general principle, the more such services can be obtained via a platform, the more attractive that platform is to existing and potential new users. In the context of traits, that means having sufficient data available to the platform for all economically valuable traits – including those that are assessed as having potential future value.

Attractiveness of the platform is critical because as a general principle, platforms whether at national or breed level will (or should) seek to maximise participation, in order to:

- Spread fix costs over a broader user audience
- Maximise scope for genetic improvement by maximising numbers of animals evaluated
- And maximise relationship to the active population, enhancing the accuracy and hence utility of genetic parameters and information

Given that data collected underpins the utility of the platform, this in turn points to the importance for platform managers of having an informed and active strategy for data – which given the fact that data collection costs money, means an investment strategy: what traits will be recorded, by whom, and on what terms made available to the platform.

This paper focussed on a first step in considering strategies for investment in data to support genetic evaluation and improvement: the assessment of the strength or balance of a data portfolio.

## Materials and methods

The value of a data “portfolio” can be summarised simply as the average or expected accuracy of genomic BVs for young animals evaluated via the platform. The balance of that portfolio can be assessed by the relationship between the array of trait accuracies and the corresponding array of trait economic weights.

Here, trait reliabilities and economic weights for the Irish national beef and dairy evaluations are considered.

ICBF data was provided by Dr Siobhan Ring (ICBF) for the following:

- Trait reliabilities (REL) for the last 6 years for the traits in the beef and dairy evaluations. Individual trait reliabilities were averaged across the 6 years (NB: while not comprehensively analysed at this stage, in general trait reliabilities were relatively constant across years).
- Trait economic weights (EW) for breeding objective traits were collated for the beef terminal and maternal indexes, and their corresponding reliabilities logged. In the dairy traits, where a trait was associated with economic weights for more than one index, the total absolute value of the economic weight was summed. Absolute values were used for all economic weights (reflecting the fact that the size of the economic weight is an indication of how much we want to change the trait, or in simple terms, its relative importance).
- These were calculated for Angus and Limousin of the beef breeds, and Holstein (HOL) of the dairy breeds.

The correlation between trait average reliability and economic weight was calculated.

Plots have been made of the average reliability against the absolute economic weight, shown as proportion of the highest absolute economic weight of the breeding objective traits (PropEW). The x-axis for these plots is sorted from highest to lowest absolute economic weight (and PropEW).

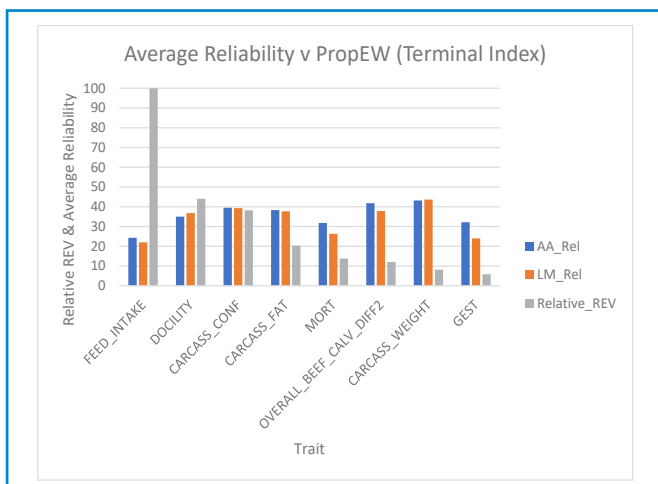
Results shown here are:

- The correlation between average reliability and absolute economic weight for breeding objective traits for each of the breeds in the categories beef terminal, beef maternal and dairy
- The respective plots of average reliability against absolute economic weight.

## Results

The correlations are -0.68 (ANG) and -0.40 (LIM) as shown in the below picture.

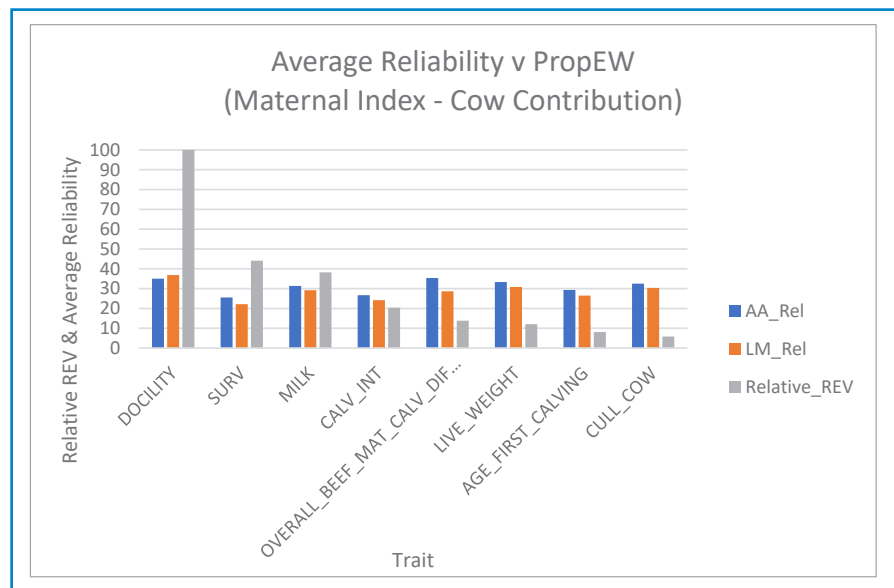
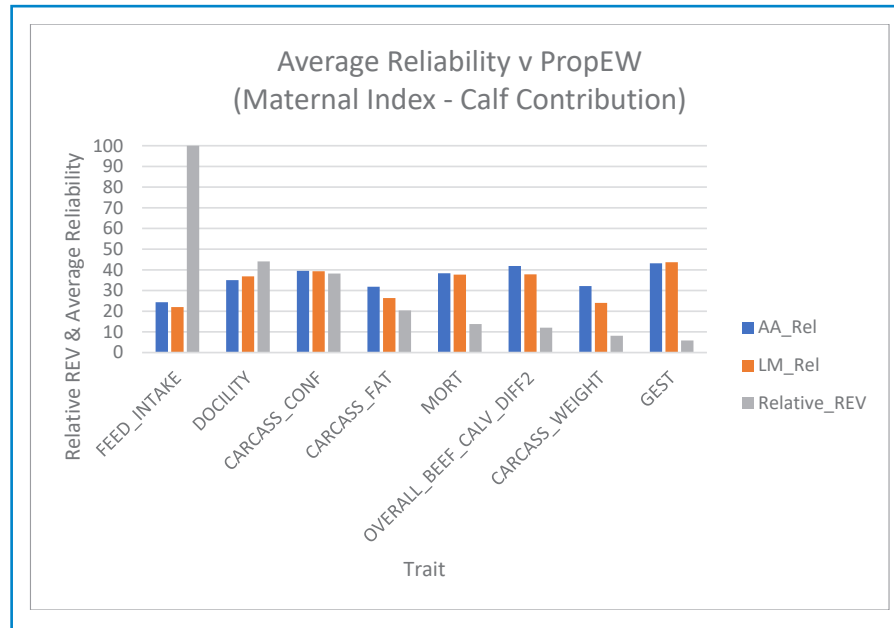
### Beef Terminal



**Beef – Maternal**

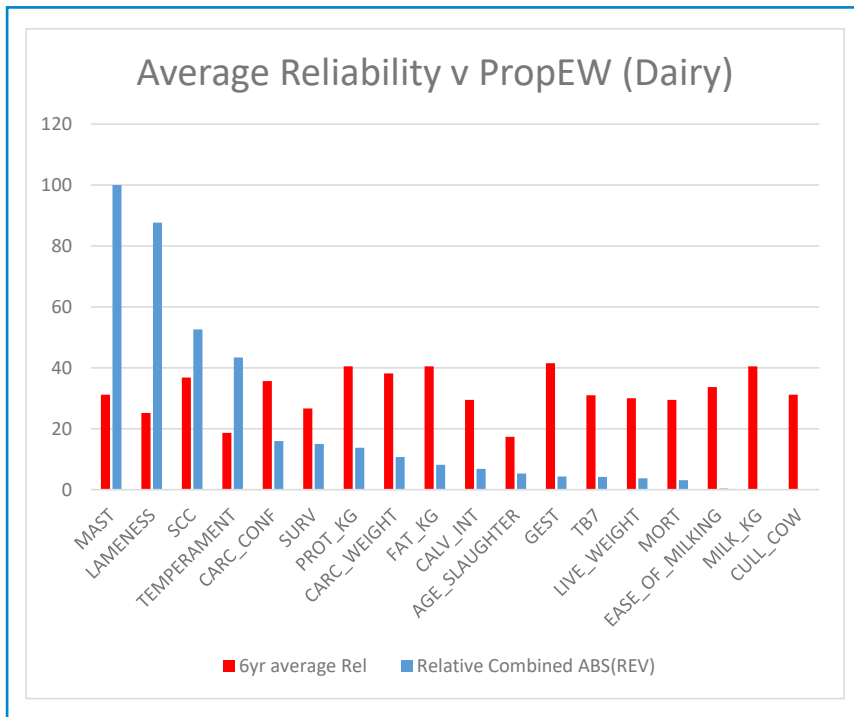
The correlations, calculated as calf traits and dam traits separately, and then together, are:

	ANG	LIM
Calf correlation	-0.69	-0.41
Cow correlation	0.38	0.69
Overall correlation	-0.05	0.15



The correlation is -0.40.

Dairy



The simple approach used here is based on the fact that accuracy (of EBVs) is one of the determinants of the rate of genetic progress ( $R = i \cdot rIT \cdot \Delta T$ , where  $rIT$  = accuracy).

Naively, the accuracies of traits in the breeding objective should correspond to their relative importance – the more valuable it is to change a trait, the more accurate the BVs would ideally be.

In principle, improved alignment of accuracy with trait importance provides the maximum opportunity to steer the genetic improvement in the trait direction implied by the EWs, with equal accuracy for all traits enabling best alignment with that direction.

Where there is variation in trait accuracy, that will likely result in genetic change being less than perfectly aligned with the optimal direction of change in trait space. (NB: this does not mean that the genetic change will not be valuable, simply that it could be more valuable).

This perspective should be modified to take account of the cost of recording different traits, but where capture of returns from genetic improvement is over a sufficiently large scale, this consideration becomes insignificant (Banks, 2022).

Given this perspective, what do the simple results show?

- In beef (terminal) for ANG and LIM, alignment between reliabilities and EWs is poor, largely reflecting the low accuracy for feed intake.
- At the same time, there is in a sense an “excess of accuracy” for Carcase Fat, Mortality, Overall Calving Difficulty, Carcase Weight and Gestation.

Discussion

The correlation is poorer in ANG than LIM, reflecting greater excess accuracy in the lower EW traits.

In beef (maternal), there is a marked difference between the calf traits and the cow traits: the correlation REL:EW is negative in the calf traits, reflecting low reliability for Feed Intake in both breeds. For the cow traits, overall the alignment is closer, but reliability for Docility is markedly lower than its relative importance.

In dairy, the overall alignment is relatively poor (correlation = -0.4) with a “deficit” of reliability for MAST, LAMENESS, SCC and Temperament, and “excess” for the remaining traits.

How significant are these findings, and what might be done in response?

The traits for which there are “deficits” of reliability include one obviously hard-to-measure trait in beef (feed intake), and several disease and ease of handling traits in dairy.

Assuming that the trait EBVs are from multi-trait analysis, then the extent of potential change in these traits is implicit in their reliabilities, and accordingly, potential responses in traits that have been defined as economically important will be constrained.

The obvious response is to invest in capturing more data for these traits. In the case of the disease and ease of handling traits in dairy, this does not seem to imply any or large investment in recording costs – simply getting the traits recorded by farmers. In the case of feed intake, off-farm or R&D investments seem the likely route in the short term, and are underway (e.g. Tully Station, Andrew Cromie, pers. comm.) – the question is whether enough data is being collected.

In the case of feed intake, it is possible that analysis of the cost-benefit of increased investment in recording should be done, although given the scale of the industry, it seems likely to be profitable (and see comment below re methane).

Some other observations prompted by these results include:

- The reliabilities seem very stable, and are not changing year to year (ie increasing) despite assumed accumulation of more data (phenotypes and genotypes).
- Reliabilities for some sets of traits are very similar – does this reflect assumed genetic parameters?
- Is Feed intake not important in dairy?
- Feed intake is a proxy for methane (Barwick *et al.*, 2019), and one would expect that the importance of feed intake would be increased if its use as a genetic correlate of methane were incorporated into the models – or more precisely, methane had its own economic value.

## Conclusions

A simple analysis of trait reliabilities for the Irish national beef and dairy evaluations shows weak relationship between trait economic importance and the reliability of the corresponding trait genomic BVs. Based on informal discussions, this observation is likely to be repeated in most breed and country evaluations.

This is not surprising, in that to date, recording has been mainly for traits that can readily be recorded on-farm. However, this limits the accuracy (reliability) that can be

achieved for hard-to-measure traits, and so limits the attractiveness of participation in the evaluation system.

Increasingly, attention will need to be paid to obtaining (or encouraging) appropriate volumes of data for hard-to-measure traits, and strategies to achieve this will become central to organisations (Banks, 2022). The simple approach used here can quickly highlight deficiencies in data, and help to focus recording efforts and investment.

**Banks, R.G.** (2022) Proc. WCGALP 12: 1804-1807 10.3920/978-90-8686-940-4

**Barwick, S.A., Henzell, A.A., Herd, R.M., Walmsley, B.J., and Arthur, P.F.** (2019) Genet. Sel. Evol. 51:18 <https://doi.org/10.1186/s12711-019-0459-5>

## References

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## Challenges and opportunities of milk recording methods in Irish dairy farms: A comparison of test day milk recordings and daily bulk collection samples

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The objective of this study was to explore and quantify the differences in fat and protein percentages between milk samples collected on test day milk recordings and those obtained from daily bulk collections in commercial dairy herds in Ireland. The dataset comprised 5,742 milk recordings from 2,841 herds in 2021, where daily bulk collections were taken on the same day as the milk recording. The predicted fat and protein percentages for test day milk were estimated using either the AM or PM milk samples and were incorporated into prediction equations approved by ICAR. The Pearson correlation between fat and protein percentages from test day milk recording and daily bulk samples was 0.82 and 0.96, respectively. These correlations correspond to mean differences of 0.1% and 0.01% for fat and protein percentages, with the average test day milk recording showing lower values for both measurements. Fat percentage correlations for herds using EDIY (electronic do it yourself meters which take a test sample automatically) and Non-EDIY (manual recordings where a milk recorder visits and takes a test sample) recording devices were 0.87 and 0.72, respectively. Herds with a higher average test day cow yield demonstrated a lower fat percentage correlation. Similarly, samples taken during peak milk production season exhibited larger differences in fat percentages compared to those taken during the off-peak milk production season. The findings of this study clearly indicate that the largest variation is observed in the fat percentage reported in test day milk recordings when compared to the corresponding daily bulk samples. The extent of this variation is affected by yield, season, recording type, or a combination of all three factors.

### Abstract

*Keywords: Milk recording; Irish dairy farmers.*

Irish dairy farming is currently facing numerous challenges, including regulatory, economic, and environmental pressures. To overcome these challenges and ensure sustainable, profitable, and efficient dairy farming practices, milk recording has become a vital tool. Milk recording organisations (MROs) provide this service during a milking, using milk meters to measure the volume of milk and take samples from each individual cow. This process grants farmers access to a significant amount of data, offering crucial insights into herd health and performance. As a result, farmers can make informed breeding decisions, identifying cows for replacements and determining which cows should be culled or not bred further.

The practice of milk recording on Irish dairy herds has been steadily increasing since the abolition of milk quotas in 2015. The percentage of milk recorded herds has risen

### Introduction

from 35% in 2018 to 44% in 2021 (ICAR, 2021). Recent years have seen a significant uptake in milk recording, with the latest data from the ICBF database showing a nearly 12% increase in the number of cows' milk recorded in 2022 compared to the same period in 2021 (ICBF, n.d., 2023a). One reason for this sudden increase is the introduction of new legislation in late 2022, which no longer permits blanket dry cow therapy. Instead, the legislation encourages the implementation of milk recording as a means of routinely monitoring mastitis levels, as well as managing dry cows and antibiotic use. Another contributing factor to the increased adoption of milk recording is the commitment made by the Department of Agriculture, Food, and the Marine (DAFM) to raise the percentage of dairy herds undergoing milk recording from the current level of 50% to 90% by 2030 (DAFM, 2020).

Traditionally, Ireland has been slower in adopting milk recording technologies compared to other dairy-producing countries such as the Netherlands and New Zealand, where 89% and 75% of herds were milk recorded, respectively, in 2021 (ICAR, 2021). The reasons for Irish dairy farmers' slower adoption of this technology are not fully understood. Despite the low total cost and labour associated with milk recording (€10 per cow to milk record four times per year with no upfront investment required), it is unlikely to be a reason for poor uptake (ICBF, n.d., 2023a). However, Balaine *et al.* (2020) suggest that the slower adoption may be due to ineffective communication of the many benefits of milk recording in previous years.

MROs offer two types of milk recording services: a) a manual recorder service, and b) an electronic "do-it-yourself" service (EDIY). A manual recorder service involves a milk recorder technician visiting the farm to conduct a manual milk recording. On the other hand, an electronic DIY service allows farmers to operate the service themselves with training and support provided by an MRO technician (ICBF, n.d., 2023a). Currently, there is limited knowledge regarding the differences in reporting between these two services. Anecdotal reports present contrasting views on which service provides a more accurate way of milk recording, as well as concerns about differences that might arise between milk samples taken during milk recording and those taken by dairy processors during bulk collections.

## Objective

The objective of this study was to explore and quantify the differences in fat and protein percentages between milk samples collected from test day milk recordings and those obtained from daily bulk collections by dairy processors on Irish commercial dairy herds. In addition, this study aimed to shed light on the factors that influence the accuracy of milk recording reporting. By doing so, intending to improve communication surrounding the advantages and limitations of milk recording as a service.

## Materials and methods

### Data

Milk recording (MR) samples were reported according to the alternative AM-PM recording scheme previously approved by the International Committee for Animal Recording (ICAR) (Berry *et al.*, 2006). Daily bulk (DB) samples were reported as recorded by the dairy processor during bulk collections. The dataset consisted of a total of 5,742 milk samples from 2,841 herds, all of which had test day MR on the same day as DB collections. All of these herds had at least 4 milk recordings in 2021 and were contracted to supply milk to dairy co-ops.

The analysis compared the fat and protein percentages of test day milk recording (MR) samples with daily bulk (DB) samples collected on the same day in 2021, where the DB sample results were assumed to be the true values, given that farmers are paid based on these values by the dairy processors. The accuracy of MR was assessed using three metrics: 1) Pearson correlation, 2) Unit difference, and 3) Percentage difference between MR and DB fat and protein percentages.

Unit difference was defined as the:

$$\text{Unit Difference} = MR_{\text{Trait}} - DB_{\text{Trait}} \quad (1)$$

If the unit difference is less than zero, the MR is underestimated compared to the DB. If the unit difference is greater than zero, the MR is overestimated compared to the DB.

Percentage difference was defined as:

$$\% \text{ Difference} = \left( \frac{MR_{\text{Trait}} - DB_{\text{Trait}}}{\frac{MR_{\text{Trait}} + DB_{\text{Trait}}}{2}} \right) * 100 \quad (2)$$

Similarly, if the percentage difference is less than zero, the MR is underestimated compared to the DB. If the percentage difference is greater than zero, the MR is overestimated compared to the DB.

The impact of recorder service type on MR accuracy was assessed across the 2,841 herds. Manual recording services were used in 1,631 of the herds, while the remaining 1,210 herds used EDIY recordings. Correlations for fat and protein percentages were calculated for both types of herds. Additionally, the impact of cow test day yields and season of recording on MR accuracy was assessed across the all herds. Average cow test day yield categories per herd (<10kg, >10-20kg, >20-30kg, and >30kg) were created, and correlations between MR and DB fat and protein percentages were calculated within each yield category. Finally, the impact of season on the accuracy of MR was assessed by comparing the correlations of fat and protein percentages between MR and DB during peak and off-peak milk production seasons. Peak was defined as any MR samples taken in April and May, whereas off-peak was defined as any MR samples taken in September and October of 2021.

When evaluating the unit difference and percentage differences at the herd level, averages were calculated for those herds that had MR and DB taken on the same day more than once in 2021.

The mean, standard deviation and min/ max values for MR and DB fat and protein percentages across the 5,742 samples are as shown in Table 1.

Table 1 Summary statistics of MR and DB milk fat and protein percentage.

Trait		Mean	SD	Min/Max
MR	Fat %	4.24	0.4	2.9/6.6
	Protein %	3.65	0.2	3/4.8
DB	Fat %	4.33	0.4	3.3/6.2
	Protein %	3.66	0.2	2.9/4.8

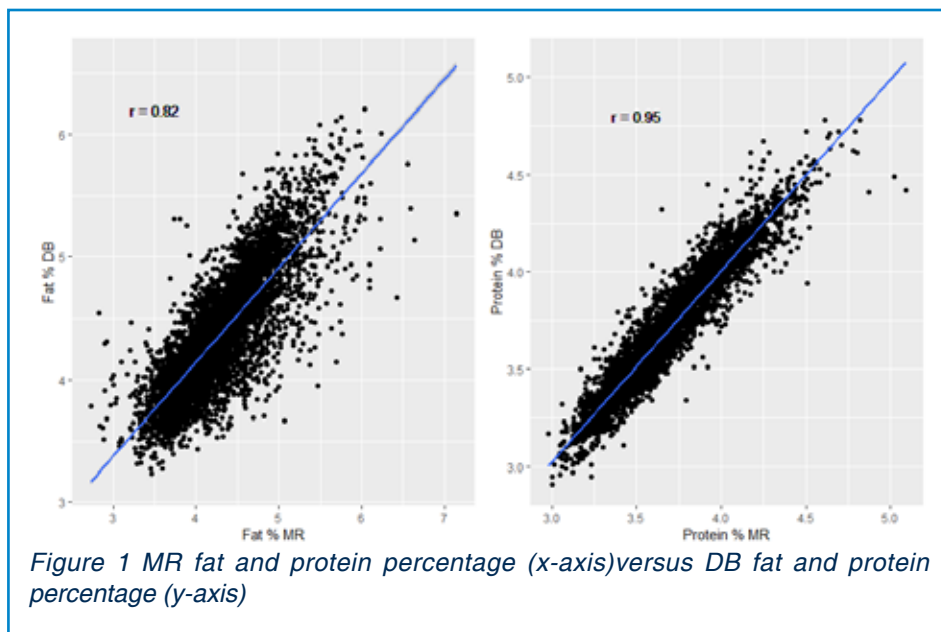
## Analysis

## Results and discussion

### Summary statistics

On average, there is a -0.09 unit difference between MR and DB fat percentage, and a -0.01 unit difference between MR and DB protein percentage. In both cases, MR is underestimated compared to DB.

These differences are reflected by the Pearson correlations, which are 0.82 for fat percentage and 0.95 for protein percentage between MR and DB.



The lower correlation observed in fat percentage compared to protein percentage may be due to the variation in milking intervals between MR and DB samples, which has been shown to impact reported fat percentages (Berry *et al.*, 2005).

Expressing these differences as percentage differences using Formula 2 provides a clearer understanding of the magnitude of the challenge. When comparing samples from two different sources, a certain level of variability is expected. To assess the accuracy of MR compared to DB samples, it can be useful to establish thresholds for acceptable error levels. Typically, a threshold of 5% is used, where any sample with a percentage difference greater than 5% is deemed an unacceptable level of error. Please refer to Table 2 for the conversion of percentage differences to percentage units for both fat and protein.

Table 2 Conversion of percent difference in unit difference.

Trait	Percentage difference	Unit difference
Fat %	1%	0.05 %
Protein %	1%	0.04%

Of the 2,841 herds analysed, the average absolute percent difference was 4.7% for fat and 1.4% for protein. In the case of fat percentage, over one third of the herds (38%) had an absolute percent difference greater than 5%. However, for protein percentage, only 2% of the herds had an absolute percent difference greater than the 5% threshold. This starkly highlights the inconsistencies in fat percentage reporting between MR and DB samples.

Table 3 presents the correlations for fat and protein percentages between MR and DB samples across different recording service types.

**Impact of recorder service type**

Across both recording service types, there was a lower correlation for fat percentage compared to protein percentage. However, the most significant finding from this analysis is the clear impact of recording type on the accuracy of MR when compared to DB samples. Herds using EDIY recordings had more accurate recordings, with a difference of nearly 0.1 in reported correlations.

It is difficult to pinpoint the exact factors driving the difference in MR accuracy between recording devices. However, it is likely that multiple factors, such as equipment type or management practices, contribute to these reporting discrepancies. EDIY devices are re-calibrated and serviced annually by ICBF at a meter calibration laboratory (ICBF, 2021). In contrast, manual recording devices rely on the farmer’s discretion regarding servicing and calibration, which may contribute to increased inconsistencies between recording service types. The regular and standardized servicing and maintenance applied to EDIY meters may contribute to increased accuracy of MR compared to manual meters, as maintenance practices for manual recording devices are likely to vary from farm to farm. Moreover, the associated maintenance cost for manual recording devices may discourage regular upkeep and maintenance, further contributing to the inconsistencies observed.

The impact of MR average cow test day yield (at the herd level) on the strength of correlations between MR and DB samples was assessed across all 2,841 herds. The results indicate that test day yield had a greater impact fat percentage correlations compared to protein percentage. Protein percentage correlations remained relatively consistent across yield categories, ranging from 0.87 to 0.93. In contrast, fat percentage correlations decreased as yield increased, dropping from 0.80 in lower yield categories to 0.58 in the highest yield category. This suggests that as cow test yields increase, the accuracy of MR fat percentage compared to DB samples decreases. These trends were consistent across both recording service types.

**Impact of yield and season**

Similarly, a seasonal impact was observed, where fat percentage correlations between MR and DB samples during the peak season (May/April) were at their lowest (0.69

Table 3 The Impact of recording service type on the correlations of fat and protein percent between MR and DBV samples.

Recording type	Number of herds	Fat % correlation	Protein % correlation
EDIY	1,210	0.87	0.95
Manual	1,631	0.78	0.96

and 0.47) compared to the off-peak season (September/October), where correlations were 0.82 and 0.73 for EDIY and manual recording herds, respectively.

## Conclusion

The analysis demonstrates that differences exist when comparing fat and protein percentages obtained from test day MR and DB samples. The largest differences are observed in fat percentage, with MR fat on average being 0.09% lower than its corresponding DB sample. The results also highlight that differences in MR accuracy compared to DB are influenced by various factors, including recording service type, test day yield, and MR season. EDIY devices exhibit a lower margin of error compared to manual recording devices. The differences in fat percentage reported by MR and DB increase as cow test day yield increases, and a similar pattern is observed during the peak milk production season.

Overall, this analysis sheds light on the complexities and challenges associated with accurately comparing MR and DB samples, emphasizing the importance of considering multiple factors that may impact the accuracy of MR measurements in dairy herds.

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## Identification of behavioural patterns associated with acidosis in dairy cows

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Acidosis is a major health and welfare issue, especially in high-producing dairy cows. During acidosis, rumination is affected, as early as a few days before diagnosis. For this reason, behavioural patterns can be considered as promising indicators for the early detection of acidosis. However, the power of behaviour analysis to predict acidosis can go much further, and behavioural patterns of healthy dairy cows, weeks or months before the onset of acidosis, could be used as a risk factor for the disease. The aim of this study was to determine whether cows that will subsequently develop acidosis, show early distinctive behavioural patterns associated with the disease compared to healthy cows. Daily hours spent standing, lying down, walking, ruminating and eating were recorded from calving to 15d prior acidosis using accelerometer collars on two commercial farms in Spain and two others in Italy. The acidosis group (n=10) included cows that suffered an episode of acidosis during lactation. Acidosis was diagnosed by veterinary records in both countries. In Spanish farms, acidosis cases were confirmed by rumen pH measured by boluses (smaXtec Animal Care GmbH, Graz, Austria; a cow was confirmed as suffering from acidosis when exhibiting a rumen pH below 5.6 for at least 50 min/day). A control group of healthy cows (n=10) balanced for parity and lactation stage was constituted afterwards. The daily milk production before the diagnosis of acidosis was similar in both groups. The group effect (acidosis vs. healthy) on the intercept and the slope of the regression curve of each individual cow for all behaviours was analyzed using ANOVA. The group significantly affected the intercept of the regression curve for the ruminating, lying down and eating duration showing that cows in the acidosis group spent more time lying (10.55 vs. 10.22 h/d) and eating (3.55 vs 3.41 h/d) 85 days before the onset of acidosis ( $P < 0.001$ ), compared to control cows. In addition, the rumination duration of acidosis cows was higher (7.73 vs. 6.86 h/d) 115 d before the onset of acidosis, but lower (7.33 vs 7.81 h/d) 35 d before the onset of acidosis, compared to the control cows. The trend in rumination duration over time prior to acidosis was also different as shown by the lower slope coefficient for acidosis cows ( $P < 0.001$ ). This study suggests that cows that will subsequently suffer from acidosis might expressed distinct behavioural patterns (e.g. rumination) already months prior to the onset of acidosis. Further research with a larger sample size is needed to confirm whether differences in behavioural patterns can be considered as promoters of acidosis. This study was conducted within the ClearFarm project which received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 862919.

### Abstract

*Keywords: Dairy cows, acidosis, behavioural activities, time-budget.*

## Introduction

Acidosis is a metabolic disease caused by an accumulation of organic acids (mainly lactic acids and volatile fatty acids) which is not counterbalanced by a sufficient rumen buffering inducing a low ruminal pH for several hours per day (Plaizier *et al.*, 2009). Acidosis is a major health and welfare issue in dairy cows since it has many negative impacts. Indeed, it affects feed intake, milk production, rumen digestion and microflora, and can lead to rumen mucosal damages, diarrhoea, lameness, and even death in the most acute cases.

Some management practices are considered as risk factors for acidosis. The ruminal pH depression observed in case of acidosis is favoured in dairy cows fed with concentrate-rich and fibre deficient diets (Plaizier *et al.*, 2009) or by fewer concentrate distribution per day (Yun and Han, 1989). This feeding practice of offering a concentrate-rich diet is commonly used to meet the high requirements of early-lactating cows, which means that this period is a risk period for acidosis (Penner *et al.* 2007).

However, even when dairy cows are fed and managed similarly, individuals can exhibit different degree of the disease because of individual factors. The individual variations for acidosis susceptibility are not totally elucidated, but behavioural factors could likely, at least in part, explain this individual variability. For instance, it has been suggested that the risk of acidosis increase in dairy cows that typically sort their feed in favour of short particles and against long fibre particles, or who exhibit a high eating rate since it results in of lower feed ensalivation (Beauchemin and Penner, 2009).

For this reason, behavioural patterns can be considered as promising indicators for the early detection of acidosis (Gomez *et al.*, 2022). However, the power of behaviour analysis to predict acidosis can go much further, and behavioural patterns of healthy dairy cows, weeks or months before the onset of acidosis, could be considered as a risk factor.

The aim of the present study was to determine whether cows that developed acidosis, show early distinctive behavioural patterns associated with the disease compared to healthy cows, that it could be considered as a risk factor for acidosis..

## Material and methods

### Data collection

This study was carried out based on data acquired from January 2021 to October 2022 in two commercial farms in Spain and two others in Italy. All cows were Holstein Friesian dairy cows. Their diet consisted of a total mixed ration or a partial mixed ration and concentrates. Dairy cows ( $n = 422$ ) were equipped with accelerometer collars dedicated to monitor their activity along the day (Ida collars, Connecterra, Amsterdam, The Netherlands). In the two Spanish farms, some cows ( $n = 17$  in the first one and  $n = 12$  in the second one) were equipped with rumen boluses (smaXtec Animal Care GmbH, Graz, Austria) which continuously measured rumen pH each 10 min (only for a duration of approximately 6 months after the placement of the bolus).

The data obtained from the dairy farms was from individual animals related to production (lactation rank, calving date, days in milk and daily milk production), dairy cow activities recorded by the accelerometer collars (daily hours spent standing, lying down, walking, ruminating and eating), and rumen pH for the cows equipped with rumen boluses. Acidosis cases were identified by veterinary records in the four farms. For the cows equipped with rumen boluses, acidosis were confirmed by rumen pH, when rumen pH was below 5.6 for at least 50 min/day



An acidosis group was constituted by including all cows that suffered from only one episode of acidosis during lactation. This group contained 10 dairy cows. A control group of healthy cows (n=10) balanced for parity and lactation stage was constituted afterwards by creating matching pairs of one acidosis and one control dairy cows. The two groups were composed of 5 primiparous and 5 multiparous each. The daily milk production before the diagnosis of acidosis was similar in both groups.

### Group constitution

For cows from the acidosis group, only data corresponding to the period from calving to 15d prior acidosis were kept for the statistical analyses in order to remove the sickness behaviour observed before the diagnosis of acidosis and analyse only the behaviour of dairy cows when they are not experiencing acidosis. In cows from the control group only data from calving to the day in milk corresponding to 15 days prior acidosis of its matching acidosis cow were kept for the statistical analyses. Before analyses, the day before acidosis and the behavioural data were mean-centered and reduced.

### Statistical analysis

All statistical analyses were performed using R software version 4.1.1. (R Development Core Team, Vienna, Austria). The evolution of the time per day spent doing each behaviour (i.e. standing, lying down, walking, ruminating and eating) relative to the day before acidosis was modelled for each cow using generalized linear mixed models thanks to the lmer function from the lme4 package. This permitted to estimate the intercept and the slope of the regression curve for the time spent doing each behaviour relative to the day before acidosis for each individual cow. Then, the group effect (acidosis vs. control) on the intercept and the slope of the regression curve of each individual cow for all behaviours was analyzed an ANOVA using the lm function from the stats package.

The statistical significance threshold was set at  $P \leq 0.05$ , and the trend-level significance was defined as  $0.05 < P \leq 0.10$ .

The intercept of the regression curves of each behaviour relative to the day before acidosis corresponded to 85 days before acidosis. The group significantly affected the intercept of the regression curve for the standing, lying, walking, ruminating and eating duration (Table 1). This showed that cows in the acidosis group spent more time lying (10.55 vs. 10.22 h/d), walking (3.50 vs. 2.83 h/d), ruminating (7.57 vs. 7.25 h/d) and eating (3.55 vs 3.41 h/d), and less time standing (9.97 vs. 10.94 h/d) 85 days before the onset of acidosis ( $P < 0.001$ ), compared to control cows.

## Results

The trend in the time spent lying and eating over time prior to acidosis was also different as shown by the lower slope coefficients for acidosis cows ( $P < 0.001$ ).

Figure 1 shows the evolution of the time spent ruminating over time before acidosis. The trends of the curves differed between groups ( $P < 0.001$ ). The regression curves of rumination duration before acidosis of both groups intersect 57 days before acidosis diagnosis. This showed that cows from acidosis group spent more time ruminating from the beginning of the lactation to 57 days before acidosis compared to cows from the control group. However, from 57 to 15 days before acidosis, they spent less time ruminating compared to control cows. We thus have re-ran the analysis of the effect on the group on the intercept for the two different periods: before 57 d prior to acidosis and from 57 d to 15 d prior to acidosis. The group significantly affected the intercept of the regression curve showing that the rumination duration of acidosis cows was

Table 1. Effect of the group (acidosis vs. control) on the intercept of the regression curve of time spent by each cow doing each behaviour. The intercept corresponds to 85 days before acidosis.

Behaviour	Group		P-value
	Acidosis	Control	
Standing	9.97	10.94	< 0.001
Lying	10.55	10.22	< 0.001
Walking	3.50	2.83	< 0.001
Ruminating	7.57	7.25	< 0.001
Eating	3.55	3.41	< 0.001

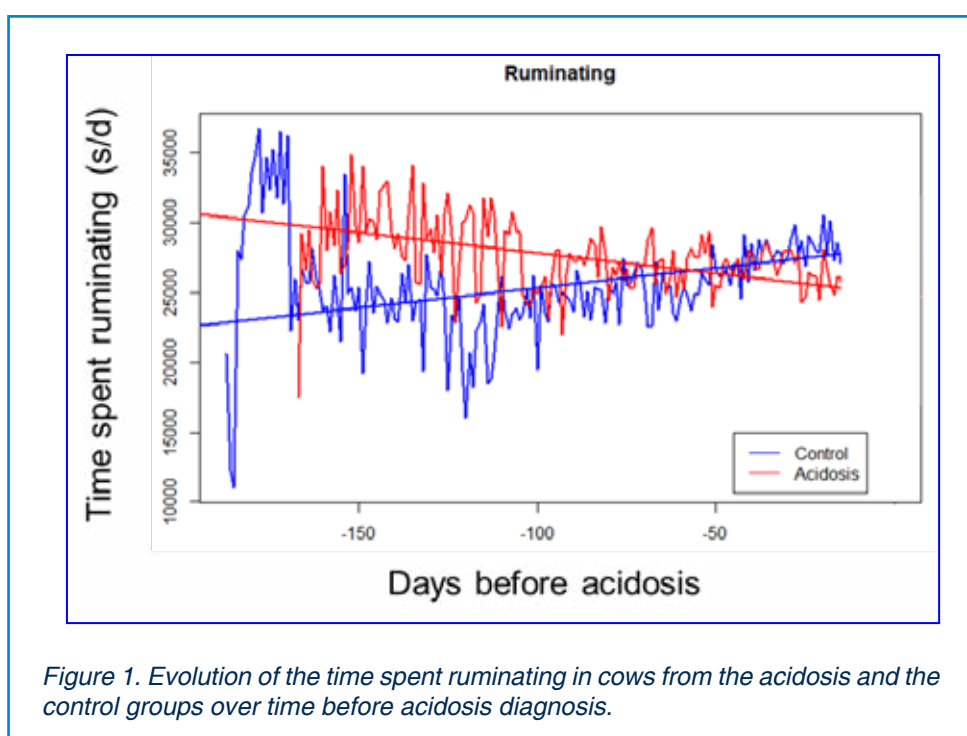


Figure 1. Evolution of the time spent ruminating in cows from the acidosis and the control groups over time before acidosis diagnosis.

higher (7.73 vs. 6.86 h/d) 115 d before the onset of acidosis, but lower (7.33 vs 7.81 h/d) 35 d before the onset of acidosis, compared to the control cows.

## Discussion and conclusions

The purpose of the present study was to analyze the differences in time-budget (time spent standing, lying down, walking, ruminating and eating per day) between dairy cows that will subsequently suffer from acidosis and dairy cows that stay healthy during the entire lactation. The present study showed that cows suffering from acidosis might expressed different time-budget already few months prior to the onset of acidosis. Therefore, the time-budget of dairy cows could be used as a risk factor of acidosis. This was already suggested by previous studies showing that cows that high acidosis risk did, on average, spend less time ruminating than the cows with a low acidosis risk (DeVries *et al.*, 2009; Coon *et al.*, 2019).

Further research with a larger sample size is needed to confirm distinct behavioural patterns associated with the subsequent development of acidosis.

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## A predictive model for hypocalcemia in dairy cows utilizing behavioural sensor data combined with deep learning

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### Abstract

(Sub)clinical hypocalcemia occurs frequently in the dairy industry, and is one of the earliest symptoms of an impaired transition period. Calcium deficiency is accompanied by changes in cows' daily behavioural variables, which can be measured by sensors. The goal of this study was to construct a predictive model to identify cows at risk of hypocalcemia in dairy cows using behavioural sensor data. For this study 133 primiparous and 476 multiparous cows from 8 commercial Dutch dairy farms were equipped with neck and leg sensors measuring behavioural elements, including eating, ruminating, lying and walking behaviour of the 21 days before calving and the day of calving. From each cow, a blood sample was taken within 48 hours after calving to measure their blood calcium concentration. Cows with a blood calcium concentration  $\leq 2.0$  mmol/L were defined as hypocalcemic. In order to create a more context based cut-off, a second way of dividing the calcium concentrations into two categories was proposed, using a linear mixed-effects model with a k-Means clustering. Three possible binary predictive models were tested; a logistic regression model, a XgBoost model and a LSTM deep learning model. The deep learning model was expanded by adding the following static features as input variables; parity (1, 2 or 3+), calving season, day of calcium sampling relative to calving (0, 1 or 2), body condition score and locomotion score. Of the three models, the deep learning model performed best with an area under the receiver operating characteristic curve (AUC) of 0.66 and an average precision of 0.53. This final model was constructed with the addition of the static features, since they improved the model's tuning AUC with 0.07. The calcium label with the cut-off categorization method proved to be easier to predict for the deep learning model and the XgBoost model, while the logistic regression model performed better using the categorization method with the k-means clustering. This study provides a novel approach for the prediction of hypocalcemia and an ameliorated version of the deep learning model proposed in this study could serve as a tool to help monitor herd calcium status and to identify animals at risk for associated transition diseases.

*Keywords: dairy cattle, hypocalcaemia, sensors, deep learning, prediction, transition period.*

## Introduction

The most challenging time in the lifespan of a cow is around parturition, more commonly known as the transition period (Grummer, 1995). The cow has to adapt homeorhetic from a pregnant state to a non-pregnant, and more importantly, lactating state (Bauman and Currie, 1980). In this period, most infectious diseases and metabolic disorders occur or originate, ranging from ketosis and retained fetal membranes to displaced abomasum and mastitis (Drackley, 1999). One of the arising problems is hypocalcemia, more commonly known as milk fever. Once a cow starts lactating, it loses more calcium in her milk, urine, and faeces than it can replenish through intestinal reuptake. The mechanisms to rebalance calcium take a while to initiate, resulting in a calcium dip right after calving (Horst *et al.*, 1994). Recently, Horst *et al.* (2021) suggested that hypocalcemia could also be explained as a result of an inflammatory reaction seen around calving. There are two forms of hypocalcemia; clinical hypocalcemia (CH), with visible clinical signs like increased heath rate, cold ears and recumbency, and subclinical hypocalcemia (SCH) which has no recognizable symptoms but is associated with impaired postpartum health and performance (Serrenho *et al.*, 2021).

The reported prevalence of hypocalcemia differs between studies, but lies between 14-40 % of the cows after parturition overall and for higher parity cows up to 69 % (Serrenho *et al.*, 2021). Since the disease is very common, many preventive measures are being applied in an attempt to reduce milk fever occurrences. For instance; feeding a pre-calving diet low in calcium, feeding a diet with a negative dietary cation-anion difference and oral calcium drenching around calving, are regularly used (Thilising-Hansen *et al.*, 2002; DeGaris and Lean, 2008). Not all risk factors of hypocalcaemia are related to nutrition, (DeGaris and Lean, 2008) and none of the measures have succeeded to fully prevent hypocalcemia (Venjakob *et al.*, 2017; Ribeiro *et al.*, 2013).

Devices that measure physiological or behavioural parameters, are increasingly used in the dairy industry. In a survey held among Dutch dairy farmers in 2015, 39% of the farmers reported using at least one sensor system (Steenefeld and Hogeveen, 2015), a number that probably has been growing ever since. Sensors can be used for oestrus detection (Firk *et al.*, 2002), lameness detection (Chapinal *et al.*, 2010), mastitis detection (Cavero *et al.*, 2008; Jensen *et al.*, 2016), and numerous other applications. Nowadays, 129 different sensor systems are commercially available (Stygar *et al.*, 2021).

Overton *et al.* (2017) has shown that there are differences in prepartum behaviour between healthy cows and those affected by metabolic disease postpartum. The authors thereby suggested that this difference could be used for disease prediction. And there have been more papers where sensors have been used to examine the association between behaviour and disease occurrence, for example by Soriani *et al.* (2012), Liboreiro *et al.* (2015), Hendriks *et al.* (2020), Gusterer *et al.* (2020). These studies also reported behavioural differences between disordered and healthy cows, suggesting a possible predictive value of behaviour for disease. There even have been studies where these sensor-based systems were used for the detection of diseases (de Mol *et al.*, 2015; Stangaferro *et al.*, 2016). This included one system made to detect metabolic disease and thereby alert the farmer of deviating behaviour (de Mol *et al.*, 2015). But at the present time, for as far as we know, models for specific disease prediction with behavioural sensor data do not yet exist (Garcia *et al.*, 2020), despite the suggestion of Overton *et al.* (2017) and the potential of precision livestock farming to perform such tasks (Garcia *et al.*, 2020; Wathes *et al.*, 2008).

In this paper therefore, it is tried to build the first prediction model for a specific disease using activity data. Given the high prevalence, impact and focus on the prevention of hypocalcemia in practice (LeBlanc *et al.*, 2006), a model was built to predict hypocalcemia.

The descriptive models using animal behaviour data described above all used traditional machine learning models, but in this paper it was chosen to use deep learning since it

often outperforms traditional data analysis approaches and results in a more accurate model (Janiesch *et al.*, 2021). Deep learning uses artificial neural networks. The system mimics the sensory processing of the animal brain and consists of multiple layers of neurons signalling to each other based on input, weight factors and threshold values (Krogh, 2008).

The goal of the study was to make a predictive model able of telling at parturition which cow is at risk for hypocalcemia after parturition. This could then serve as a tool for the prevention and control of metabolic disease.

This study was a part of the Sense Of Sensors project. In this project, cows of eight Dutch dairy farms were equipped with leg and neck sensors measuring animal behaviour. The Smarttag sensors were provided by the company Nedap (Nedap, Groenlo, the Netherlands) and measured nineteen different features throughout the day. The leg sensors measured lying and walking behaviour, while the neck sensors measured eating and rumination behaviour. On top of the total minutes spent on a certain behaviour per day, the sensors provided the number of bouts per behaviour per day, the average time per bout for each day and the average time between bouts for each day. For this study, the sensor values recorded during the 21 days before calving and the day of calving were used. So for each cow there were 19 different values for each day and this for 22 days, resulting in 418 values per cow in total. In a paper by Hut *et al.* (2021), the farms and sensors used are described elaborately. The data was collected between the twentieth of November 2016, until the tenth of May 2018 and included both data of dairy cows in the transition period as pre-fresh heifers. The behavioural features measured are noted in table 1.

## Materials and methods

### Data collection

Table 1. Behavioural features measured by the neck and leg sensors calculated for each day.

Behaviour	Feature
Walking	Minutes per day
	Number of bouts
Standing	Minutes per day
	Number of bouts
Eating	Minutes per day
	Number of bouts
	Average minutes per bout
Rumination	Average minutes between bouts
	Minutes per day
	Number of bouts
Lying	Average minutes per bout
	Minutes per day
	Number of bouts
Leg activity	Average minutes per bout
	Number of steps
Inactivity	Minutes per day
	Number of bouts
	Average minutes per bout
	Average minutes between bouts

Blood samples were taken from the cows by a veterinarian on the day of calving (0), the day after calving (1) or two days after calving (2), in order to measure the blood calcium concentration. The samples were taken from the coccygeal vein using a vacutainer and collected into a heparinized blood collection tube. At the same day, the collected samples were centrifuged for ten minutes at 4500 rpm (Centrifuge 5804 R; Eppendorf Germany) and afterwards manually pipetted into Eppendorf cups. The samples were stored at -20 degrees Celsius, awaiting quantitative analysis of total calcium serum concentration using the Calcium Arsenazo method (Leary *et al.*, 1992). This method was executed by the Olympus AU680 with a limit of quantitation of 1 mmol/L and an end point determination of 660 nm. One cow was removed from the dataset because of an extraordinary high blood calcium value of above 3,4 mmol/L due to the administration of a calcium infusion just before sampling. Since the research was conducted over a longer period of time, 21 cows participated multiple times, but with a different parity. However, each unique animal calving date combination was seen as a different test subject. This selection process resulted in 609 unique dairy cow calving date combinations deemed appropriate for this research.

For 416 cows, the body condition score (BCS) was determined by a trained veterinarian at the end of the dry period. The scores were described on a scale between 1 and 5, with 0.25 increments, as defined by Ferguson *et al.* (1994). At the same observation for 414 cows, the locomotion score was determined on a scale between 1 and 5 based on posture and gate, but with the use of integers only, according to Sprecher *et al.* (1997).

Calving seasons were extracted from the recorded calving dates and were defined as 3-month periods according to Sanders *et al.* (2009). Summer, for instance, was defined as the months of July, August, and September.

### Label preprocessing

Cows were divided into two categories; hypocalcaemic cows with a blood calcium concentration equal to or lesser than 2.0 mmol/L and normocalcaemic cows with a blood calcium concentration above 2.0 mmol/L. This threshold was chosen according to Reinhardt *et al.* (2011). There is, however, increasing discussion whether the cut-off value of 2,0 mmol/L is a valid number to define SCH, or is in fact chosen arbitrarily in the past and therefore not evidence based (Serrenho *et al.*, 2021). Therefore, a second way of splitting the two categories was proposed using a linear mixed-effects model, in combination with k-means clustering. This method corrects for parity and day of measurement and results in a more fluent context based cut-off, as recommended by Serrenho *et al.* (2021). The calcium concentration was used as the response variable for the linear mixed effect model and the day of blood sampling relative to calving (0,1 or 2), parity (1,2 or 3+) and farm were the predictor variables. Since the measurements of cows from the same farm are correlated, the grouping of the data must be taken into account. Therefore, a linear mixed effect model was chosen, which is a hierarchical multilevel model, and allows for different regression coefficients for each predictor per farm and thereby includes both the variation within a farm and between farms. Then the k-means clustering method was used based upon the residuals of the calcium predictions and the absolute values of the calcium concentrations. This resulted in two clusters, still one group with relatively low calcium concentrations and the other group with relatively high calcium concentrations. The categories were defined as low calcium equals one and high calcium equals zero.

From the 609 cows, 365 cows were randomly attributed to the train set, which equals approximately 60%. Of the remaining 244 cows, 50% were attributed to the validation set and the remaining 122 cows were attributed to the test set.



Since neural networks cannot function with incomplete data (Ennett *et al.*, 2001), missing BCS and locomotion values were imputed using the sklearn SimpleImputer, which replaced null values by the score that was most frequent in the train set.

Min-Max scaling was used to normalize the sensor data. The normalization was fitted on the values of the train set per day (-21,0) before calving and per feature. The resulting normalization was also used for the test and validation set without resetting the minimal and maximal value.

For numerous reasons, including sensor malfunctioning and administrative errors, data points were missing from the dataset. In fact, approximately 21.4% of the sensor values were misrecorded. The day of parturition had significantly the most missing values, this can be explained by In

order to replace those missing values, a SimpleImputer was used based upon the train set, which transformed the missing values into the mean per feature of the according day in the train set similarly as the previously described normalization.

In order to facilitate analysis of behavioural measurement in a chronological order and to promote discovery of temporal patterns for each cow individually. The sensor values were placed into a three-dimensional matrix of 22 days x 19 sensors x the number of cows. The calcium categories and static features were also extracted per cow and put into separate lists with an index matching the sensor matrix.

There were approximately 2.7 times more cows in one of the calcium categories, causing class-imbalance. However, most prediction models give the best results with a balanced dataset (Johnson and Khoshgoftaar, 2019). Therefore, upsampling was performed whereby the cows with value 1 were extracted from the train set and randomly sampled with replacement until there were as many cows with the calcium category value 1 as cows with value 0. This upsampling was only performed on the train set.

In order to predict the probability to fall within a specific category of calcium concentrations, three kinds of models were built: a logistic regression model, a XgBoost model and a LSTM deep learning model.

For the logistic regression model, the 3D sensor value array was flattened to an array with the shape (number of cows, 22 days\*19 features). This array served as the input feature of the model, while the calcium group served as the output label. For the model, the liblinear solver algorithm was used. Due to the limited amount of hypocalcaemic cows, it was hypothesized that the model could focus too much on the healthy cows, therefore the model was trained both with and without random upsampling of the trainset. And a second way to deal with class imbalance, namely adding class weights, and therefore introducing cost-sensitive learning (Johnson and Khoshgoftaar, 2019), was also tested. The accompanying cost matrix was defined by a grid search of a range of possible weights.

The second model used was a XgBoost model; a relatively recent developed machine learning model using tree boosting (Chen and Guestrin, 2016). The input features and output labels used for this model were the same as for the logistic regression model. The validation set was used for early stopping and hyperparameter tuning, which was performed automatically using random search. The hyperparameters tuned are noted

## Feature preprocessing

## Model building

in table 2. One parameter to point out is a positive class weight as a possible solution to class imbalance, set to be the total number of cows in group zero divided by the total number of cows in group one. This method was used since it is the default solution for class imbalance in a XgBoost model.

The third model was a LSTM deep learning model. A LSTM model was chosen, since this network can analyse sequential data and has the ability to recognize temporal patterns (Hochreiter and Schmidhuber, 1997). A LSTM model consist of cells, that next to providing an output, also provide a cell-state which functions as the memory for the next cell. The matrices of the 19 behavioural features were used as model input, where for each time step a LSTM cell was formed. The cells were aligned in a chronological order, connected by the cell state. Each cell has three gates; a forget gate, to forget unnecessary information passed on by the previous cell, an input gate layer, to process the new input and add it to the cell state, and an output gate, which provides a filtered version of the acquired cell-state as output. In the end, this resulted in a one dimensional vector containing a summary of the information the LSTM layers filtered from the sensors. This vector was passed on to a classic multilayer perceptron (MLP) layer, which converted the vector to a value between 0 and 1 using a sigmoid function. This value was used to predict the calcium group dependent on the chosen threshold.

## LSTM model training

The training process of the LSTM model started with random initiation of all the weights of the equations in the deep learning model, thereby forming the untrained version of the model. The calcium category acquired with these random weights was compared with the desired result. This difference is expressed as the loss function. The LSTM model was then trained using the backpropagation algorithm (Rumelhart *et al.*, 1986) in combination with the Adam gradient-based optimization algorithm (Kingma and Ba, 2015), thereby adjusting the weights and biases in order to minimize the loss function, using the training data. The model was programmed to stop training when the loss of the validation set did no longer decrease for three consecutive rounds, a process which is called earlystopping. This was used to prevent overfitting on the training data.

## Static cow features

In order to improve the sensor based model performance, static cow features were added as input features. The following features were included: calving season, parity (1,2 or 3+) and the day of blood sampling compared to calving. Since neural networks can only process numerical data as input, calving season and parity were converted to a binary variable using the sklearn OneHotEncoder. This process resulted in 8 static features to include in the model. The model was also tested with the addition of BCS and locomotion score measured at the end of the dry period to the static cow features, resulting in 10 static features to include in the model. The addition of static cow features was only performed for the LSTM model. In order to combine static and sequential input, a functional model was build where the output vector of the LSTM layer was combined with the static features in a concatenation layer, which was then processed using a traditional MLP layer with a ReLu activation function and converted into a binary output using an MLP layer with a sigmoid activation function.

Since neural networks are prone to overfitting (Krogh, 2008), measures were taken in order to prevent overfitting. The number of nodes were kept low to limit the size of the models, and a dropout and batch normalization layer were added. Among others, these hyperparameters were tuned and can be found in table 2. The tuning process of the models was based on random search, using the validation set to compare the different hyperparameter combinations. All the combinations of the different sets of input features with the calcium category to predict, based on either the clusters or on the cut-off value, were seen as separate models. These combinations are listed in table 3. Each model was individually tuned and for each model 200 random combinations of hyperparameters were tried, selecting the hyperparameter settings with the best results. Only the logistic regression model was tuned using grid search, thereby testing all twenty possible combinations. This method was more appropriate since there were little possible combinations of hyperparameters.

### Model tuning

The best performing hyperparameter configuration was evaluated using bootstraps in order to quantify model consistency. A bootstrap is a random sample with replacement of cows from the validation set with the same size as the validation set. Fifty bootstraps were created, each consisting of a unique combination of re-sampled cows. First, the best hyperparameter configuration based on the area under the receiver operating characteristic curve (AUC) while predicting the calcium category of the validation set, was selected for each model. Then this model was tested using the bootstraps rendering fifty AUC values each. Finally, the mean AUC and standard deviation (SD) of the AUC were calculated and used to compare the different models. Models were compared initially using the mean AUC score and when this value was equivalent between two models, the SD was used to differentiate between model performances. The SD is a metric for consistent model performance, thus models with a lower SD are more precise. The three models with the best mean AUC and SD score were evaluated on the test set. In Figure 1 a schematic overview of the methodology for the model evaluation is given.

### Model evaluation and comparison

In order to test the difference between mean AUC of the models for statistical significance, 95% confidence intervals for the difference in mean were calculated utilizing the one-sided t-score.

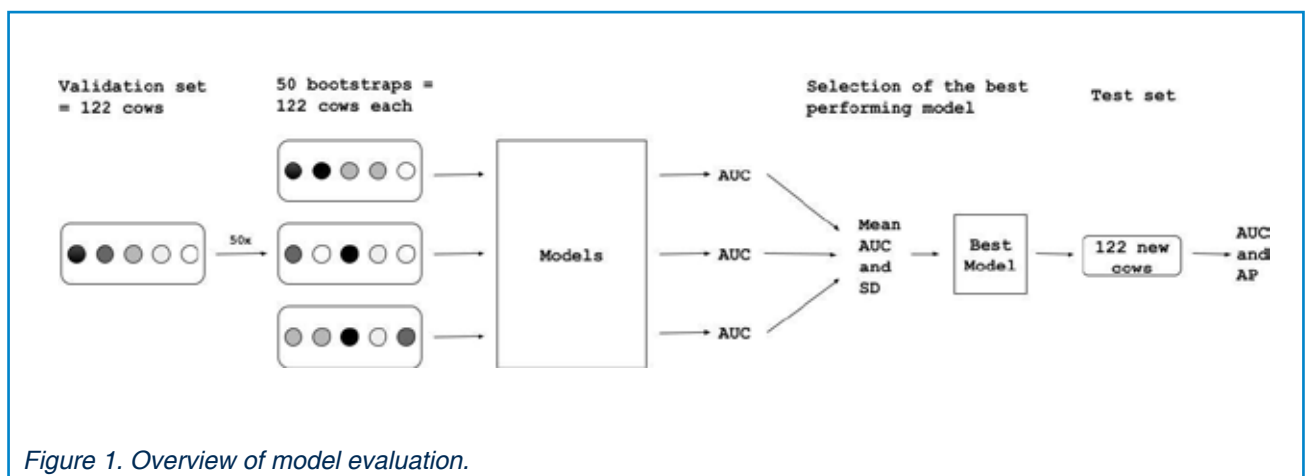


Figure 1. Overview of model evaluation.

Performance of the final models was evaluated on the test set using the AUC and the average precision (AP); the area under the precision-recall curve, using the predicted and true calcium categories of the test set. These metrics were chosen since they are threshold independent and therefore more suitable to compare between models. The sensitivity (true positive rate) and specificity (true negative rate), of the best performing model were calculated but are reported for multiple thresholds since they are dependent on the chosen threshold.

## Ontology

To contribute to open science as defined by the UNESCO recommendation on Open Science (UNESCO, 2021) the data used in this research is made publicly available. The data is published in the form of an ontology. This was developed in order to make the structure and concepts of the data more comprehensible and to make it easier to extend the dataset with external data, thereby facilitating future research. The populated ontology, the code written for the ontology and a figure to visualize the structure of the ontology can be found on <https://github.com/Bovi-analytics/van-leerdam-et-al>.

## Programming framework

Data processing and analysis was performed using the programming language Python (Python Software Foundation, version 3.8.10, <http://www.python.org>) with the add-on packages Pandas (Pandas Development Team, 2020; Wes McKinney, 2010), NumPy (Harris *et al.*, 2020), scikit-learn (Pedregosa *et al.*, 2011), XgBoost (Chen and Guestrin, 2016), Ray Tune (Liaw *et al.*, 2018), TensorFlow (Abadi *et al.*, 2016), Keras (Chollet *et al.*, 2015) and Matplotlib (Hunter, 2007). For the linear mixed-effects model, the programming language R was used (R Core Team, 2013) version 4.1.1, with the following packages: 'lme4' (Bates *et al.*, 2015), 'dplyr' (Wickham *et al.*, 2015), and 'ggplot2' (Wickham, 2016). For both R and Python the Apache Spark (Zaharia *et al.*, 2016) cluster-computing framework was used. All written codes can be found on <https://github.com/Bovi-analytics/van-leerdam-et-al>.

Table 2. For each of the three model categories, hyperparameters were tuned in order to select the hyperparameter configuration with the best results. The different hyperparameters are listed below, next to their possible settings, wherefrom 200 random combination were picked and tested. For the Logistic Regression model, all the combinations of settings were tested.

## Results

### Calcium measurements

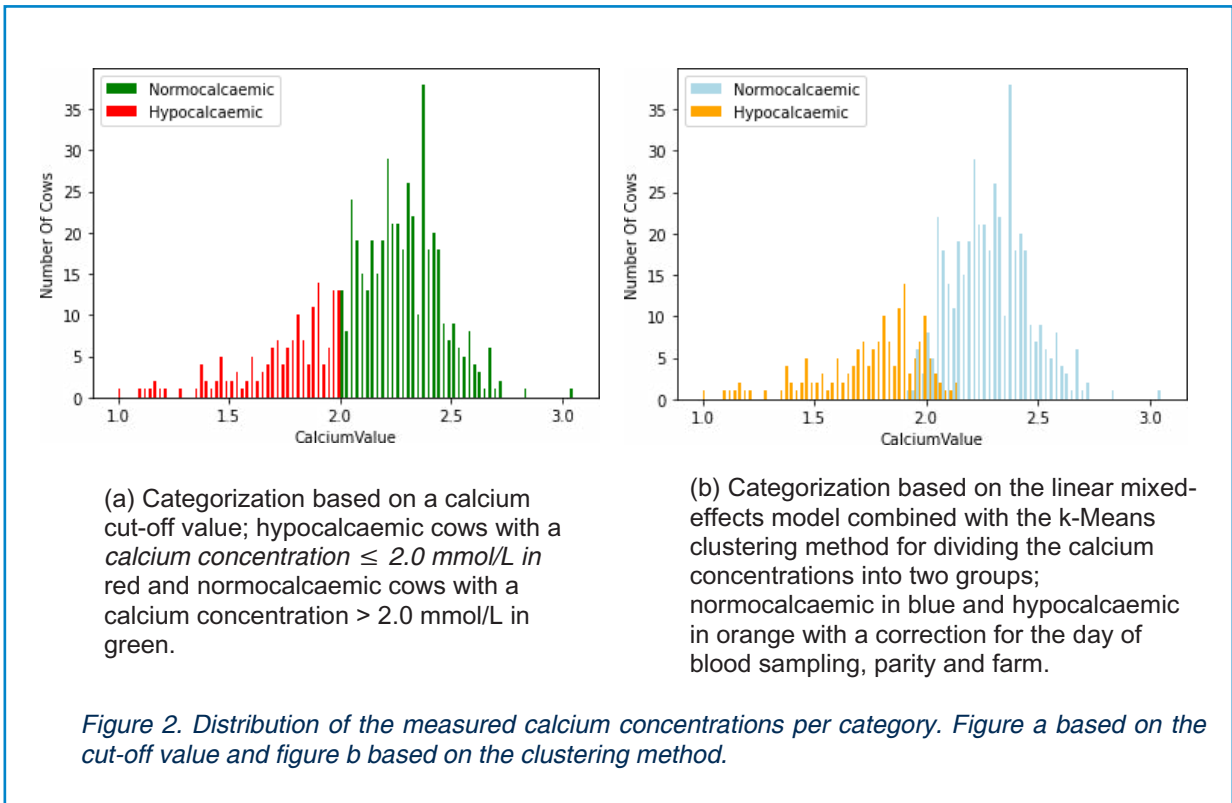
The measured mean calcium concentration was 2.15 mmol/L. Approximately 26.3% of the cows had a calcium concentration lower than or equal to 2.0 mmol/L. The results of the measurements on the blood samples taken from the cows after calving, as well as the division between the two categories, are presented in figure 2a. The k-Means clustering of the measured calcium concentrations and residuals produced by the linear mixed-effects model resulted in two clustered categories, visualized in figure 3. Figure 2b shows the distribution of measured calcium concentrations per clustered category. The percentage of cows attributed to the low calcium cluster was approximately 26.8%, thus the group sizes between the two methods were comparable.

*Table 2. For each of the three model categories, hyperparameters were tuned in order to select the hyperparameter configuration with the best results. The different hyperparameters are listed below, next to their possible settings, wherefrom 200 random combination were picked and tested. For the Logistic Regression model, all the combinations of settings were tested.*

Model category	Hyperparameter	Possible settings
Logistic Regression	Upsampling	True or False
	Class weights	1:1, 1:2, 1:3, 1:4, 1:5
	Predicted variable	Based on Cut-off or Based on Cluster
XgBoost	Learning rate	Log-Uniform between 0.0001 and 0.1
	Minimum loss reduction required for partition	0 or 1
	Maximum depth of a tree	2, 3, ..., 10
	Minimum sum of instance weight needed in a child	1, 2, 3, 4
	Class weights	1:1 or 1:2.7
	Upsampling	True or False
	Predicted variable	Based on Cut-off or Based on Cluster
Deep learning model	Number of LSTM layers	0, 1, 2
	Size of hidden state	10, 20, ..., 100
	Use of ReLu activation LSTM	True or False
	Dropout Rate	0, 0.1, ..., 0.4
	Batch Normalization	True or False
	Batch Size	12, 22, 32, 42
	Use of static features	None, All, Without BCS en locomotion score
	Upsampling	True or False
	Predicted variable	Based on Cut-off or Based on Cluster
	Class weights	1:1, 1:2, 1:3, 1:4
	Size MLP layer for static features	10, 20, ..., 80

When plotting the average values for each behavioural feature as listed in 1 per day relative to calving per calcium category based on the clustering method, the following observations can be made. High calcium cows walk more than low calcium cows during the entire 21 days. Low calcium cows spent more minutes inactive per bout for the entire 21 days, and from 15 days antepartum they have on average fewer minutes between bouts. Eating differs from day 15 antepartum, when low calcium cows spent fewer minutes per day eating, with fewer minutes per bouts and with more average minutes between bouts. This difference is biggest on the day of parturition. The low calcium cows spent fewer minutes lying per day and have fewer steps, and as a result

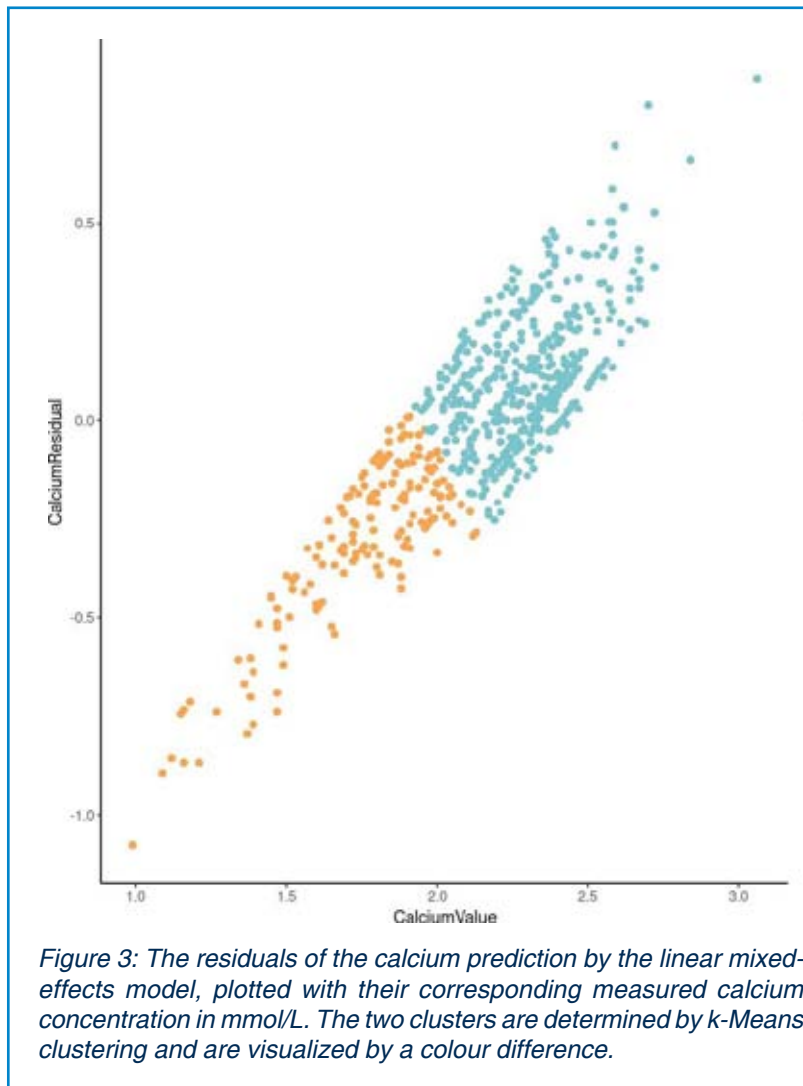
***Difference in behaviour between the two calcium categories***



spent slightly more time standing. Rumination does not seem to differ between high calcium and low calcium cows except for the day of calving when the low calcium cows spent more minutes ruminating in more bouts, with less average minutes between bouts. The number of bouts for inactivity, lying and eating also only differ on the day of calving, whereby the biggest difference can be found in an increase on average of five eating bouts more for high calcium cows. The corresponding figures can be found on Github on <https://github.com/Bovi-analytics/van-leerdam-et-al>.

### Selecting the best model

The XgBoost and the deep learning model predicted best using the cut-off categorization (XgBoost: mean AUC 0.58 and SD 0.068), while the logistic regression model predicted best using the clusters for the calcium categorization (mean AUC 0.51 and SD 0.066). For the deep learning model, the results of the bootstrapping for the different combinations of input features and labels to predict are presented in table 3, sorted by performance. The static cow features improved the model performance by  $\approx 12\%$ . The 95 % confidence interval of the difference in mean AUC between the best performing model with and without the addition of the static cow features, did not include zero. The improvement in model performance was therefore significant. The addition of the BCS and locomotion score did not significantly improve the model. When comparing the mean AUC of the best model without the BCSs and locomotion scores to the best model with the BCS and locomotion scores, the 95 % confidence interval of the mean difference included zero. In figure 4 the confidence intervals of the differences between models are visualized.



The model performance on the test set of the best hyperparameter configuration of each model is presented in table 4. Both the AUC and the AP were highest in the deep learning model, with the LSTM layer and all static features. Table 5 describes the sensitivity and specificity of the best performing deep learning model for different thresholds.

Table 3. Mean AUC of the LSTM deep learning models evaluated on the bootstraps of the validation set for different combinations of input features and the calcium category to predict, based either on the clustering method or on the cut-off value of 2.0 mmol/L. The models are sorted based on their performance. The small set of static feature comprises the day of blood sampling compared to calving, parity and calving season. The complete set of static features contains next to the day of blood sampling compared to calving, parity and calving season also BCS and locomotion score.

### **Performance of the final models**

Table 3. Mean AUC of the LSTM deep learning models evaluated on the bootstraps of the validation set for different combinations of input features and the calcium category to predict, based either on the clustering method or on the cut-off value of 2.0 mmol/L. The models are sorted based on their performance. The small set of static feature comprises the day of blood sampling compared to calving, parity and calving season. The complete set of static features contains next to the day of blood sampling compared to calving, parity and calving season also BCS and locomotion score.

Categorization method	Upsampling	Static. features	AUC	SD
Cluster	+	-	0.49	0.071
Cluster	-	-	0.59	0.061
Cut-off	+	-	0.61	0.062
Cluster	+	Small	0.61	0.058
Cut-off	-	-	0.64	0.074
Cluster	-	Small	0.66	0.054
Cluster	+	All	0.66	0.043
Cut-off	+	All	0.67	0.069
Cluster	-	All	0.68	0.060
Cut-off	+	Small	0.70	0.063
Cut-off	-	All	0.71	0.067
Cut-off	-	Small	0.71	0.057

Table 4. Performance of the best models tasked to predict the calcium categories of the test set. Selection of the best model was based on the mean AUC values and the SD on the bootstraps of the validation set per model type.

Model	AUC	AP
Logistic Regression	0.57	0.45
XgBoost	0.58	0.43
LSTM model	0.66	0.53

Table 5: Sensitivity and Specificity of the best performing LSTM deep learning model for different classification thresholds when predicting the calcium categories of the test set.

Threshold	Sensitivity	Specificity
0.1	1	0.15
0.2	0.93	0.30
0.3	0.77	0.49
0.4	0.49	0.67
0.5	0.35	0.78
0.6	0.26	0.92
0.7	0.09	0.98
0.8	0.02	1



The proposed model predicts at parturition the probability that a cow will be in the low calcium category in the days post-partum, and thereby the risk of hypocalcemia. This is the first study that shows that prediction of the risk of hypocalcemia with behavioural sensor data is possible. However, the best performing model still performs far from perfect. An AUC of 0.5 can be expected when a coin is tossed in order to predict whether a cow has hypocalcemia or not, while a perfect model assigning each cow to the correct group would have an AUC of 1. So with an AUC of 0.66 the model performs better than random, but still far from 1. In order to be able to use this model in practice, the model performance will have to increase.

The difference between traditional machine learning models and the deep learning model is that the LSTM can discover sequential patterns in the data. The applied machine learning models use flattened data, therefore the time dimension is lost. The deep learning model was the best performing model. This finding means that the temporal patterns in the sensor data, and not only the absolute occurrence of behaviour, differ between normocalcaemic and hypocalcaemic cows. It also shows that these patterns have a predictive value. A finding corresponding with Hendriks *et al.* (2020), who found that relative changes in daily and hourly daytime lying time in the two weeks before calving, were negatively associated with the blood calcium concentration within 24 hours after calving, in contradiction to the relative change in daily and hourly daytime steps, which were positively associated with the blood calcium concentration after calving.

Clinical hypocalcemia impairs animal welfare, farm economics and has a long-lasting impact on transition success. It is associated with numerous postpartum health events including; dystocia, retained placenta, ketosis and mastitis (Curtis *et al.*, 1983; Erb *et al.*, 1985; Correa *et al.*, 1990; Klerx and Smolders, 1997) In addition, CH affected cows produce less milk and have an increased time to pregnancy (Venjakob, 2018). On the other hand, for subclinical hypocalcemia, the effect on transition success is not as easily defined and depends on the day of calcium sampling, the duration of low blood calcium values and parity (Neves *et al.*, 2018). Transient hypocalcemia; only at 1 day after calving, does not lead to increased disease events and is associated with higher milk yield than normocalcaemic cows, while chronic or delayed SCH does lead to adverse events (McArt and Neves, 2020). It has been hypothesized that hypocalcemia beyond 48 hours after parturition is not caused by a primary problem of adapting to a new calcium demand, but rather by reduced feed intake or/and inflammation (Serrenho *et al.*, 2021; Horst *et al.*, 2021). This turns hypocalcemia into a symptom rather than an individual disease, and it could therefore be an indicator of an impaired transition period.

There is, however, no distinction made by the model between transient, chronic or delayed hypocalcemia, since blood measurements were not taken at the exact same moment each time, but instead somewhere in between the 48 hours after calving and only one blood sample was taken from each cow. It is therefore plausible that a cow is assigned to the risk group while in fact be a healthy, high producing cow with transient hypocalcemia. The model also does not distinguish between subclinical and clinical hypocalcemia, because there were too few clinically affected cows to train the model this distinction.

We believe that, for future research, it is important to change the way of calcium categorization. Multiple blood samples should be taken instead of only one calcium measurement. This makes it possible to differentiate between chronic, delayed and transient hypocalcemia and then train the model to predict clinical, chronic or delayed hypocalcemia only. We hypothesize that this method will greatly improve the predictability of hypocalcemia. Moreover, from a practical point of view, it would be

## Discussion

### *The meaning of the results*

### *Back to hypocalcemia*

useful to differentiate between hypocalcemia forms, since the best course of action for disease prevention could differ between variants.

### Suggestions to improve model performance

As stated before, this study showed that it is possible to predict hypocalcemia, but the AUC value is too low for practical implementation. Fortunately, there are multiple ways to improve model performance. The first element of a good performing model is high input and output quality. The behaviour as recorded by the sensors agrees with true behaviour, but with a range of error (Borchers *et al.*, 2021; Nielsen *et al.*, 2018), in combination with uncertainty in calcium measurements, BCS scoring and locomotion scoring this results in an overall relatively low precision due to the propagation of uncertainty. On top of this, 21.4% of the sensor values were missing from the dataset and had to be imputed. The imputation uses a mean value and does not take into account the values before and after the missing value. The imputation therefore causes a disruption in the sequential patterns of the sensor data, which makes them more difficult to analyse. In a study by Liseune *et al.* (2021) an improved way of missing value imputation was proposed using deep learning to fill in missing values based on the values observed in the same sensor sequence as well as the recorded values of the other features. This method led to a significant increase in model performance for a methodology-wise similar predictive model, and is therefore a promising method to use for model improvement.

A second important factor in model performance is the number of animals. As stated before, neural networks are prone to overfitting. A neural network quickly becomes very complicated compared to other models due to its many connections and weights. In the proposed model, many input variables were used; 19 different sensor features and 10 static features, complexing the model even more. The general rule is that as the complexity of a model increases, the noise of the training set is better memorized and the model performance on new data decreases (Alpaydin, 2020). Many measures were therefore taken in order to prevent overfitting, but another effective way to prevent overfitting is to increase the amount of training data. Besides, with 365 cows in the training set, there is a chance that the sample is not a correct representation of the behaviour of cows in the Netherlands. The model is trained to classify cows similar to the training data. It could therefore come to pass that there are cows in the test or validation set behaving very unlike those in the train set, and since there are only 122 cows in the test or validation set, this could result in low performance scores. The opposite could also be true; the model to evaluate could accidentally fit the test or validation set very well leading to high performance metrics, while when confronted with a more representative population sample would perform very poorly. Increasing the number of participants could therefore increase model performance and reliability, but unfortunately is also expensive and labour-intensive.

Another possibility to assess model performance for representativeness would have been to leave one of the eight herds out and use the cows of this herd as a test set. Because then it would have been known how the model would perform on a new farm, and thus it would have said something about the generalizability of the model. This is a difficult trade-off because on the other hand to make the model as generalizable as possible, it is beneficial to train the model on cows from as many herds as possible. And therefore this method was not applied in this study.

It was already mentioned that differentiating between different forms of hypocalcemia is useful. This was, however, not the only issue with the calcium categorization in need of fine-tuning. Serrenho *et al.* (2021) already pointed out that the cut-off value of 2.0 mmol/L is dubiously evidence based. The clusters proposed in this study are already an improvement compared to the cut-off value, since they correct for parity and day

of measurement and have a more fluent context based cut-off. But this clustering is not founded upon an association with post-partum disease and therefore could lack clinical relevance. Besides, it proved to be more difficult to predict the clusters by the XgBoost and the deep learning model compared to the cut-off grouping. On the other hand, the logistic regression model performed better using the clusters and the difference in model performance is minimal. For future research, it would be advisable to use the association with the outcome of interest to define a cut-off value. And in addition, take multiple blood samples from each cow on day 1, 2 and 4 after calving in order to differentiate between different hypocalcemia variants (Serrenho *et al.*, 2021). Both suggestion can be used to increase the clinical relevance of the prediction, and we believe that improving the calcium categorization will ameliorate the AUC values of the models the most.

Lameness is a known risk factor for SCH (Neves *et al.*, 2017) and can be quantified using the locomotion score. In addition, there is a known correlation between BCS and hypocalcemia (Heuer *et al.*, 1999; Roche and Berry, 2006). One would therefore expect that adding BCSs and locomotion scores as input variables would improve model performance. It turns out, however, that this is not the case, since there was no significant difference in mean AUC between the best performing model with BCSs and locomotion scores and the best performing model without, indicating no additional predictive value. This contradiction could be explained by two phenomena. Firstly, as stated before, when the complexity of a model increases, the noise of the training set is better memorized and the model performance on new data decreases. Adding two extra input features leads to many extra connections and weights, and therefore adds extra complexity, causing overfitting. The second reason could be that the model already recognizes lameness through the sensor data for instance from the walking, standing and lying features and as a consequence the locomotion score does not provide extra information to use for the prediction. Besides, in this study the locomotion score and BCS were scored by the same trained veterinarian, but when implementing this model in practice there is not always a trained veterinarian present to assess these scores. A problem already prevalent during the course of this research, which resulted in missing values. But even if it were possible for each cow to be scored by a veterinarian, there still would be quite a high variability due to imperfect inter-observer reliability (March *et al.*, 2007). All of the reasons above combined make the BCS and locomotion score inconvenient to use in a future model.

### Lameness and BCS

The model provides as output the probability for a cow to belong in the low calcium category. An ameliorated version of this model could serve as a tool to identify high-risk animals. A high-risk animal would be one with a high probability to fall within the low calcium category. As stated before, there is a known correlation between hypocalcemia and other transition diseases. Early detection of high-risk animals could therefore augment early detection of other associated diseases or underlying causes of reduced transition success. From a management perspective, this tool could serve as a method to keep track of calcium status of the herd as a whole. Nowadays, the only tool to evaluate calcium values is to regularly take blood samples and measure calcium in the laboratory. A method not often applied due to costs and labour. An improved model could give insight in calcium status and therefore be used to, for instance, evaluate preventive measures, review diet changes or as a screening tool to direct the focus of a herd analysis.

### Use in practice

Although many model improvement options, including the suggestions mentioned above, have not yet been tried out, the question remains whether a practically applicable model, with both high sensitivity and specificity, can be made with the input features proposed in this study. A cow is a living being rather than a predictable milk producing robot and maybe only seven types of behaviour, combined with some static features, are a too simple description of something as complex as a living cow. A cow displays many more kinds of behaviour, for instance social behaviour, which are not included in the research. Besides, a lot of different other variables are associated with the risk of hypocalcemia and could therefore explain a part of the variability between cows. For instance, the weather is associated with the risk of hypocalcemia (Roche and Berry, 2006) and the cow's diet (Thilsing-Hansen *et al.*, 2002). In the future, these variables could be added to the predictive model to enhance its performance. At the same time, previous research suggested that sometimes a model with equal reliability can be made while using fewer features, provided that for each feature a correlation with milk fever was previously proved (de Mol *et al.*, 2015). In this study, standing and lying bouts are now used as separate input features although providing the same information; every time a cow lies down it has to stand up after in order to be able to lie down again, suggesting possibilities for exclusion of features. Critically reviewing the behavioural features and then omit some could decrease model complexity and therefore improve model performance. For now, if the model proposed in this study would be used in practice, results need to be interpreted with caution. Although the model predicts better than random, still many false positives and false negatives are present. Especially false negatives can be dangerous since they could delay the detection of clinical hypocalcemia and other transition diseases, thereby quickly lowering the user's confidence in the model (Petticrew *et al.*, 2000).

### Similar studies

There have been others to propose a predictive model for hypocalcemia using different kinds of data. Ma *et al.* (2022) proposed a multivariate logistic regression model using blood analytes to predict the risk of subclinical hypocalcemia. The authors report a very high model AUC of 0.90, suggesting high model performance. But the model does not seem to be evaluated using a test set and the results can therefore not be interpreted as predictions since they were used to fit the logistic regression model and the model is therefore likely overfitted. Besides, from each cow 2 blood samples needed to be taken at different time points antepartum, making it a very labour-intensive method for prediction and therefore not suitable in practice. Using genomic information, Cavani *et al.* (2022) employed a multiple linear regression model to predict blood calcium concentration after calving. The authors reported a predictive correlation average of  $0.463 \pm 0.056$ ,  $0.396 \pm 0.052$ , and  $0.297 \pm 0.057$  for blood calcium concentrations on day 1, day 2, and day 3 after calving, respectively. The strongest association was observed on day 1, indicating the highest predictability. Although the model by Cavani *et al.* (2022) did not achieve high accuracy, it demonstrated the potential of genomics for prediction, making it a valuable addition to future models. However, as the study predicts calcium concentration as a continuous variable, it is not directly comparable to the binary variable predicted in this study.

### Suggestion for future research

When building a new model to predict transition success, the metric hypocalcemia as an indirect indicator of an impaired transition period could be abandoned and instead a more direct metric to evaluate a general transition success could be developed. As stated before, taking blood samples is expensive and labour-intensive, and it is therefore difficult to collect a large data set. For future research, an approach could be

to develop a score for transition success evaluated at the end of the transition period, when it is known how successful a cow has transitioned. The sensor data can then be used to predict this score on parturition and therefore give insight in the direction the transition period is heading at that point in time. This model could then still be used for the identification of animals at risk of disease, but might even be more powerful as a metric for herd health status and could therefore possibly be implemented as a management tool.

It has proved to be possible to predict the risk of hypocalcemia using behavioural sensor data and measured calcium concentrations, with an AUC value of 0.66 and an AP of 0.53. The behavioural patterns of the 21 days before calving contain valuable clues for the prediction of hypocalcemia after parturition, as do the static features: parity, calving season and day of measurement. The predictions of an ameliorated version of the model can be used to monitor herd calcium status and to identify animals at risk for transition diseases. Although there is still a long way to go to develop a model suitable for widespread practical implementation, the proposed model could be the first step towards achieving that goal.

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## Conclusion

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## Exploration of animal-based parameters (activity level and respiratory health status) collected by sensors to monitor pig welfare on farm

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Using sensor technology to support monitoring the environment and animal welfare on farm is becoming a common practice. An EU-funded project, ClearFarm, aims to use the data collected by PLF (Precision Livestock Farming) sensors to build an algorithm to assess animal welfare continuously across the value chain, with the focus on pig and dairy cattle farming. To achieve this, parameters collected by PLF sensors should first be contrasted with reference indicators reflecting the welfare status of the animals. The objective of the present study was to investigate the potential of animal-based parameters (in pigs) measured by PLF sensors, by contrasting them against aggression-related lesions and physiological biomarkers. The study was conducted in two Spanish commercial pig farms, one nursery farm and one fattening farm. Two commercial PLF sensors were used on both farms: Peek Analytics (Copeeks SAS, France) and SoundTalks (SoundTalks NV, Belgium). Peek Analytics collected environmental (temperature, humidity, NH<sub>3</sub>, and CO<sub>2</sub>) and animal-based data (activity level and number of active/inactive animals). SoundTalks collected environmental (temperature) and animal-based data index on respiratory health (ReHS: respiratory health status). Activity level was calculated by tracking the movement of each pig per unit of time. ReHS is a score from 0 to 100: <40 indicates high risk of respiratory health problems; 40-60 indicates potential respiratory health problems; and >60 indicates healthy animals. Sixty males and females (30+30) of each farm were randomly selected for skin lesion scoring (ear, head to fore legs, and trunk) and saliva sampling. There were two sampling points (beginning and end of the stage) in the nursery farm, and three (beginning, middle, and end of the stage) in the fattening farm. Stress- (cortisol, sAA: salivary  $\alpha$ -amylase, BChE: butyrylcholinesterase, and oxytocin), inflammatory- (Hp: haptoglobin), and immune system-related biomarkers (ADA: adenosine deaminase) were analysed from the saliva samples. Preliminary results showed that when temperature increased, and humidity or NH<sub>3</sub> decreased, pigs increased their activity ( $P<0.05$ ). On the other hand, ReHS was higher when humidity decreased, or when CO<sub>2</sub> increased ( $P<0.05$ ). The increase of activity was linked to the increase of ear lesion counts ( $P<0.05$ ), total lesion counts ( $P<0.07$ ), Hp ( $P<0.05$ ) and BChE ( $P<0.05$ ), and the decrease of oxytocin ( $P<0.05$ ). ReHS was positively associated with Hp ( $P<0.05$ ) and sAA ( $P=0.06$ ). Overall, the change of activity or ReHS may reflect the change of the environmental conditions, which can affect the aggression level and the physiological status of the pigs. In conclusion, continuously recording animal-based parameters collected by PLF sensors, such as activity level and ReHS, may be useful to monitor

### Abstract

animal welfare. Nonetheless, exploring more relevant animal-based parameters by sensor technology, especially covering parameters reflecting other welfare domains, will provide a better picture of the real-time welfare status of the animals.

*Keywords: activity, animal welfare, pig, Precision Livestock Farming, respiratory health, salivary biomarker, sensor.*

## Introduction

An increasing herd size with minimum labour input has become common lately in the last few decades in modern livestock farming. Exploiting sensor technology together with artificial intelligence may facilitate producers for rapid decision-making to optimize herd management in an objective way. To replace in-person animal welfare assessment, which is time- and labour-consuming, an EU-funded project, ClearFarm, aims to build a platform to monitor animal welfare in real-time and continuously along the production chain through sensor technology. The foundation of this project relies on the selection of relevant welfare parameters collected from the sensors, in order to be built in the platform algorithm. To achieve this goal, it is fundamental to first contrast these parameters with reference indicators which can reflect animal welfare status and are collected by humans. The objective of the present study is therefore to investigate the potential of animal-based parameters measured by the sensor, by contrasting them against two reference indicators, which are aggression-related skin lesions and salivary biomarkers.

## Material and methods

### Animals and housings

The study was conducted in two commercial pig farms in the Region of Murcia (Spain), one was a nursery farm and the other one was a fattening farm. Two pens of the nursery farm and four pens of the fattening farm were followed. There were 100 weaners in each nursery pen and 13 fatteners in each fattening pen. The nursery pen size was 2.5 m x 10.5 m, and the fattening pen size was 2.5 m x 4 m, providing the stocking density of 0.26 and 0.77 m<sup>2</sup>/pig, respectively. Both farms raised commercial crossbred pigs, which were (Piétrain x Duroc) x Danbred in the nursery farm, and (Danbred x Duroc) x Danbred in the fattening farm. It was 100% of fully slatted floor in the nursery pen, and 50% of slatted and 50% of concrete floor in the fattening pen. The study period of the nursery farm was between 29/September and 22/November 2022, and that of the fattening farm was between 26/October 2022 and 12/January 2023.

### Precision Livestock Farming (PLF) devices

#### Features of Peek Analytics and SoundTalks devices

Two commercial PLF devices were used in the study: Peek Analytics (Copeeks SAS, France) and SoundTalks (SoundTalks NV, Belgium). Peek Analytics was a multi-sensor device which consists of two main parts, the camera and the sensor. The camera measured the activity level of the animals and the number of active/inactive animals, calculated by a built-in algorithm, whereas the sensor monitored the environment of the pen, including temperature, humidity, and CO<sub>2</sub> and NH<sub>3</sub> concentrations. It was not possible to access the raw data and the algorithm to know how Peek Analytics calculated activity level for the confidentiality reason. However, the concept of it was to track the dots of each pig when the pig moves. As it did not monitor the distance and the speed of the movement, activity level did not have a unit and was provided with a numeric value (being the minimum value of 0). The sensor measured the environmental parameters every 2 minutes from 00:00 to 23:59, whereas the camera measured the

animal-based parameters every 30 minutes from 07:00 to 22:00. Due to the darkness of the night-time, the camera could not measure the animal-based parameters. As for SoundTalks, it measured the temperature of the pen and a respiratory health status (ReHS) daily, which was a score also calculated by a built-in algorithm mainly based on the coughing sounds of the pigs. It was also not possible to access the exact parameters the algorithm collected and how it calculated the ReHS score due to confidentiality reason. ReHS score had three levels and was presented in two forms: the numeric value or colour scale value, in which 0-40 (colour red) indicated a 'high risk of respiratory health problems', 40-60 (colour yellow) a 'potential respiratory health problems', and 60-100 (colour green) 'animals are healthy'.

Two Peek Analytics and two SoundTalks were used to monitor two nursery pens (n=200). Peek Analytics monitored the same two pens, with one installed at the front and the other at the back side of the pens, approximately 2.4 m height from the floor. SoundTalks were also installed in a similar way, with one at the front and the other at the back side of the pens, approximately 1.8 m height from the floor. On the other hand, two Peek Analytics and one SoundTalks were used to monitor four fattening pens (n=52). Each Peek Analytics monitored two pens (2.5 m height) and the SoundTalks was installed in the middle of these four fattening pens (2 m height). The views taken by Peek Analytics are shown in Figure 1.

Installation of Peek Analytics and SoundTalks devices

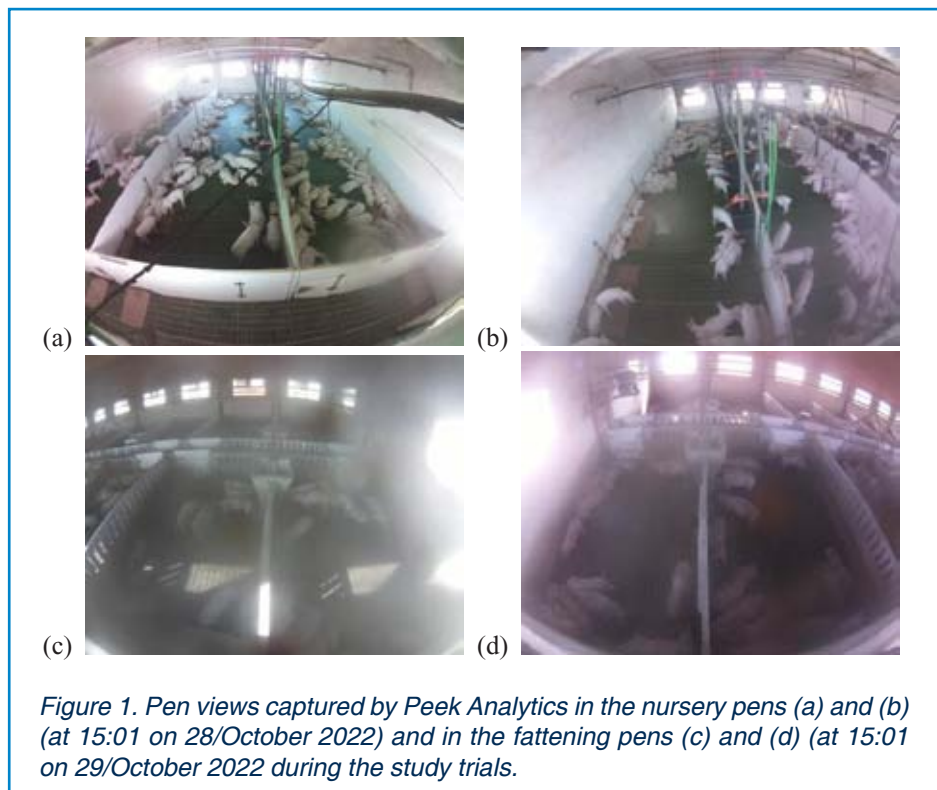


Figure 1. Pen views captured by Peek Analytics in the nursery pens (a) and (b) (at 15:01 on 28/October 2022) and in the fattening pens (c) and (d) (at 15:01 on 29/October 2022) during the study trials.

### Reference indicators: Salivary sampling and skin lesion assessment

Accumulation of skin lesions and salivary biomarkers were selected to be the reference welfare indicators for this study. Accumulation of skin lesions can be an indicator of aggression at group level in pigs (Turner *et al.*, 2006) and salivary biomarkers are suggested to adequately reflect the physiological status of a pig (Cerón *et al.*, 2022). A subgroup of pigs was selected for saliva sampling and skin lesion assessment: 60 weaners (30 males and 30 females) and 60 fatteners (30 males and 30 females). Aggression-related skin lesions were counted when the lesions were fresh, red, and linear (Turner *et al.*, 2006). The body of a pig was divided into six parts: ear, head to fore legs, and trunk, of left and right sides. As for saliva sampling, there were two sampling points in the nursery farm (i.e., the beginning and the end of the nursery stage) and three in the fattening farm (i.e., the beginning, the middle, and the end of the fattening stage). Stress (i.e., cortisol, sAA: salivary  $\alpha$ -amylase, BChE: butyrylcholinesterase, and oxytocin), inflammatory (i.e., Hp: haptoglobin), and immune function (i.e., ADA: adenosine deaminase) biomarkers were analysed from the saliva samples, following the procedures of sampling and determination of these biomarkers mentioned in Ortín-Bustillo *et al.* (2022).

### Statistical analysis

Statistical analysis was performed in RStudio ver 2023.03.1+386. Pearson correlation tests were conducted: environmental parameters of the PLF devices (i.e., temperature, humidity, CO<sub>2</sub>, and NH<sub>3</sub>) vs. animal-based parameters of the PLF devices (i.e., activity level, number of active/inactive animals, and ReHS score). Additionally, generalized linear models were conducted, having animal-based parameters/skin lesions/salivary biomarkers as response variable, date, sex, and environmental parameters as fixed factors. Statistical significance was set at  $P \leq 0.05$ , whereas the tendency was considered when  $0.05 < P \leq 0.10$ .

## Results

The preliminary results of the correlation tests between the environmental parameters and animal-based parameters are presented in Table 1. Activity level and number of active animals followed similar correlations with the environmental parameters. When temperature increased, the activity of the animals increased ( $P < 0.05$ ), whereas when humidity and NH<sub>3</sub> increased, the activity of the animals decreased ( $P < 0.05$ ). On the other hand, when temperature increased, ReHS decreased ( $P < 0.05$ ), and when CO<sub>2</sub> and NH<sub>3</sub> increased, ReHS increased ( $P < 0.05$ ). In general, temperature showed a

Table 1. Pearson's correlation coefficients (*r*) between the environmental parameter and animal-based parameter collected by the PLF devices (Peek Analytics and SoundTalks) in the nursery and fattening pigs. The correlation coefficients with no specified *P*-values indicate  $P < 0.05$ . The correlation coefficients that are not available indicate the insignificant results.

	Temperature	Humidity	CO <sub>2</sub>	NH <sub>3</sub>
Activity level	0.52	-0.30	-0.20 ( <i>P</i> =0.06)	-0.39
Number of active animals	0.52	-0.32	-0.18 ( <i>P</i> =0.08)	-0.24
Number of inactive animals	0.21	-0.36	-	-0.33
ReHS <sup>1</sup>	-0.50	-	0.44	0.22

<sup>1</sup> ReHS: Respiratory health status.



rather stronger correlation with the animal-based parameters (especially activity level, number of active animals, and ReHS), compared to other environmental parameters.

In terms of the association of skin lesion counts and salivary biomarkers with the parameters collected by PLF devices, the increase of activity was linked to the increase of ear lesion counts ( $P=0.02$ ), total lesion counts ( $P=0.08$ ), Hp ( $P<0.01$ ) and BChE ( $P<0.01$ ), and the decrease of oxytocin ( $P<0.01$ ). On the other hand, ReHS was positively associated with Hp ( $P<0.01$ ) and sAA ( $P=0.06$ ).

In the present study, activity level of pigs increased when the environmental condition was better (i.e., higher temperature, and lower humidity and CO<sub>2</sub> concentration in this case). On the other hand, the ReHS score was higher (i.e., healthier pigs in terms of respiratory) during lower humidity but higher concentration of CO<sub>2</sub> and NH<sub>3</sub>. Positive correlations between activity level with skin lesion counts and some salivary biomarkers may indicate that a higher activity level can be interpreted as higher agonistic interactions within the group, which therefore caused higher aggression-related skin lesion counts, higher concentrations of biomarkers related to inflammation (i.e., Hp) and stress in pain or discomfort (i.e., BChE), lower concentration of biomarker related to positive emotions (i.e., oxytocin) (Cerón *et al.*, 2022). In addition, a higher ReHS score was found to correlate with higher concentrations of biomarkers related to stress (i.e., sAA) and inflammation (i.e., Hp). However, a higher level of sAA was known to be associated with the activation autonomous nervous system (Cerón *et al.*, 2022), which is the system that also regulates the respiratory system, and this may be the reason for this positive correlation between ReHS and sAA. A deeper look on the data for each parameter and their associations are needed and are the current work of this project. To conclude, the change of the animal-based parameters collected from PLF sensors, such as activity level and ReHS score, may reflect the change of the environment, which can affect the aggression level and the physiological status of the pigs. There is potential to use these two animal-based parameters as an animal welfare indicator for continuous monitoring, but further confirmation and validation such as using farm or veterinary records as a 'ground truth' for another reference is necessary.

## Discussion and Conclusion

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## Searching for phenotypes to improve welfare in Avileña-Negra Ibérica beef cattle breed: preliminary results

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Animal welfare is of big concern for the civil society, and we know that animal under stress is an animal with lack of welfare, and so, with losses in its productive life. The Avileña-Negra Ibérica (ANI) breed is a Spanish local beef cattle reared under extensive conditions. This breed has shown a large variability in their reactivity when exposed to common triggers of stress. The objective of this work was to establish a procedure to evaluate temperament in ANI calves in the post-weaning phase and estimate the repeatability of temperament indicators. Eighty data were recorded from 25 male calves under commercial conditions. The number of records per animal varied between three and four. Temperament indicators were flight time (FT) which is the time (in second) needed to cover a distance of 1.83 m. FT was recorded digitally, using a timing system with two infrared sensors (FarmTek, North Wylie, TX) or manually, using a conventional chronometer. In addition, two subjective scores were assigned: flight score (FS) measured in four categories and restraint score (RS) in five categories. The repeatabilities of measurements were 0.29, 0.55 and 0.26 for FT, FS and RS, respectively. The objective indicator (FT) showed lower repeatability than expected. The manual system tended to increase FT. Some discrepancies were observed in the inter-observer consistency.

*Keywords: animal welfare, stress, behaviour, temperament, repeatability.*

Nowadays, animal welfare is of big concern for the civil society, and is increasing (Alonso, *et al.* 2020). Regardless the ethical issues, welfare is also a main priority for beef farmers because lack of welfare in an animal is caused by stress as the result of its reaction to internal or external factors such as high temperatures, diseases, social isolation or changes in social groups, handling and injuries (Salvin *et al.*, 2021) Stress affects any aspect of an the productive life of an animal as well as increases the maintenance requirements (Collier *et al.*, 2017) due to physiological changes resulting from disturbed homeostasis.

### Abstract

### Introduction

The reactions of animals when they are exposed to different sources of stress (social, handling environmental, etc) trigger an emotional response that changes their behaviour. Changes in behaviour generated as an emotional response to stressors is what is understood as the temperament of the animal (Burrow and Dillon, 1982, Grandin, 2000). Temperament is a very complex trait and comprise a number of attributes such as shyness, aggressiveness, sociability, avoidance, fear (Reale *et al.*, 2007). Poor temperament in an animal is a risk factor, an indicator of lack of welfare. Temperament affects production, reproduction, immunocompetency as well as meat quality therefore, it could be considered an indicator of poor performance to be selected against (Yu *et al.*, 2020).

Several measurements of temperament have been proposed to evaluate temperament in beef cattle, such as chute score (Tulloh, 1961), flight speed (Burrow *et al.*, 1988), exit score (Vertter *et al.* 2013), among others. All of these measurements are easy to record. All of them are an attempt to summarize all the different attributes of temperament. Except flight speed, the other two traits are subjective evaluations of temperament. Thus, the recording of these traits requires trained observers in order to obtain consistent measurements.

Calves from Avileña-Negra Ibérica (ANI), are an interesting population to understand temperament in animals. These animals show a large variability in their reactivity when they are exposed to a common stress factor (Meneses, *et al.*, 2022). Because of this variability, it is a trait of interest for the selection program of the breed.

The objective of this work was to set up a procedure to evaluate temperament in Avileña Negra Ibérica (ANI) calves at the Control Center of the Breed in the postweaning phase and to evaluate for the first time, the repeatability of these traits in this breed.

## Material and methods

Data were recorded from twenty-nine male ANI calves every month, between 200 and 400 days of age. Animals were passed through the chute to be weighted in the Control Center of the Avileña-Negra Ibérica Breed. Data were taken under commercial conditions. Animals were handled in two different batches or groups. Depending upon the group, the number of controls per animal varied from three to four; three for group 1 and four in group 2 (Table 1).

The protocol to evaluate temperament comprised three measurements: flight time (FT), exit or flight score (FS) and chute or restraint score (RS). The FT is the time taken by an animal to cover a distance of 1.83m (6 feet distance). Since that distance is measured at exiting from the weighting chute, we refer to it as flying score. FT was taken in two ways; in the first recording, FT was manually measured with a chronometer (control 1 and 2); for the third and fourth recordings, data were automatically recorded with an infrared system (control 3 and 4). The infrared equipment (FarmTek, North Wylie, TX) was made up of two wireless electric eyes that were turned on and off when the animal passed, and the time was registered in a timer console. The two subjective scores were FS and RS, with four and five categories, respectively. Categories for FS were; 1: walk, 2: trot, 3: canter and 4: run, the same categories as described by Vettters *et al.* (2013). RS was recorded in the weighting chute in five categories: 1: for quiet animals, 2: for animals with slow movements, 3: for animals with frequent movements with vocalization, 4: for animals with constant movements, lateral displacements and vocalization, and 5: for animals with violent movements, and continues intention to leave. Two observers recorded FS and RS. A total number of 80 observations were obtained (Table 1). In addition to total number of observations, Table 1 also show the distribution of number of observations in groups and control number.

Table 1. Number of animals by group and control number.

Group		G1	G2	Total
Nº animals		14	11	
Control	C1	14	11	25
	C2	14	11	25
	C3	14	8	22
	C4		8	8
Total		42	38	80

G: group or batch of management, C: control number.

Pearson correlations were obtained between successive controls for FT. Spearman correlations within and across observers were obtained for FS and RS between controls. These correlations are interpreted as a measurement of consistency between controls and observers. Remlf90 estimates of repeatability and correlations were obtained (Misztal, *et al.*, 2009) in a multi-trait model where the animal effects were assumed to be independent within traits. Covariances between traits were assumed. In addition to random effects, the models included age of calves at recording as covariates and batch (3 levels) for all traits, in addition, observer (2 levels) was included for FS and RS, and recording device (2 levels) for FT. The repeatability was estimated as the ratio between the variance associated to the animal and the total variance.

On average ANI, calves took 1,5 seconds (0,618) to cover the 1.83 m of distance. The average FS and RS were 1.89 (0.79) and 2.26 (0.97), respectively. We analyzed the inter-observer consistency for FS and RS. Results are shown in Table 2 and Table 3, respectively. As it can be observed in both tables some discrepancies between observers occurred. For FS both observers used all levels of the scale from 1 to 4, however only observer 1 used the whole scale for RS. The number of discrepancies per traits were 9 out of 80 (Table 2) and 15 out of 80 (Table 3) for FS and RS. Thus, it is clear that evaluation of RS is more complex than FS and it seems to involve a larger degree of subjectivity than FS. In general, observer 2 tended to assign higher scores for RS than observer 1 while the opposite occurred for FS.

Correlations between consecutive measures of FS and RS are shown in Table 4. The magnitude of correlations between subsequent measures for FS were much higher than the correlation for RS. This was so, on average as well as within observer. In both traits, the correlations between controls two (C2) and three (C3) were higher than the ones between control one and two. Control 1 was a new experience for the calves, while C2 and C3 involved a learning process for calves (Kamel *et al.*, 2006; Veters *et al.*, 2013)

## Results and discussion

Table 2. Contingency matrix for categorical classification Flight Score by observer.

		Cat Observer 2			
		1	2	3	4
Cat Observer 1	1	26	2		
	2	2	36	4	
	3			12	
	4			1	1

Table 5 shows estimates of repeatability and correlations between traits. Repeatabilities were 0.29, 0.55 and 0.26 for FT, FS and RS, respectively. The objective indicator, FT, showed lower repeatabilities than expected according to literature, but there are a number of factors that may be causing this result. Firstly, the sample size is very small; secondly, in order to measure FT, we used two different systems, manual vs automatic and the correlation between them were lower than expected (0.207). The manual system overestimated FT (1.77 vs 1.07 sec. for manual versus automatic systems.) In addition to those reasons, we have to take into consideration that the animals were growing while the trials were performed therefore, it is not strange that the time required by an animal to cover the same distance decreases over time what affect variability. In any case, the repeatability for FT was lower than others published in the literature 0.40 (Kamel *et al.*, 2006) and 0.5 (Vetters *et al.*, 2013) Correlation between traits are also shown in Table 4. Correlation between FT and FS was very high and negative (-0.98). Thus, animals that took more time to cover the distance also received a lower FS. This finding if confirmed, is a positive result because it means that both, FT and FS are pointing out toward the same trait. From a recording point of view, it could help to record more data in the absence of an automatic recording system. The correlations between FT and RS, and between FS and RS were in the expected sense and very high, -0.95 and 0.92, respectively. These results may suggest that all these temperament indicators are measuring the same trait however, the magnitude of correlations between FT and FS with RS are much higher than estimates of genetic

Table 3. Contingency matrix for categorical classification Restraint Score by observer.

		Observer 2			
		1	2	3	4
Observer 1	1	18	3		
	2	3	24	3	
	3		2	19	
	4			3	8
	5				1

Table 4. Correlations between subsequent evaluations of Flight speed (FS) and restraint score (RS) by observer and on average.

Observers	FS		RS	
	C1-C2	C2-C3	C1-C2	C2-C3
1	0.542	0.632	0.052	0.152
2	0.426	0.621	0.005	0.134
Average	0.489	0.635	0.052	0.149
N	25	22	25	22

Table 5. Repeatabilities of the traits (diagonal), and correlations between traits (off diagonal).

	FT	FS	RS
FT	0.29	-0.98	-0.95
FS		0.55	0.92
RS			0.26

FT: flight time, FS: flight score and RS: restraint score.

correlations between those temperament indicators found in the literature (Kadel *et al.*, 2006). Kadel *et al.* (2006) found a genetic correlation between FT and FS with of -0.37 and 0.39, respectively.

Our main objective was to set up a protocol to evaluate temperament in this breed as an indicator of welfare. Regardless the magnitude of repeatabilities our main objective was achieved. However, taking into consideration the sample size of the assay, we cannot draw many conclusions from the results. Moreover, we need to understand if results are due to the protocol in itself or caused by changes in the animals to deal with stress when they are exposed several times to the same management/stressor. The consistency between observers needs to be periodically revised. We are now recording more data as well as additional information to understand the underlying physiological processes in response to stress.

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## Conclusions

## Acknowledgments

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## Sensor data for animal health and welfare: present perspectives and future applications

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Sensor technologies measuring individual animal behaviour and physiological parameters are increasingly used in dairy farms to improve fertility and health management. These technologies produce a large amount of high-resolution data at individual cow level and thus interest in using these data exists beyond herd management. In this study, which was conducted within ICAR's Brian Wickham Young Persons Exchange Program (BWPEX) five representatives from ICAR member organisations and research institutions were interviewed to gain more insights into benefits and challenges of the use of sensor data beyond its intended purpose. The topics addressed in the interview were about

1. The greatest potential of using sensor data in general and for the interview partner's organisation specifically,
2. How sensor data is currently used in the interview partner's organisation and planned to be used in the future,
3. Which challenges exist and how they can be overcome,
4. How sensor data can be used for animal health and welfare improvement and for breeding, and
5. How important sensor data will be for the dairy industry in the future.

All interview partners attributed great potential to the use of sensor data beyond herd management and were interested in using it also in their organisations. However, several challenges were identified and although ideas on how to overcome them exist, it was concluded that the development of third-party applications or other products based on sensor data is not ready yet. Some aspects of how the data may contribute to enhancement of animal health and welfare and in a breeding context were mentioned and there was consensus that these data will play an important role for dairy industry in the future.

*Keywords: cow, sensor data, animal health and welfare, breeding, interview.*

Dairy farms increasingly use new technologies such as automatic milking systems (AMS) and wearable sensor devices measuring behaviour such as activity or rumination and physiological parameters (e.g. rumen temperature) in dairy cows. Manufacturers offer these technologies in combination with software programmes for certain management purposes such as notifications for oestrus or calving detection or for health monitoring. These notifications are based on algorithms, which identify for example changes in movement patterns and relate them to a potential heat event.

### Abstract

### Introduction

However, the large amount of high-resolution data at individual cow level offers a huge potential beyond its intended purpose and may be used in research, for breeding or by milk recording organisations. ICAR is working on several aspects of the use of sensor data to investigate their potential. Currently, guidelines on the validation of sensor systems are developed by the ICAR Measuring, Recording and Sampling Devices Sub-Committee and on the use of sensor data by the ICAR Functional Trait Working Group together with the IDF Standing Committee on Animal Health and Welfare. Furthermore, the ICAR Animal Data Exchange Working Group deals with technical issues and requirements for interfaces between sensor companies and milk recording or breeding organisations, who want to obtain the data. There may still be some legal and technical challenges, which have to be overcome, but nonetheless it is important to think about use cases and added benefit of these data. Thus, the aim of this study was to disclose potential fields of applications within ICAR member organisations focusing on the aim of animal health and welfare improvement.

## Material and methods

This study was conducted within ICAR's Brian Wickham Young Person's Exchange Program (BWYPEX), which supports young researchers, who work on topics important to ICAR and its member organisations, in building a network and gaining experience through visiting different member organisations in various countries. Guided interviews were conducted with five persons related to ICAR, its member organisations or research institutions between March and May 2023. Two persons were working in research institutions, one person in a breeding organisation, one person in an artificial insemination (A.I.) company, and one person for ICAR. Interviews were recorded, transcribed, and subsequently coded based on five categories, some including subcategories, which are listed in Table 1. The categories were deduced based on the interview guide. For content analysis, interview transcripts were printed, and colour coded to assign them to the single categories. Finally, each category was analysed on its own using the text snippets.

Table 1 Categories for analysis of guided interviews

Category	Subcategory
Greatest potential of using sensor data in general and specifically for the interview partner's organisation	
Use of sensor data in the interview partner's organisation	Status quo and purpose Plans for the future
Challenges	Identified challenges Ways to overcome them
Use of sensor data for animal health and welfare and breeding	
Future perspectives of sensor data for the dairy sector	Expectation for the future and possible developments Importance of sensor data for the dairy industry in the future

Two major aspects were mentioned. First, integrating the data with other (historical) farm and cow-specific data, may increase the value of these data for farm management. Whereas these devices create a large amount of data and offer decision support in certain areas (e.g. heat detection) the real benefit only emerges when integrating it with historical information and other farm data. This way predictions for diseases, behaviour, or milk yield may improve. Based on that, smart insights for farm management may be created and if data are used across farms, this may enable benchmarking applications. Starting from that and using various other sources, added value may also be generated beyond farm level. Additionally, DHI and/or milk recording organisations may benefit by adding value to existing services or even broaden their service portfolio for farmers.

Furthermore, these data bear a great potential for use in genetic evaluation, which in most cases may be regarded as by-product of data recording programs. Given that it serves its initial purpose of herd management improvement, so farmers keep using it, data can be used for large scale phenotyping and trait definition. These may comprise completely new traits based on what the sensor is measuring or what can be predicted using integrated data sets on the one hand or the development of new proxies for complicated traits such as feed efficiency, resilience, or health traits on the other hand. Furthermore, phenotypes based on these sensor-derived data may be closer to the animal's physiology and thus improve genetic evaluation. Generally speaking, three main technologies can be used for the development of new traits - vision, accelerometer-data and mid-infrared. The latter, however, will not be able to do sensing around transition or in the dry period.

On ongoing initiative aims at a validation for sensor systems, which may be regarded as a check or a guideline for several aspects of the sensor systems. It should help users to understand what kind of data changes happen between the sensor measurement and changing the measurement into an observation and how big the black box in between is. Eventually, the user should be able to understand what the system is able to do, and for which purpose the data can be used (e.g. for management or for genetic evaluation). From a scientific perspective, showing variation in the different behaviours and trying to scientifically define ranges for normal behaviour and for deviations from normality are currently interesting fields of research. Other aspects mentioned comprise how useful these data are for predictions on animal health and welfare and how they can be integrated with other data for this purpose. These basic understandings and predictions should also form the basis for the development of new traits for different purposes. For one, new technologies such as AMS may pose new demands to cows in terms of milking behaviour and thus, these can be recorded using these technologies. Furthermore, there is research being carried out on using the sensor data for fertility related genetic evaluation. The idea behind is that sensor data may be closer to the physiology of the animal and may thus yield more heritable traits than those based on breeding records.

However, more research is needed in terms of data editing and trait definition because in the current form they do not meet the quality standards. Furthermore, sensor data allow to characterize intensity of heat expression, which was shown to positively correlate with retainment of a successful pregnancy in embryo transfer recipient cows in one study. In addition to research on sensor data applications, also technology development is of interest and particularly the development of sensors based on computer vision is currently boosted. Its benefits are that it can offer a way to mimic what is otherwise visually assessed by an observer and that the sensor does not have to be attached to the animal itself, which may in some cases be considered as a painful intervention.

## Potential applications of sensor data

## Use of sensor data in the different organisations

### Current use of sensor data

### **Use of sensor data planned in the future**

Future plans address approaches for standardizing data across manufacturers and join forces among organisations in a collaborative effort to access sensor data. In this context, ICAR is expected to take the initiative to speak on behalf of its partner organisations. Furthermore, the definition of new traits and the possibility of introducing new evaluations (e.g. fertility or heat stress related) based on sensor data will be investigated. Further research will – if existing – build on preceding work revising data editing and amplifying the data set by for example increasing the number of herds. Implementation of these new traits, indices or evaluations depends not only on their performance and suitability for genetic evaluation, but also on their superiority compared to current ones in terms of higher heritabilities or lower costs for phenotyping, etc. Further ideas are to develop (genetic) models for an on-farm use, so the data does not have to leave the farm anymore. This approach is inspired by the use of phone data by companies such as Google, who passed from training their models in the cloud to directly training them on the user's phone. However, these initiatives are still at the very beginning, and it is difficult to receive funding for it.

## **Challenges**

### **Data security and ownership**

Before thinking about any further use of data from sensor systems these data must be available to the person or institution interested in working with it. First, there is the legal side of data ownership. Who is the owner of the data and what are the conditions for using the data? This is not as straightforward as it may seem. Farmers using the sensor systems are the supposed owner of the data generated on their farms and thus, data cannot be shared without the farmer's consent. Furthermore, data must be treated confidentially – it must be clear who is using the data for what. Farmers may be hesitant in sharing these data due to the fear of misuse, unauthorized sharing with third parties or the emergence of disadvantages for them. However, even with farmers' consent access to the data is still depending on the agreement of the manufacturer companies, who may be reluctant to share these data. Most of them are large companies acting at a global scale, which may sometimes imply the lack of a clear policy on how to handle data sharing with interested third parties or changes in existing policies due to takeovers by other companies. Another scenario is that manufacturers charge a fee for data provision, which may present a challenge for research initiatives and business opportunities with unknown outcomes. In many countries, companies doing genetic evaluation used to have access to a lot of data at no or low cost (e.g. milk recording or classification data) because they were recorded at national levels with a lot of subsidies and genetic evaluation was often considered a by-product of these (herd management) data. However, this may change when it comes to sensor data from private companies and breeding organisations may have to pay for these data in the future.

### **Validity and quality of sensor data**

Assuming that access to data is granted, further challenges arise. Parameters and values generated by the sensor are not clearly defined and information on how the raw measurements of a sensor (e.g. an accelerometer) are changed into the output parameter are mostly not accessible due to intellectual property. This is even more true for alarms, which are generated based on the measurements without disclosure of thresholds or algorithms. However, for deriving traits for breeding from these data it may be important to get access to this information to better understand what exactly is being measured. Furthermore, information on the accuracy of alerts generated by the system is also lacking. For the sensor parameters themselves it is not clear how accurate the data is and if the output parameter is measuring what it claims to measure. Hence, further use of the data may require validation tools or regular calibrations of the sensor. This opens up another important issue concerning reference values and

what can be considered normal, let alone which expression is desirable – more or less active cows for example. The sensor information is often very generic, and the challenge is to associate these non-specific measurements to anything related to e.g. welfare. Irrespective of the final purpose of the sensor data application – be it herd management, breeding, or welfare assessment – there is need for a solid reference value and gold standard. This relates to the sensor parameter itself in terms of what exactly the output parameter is measuring but also to the definition of the trait or disease, which should be predicted by the sensor values. Having a clear understanding of the predicted phenotype is particularly important if this information is further used for genetic or genomic prediction models. However, often the real diagnosis is hard to get or difficult to detect (e.g. silent estrus) and additionally frequencies are too low to obtain enough data and reliable results. On top of these cross-sectional data, there is need for longitudinal data to assess repeatability over time.

Besides those general requirements of data validity and accuracy there is another important aspect of data quality – measurement errors and outliers. The system may break down or the sensor may run out of battery generating faulty data or no data at all. In commercial farms there are also a lot of management related sources for missing or erroneous data. The replacement of an empty battery or a lost device on a cow will be depending on available time of the farmer or the urgency of the sensor to work because for example the cow is going to be up for breeding soon and thus the heat alarm system is needed. However, if the cow was already bred then changing the sensor may not be first priority to the farmer.

Furthermore, these systems are intentionally created as a management tool and thus alerts, or other information is optimized to serve this purpose rather than to correspond to the correct physiological trait. Taking heat alarms as an example, they may be intentionally prolonged indicating the time window for a successful breeding rather than the physiological duration of an oestrus. Moreover, an alert may be generated based on changes in the sensor parameters although the indicated event should be impossible (e.g. heat alerts during pregnancy). Other disturbances than heat or calving events or diseases such as social interactions between animals or the use of synchronization protocols, respectively can influence cow behaviour or physiological states and thus create system alerts. Thus, correctly identifying these irregularities in the data is another challenge for any further application and in addition to adequate skills in data science it requires a lot of domain specific knowledge.

One important step towards solving these issues and creating added value is the integration of the sensor data with other farm (management) records and historical data. However, this entails several other challenges. First, integrating these animal-individual data, which are available at daily or even hourly resolution, with additional data and using them in smart applications requires a lot of space for data storage as well as computational capacity. Furthermore, correctly matching the sensor data with other animals-specific records can also be quite challenging, particularly regarding the correct animal identification. Whereas this may be easier to solve for wearable devices, which can be assigned to an individual animal, this is more challenging for installed systems working for example with computer vision techniques. While these are well performing in assessing lameness in cows, the positive identification of the correct animal is much harder.

Even if those requirements were met for individual sensor systems, there is still the issue of lacking standardisation between systems of different manufacturers. Parameters may be called the same and intentionally measuring the same thing (e.g. rumination or activity), but they differ in measurement, definition and algorithm between devices

**Standardisation  
between sensor  
systems**

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or even between devices by the same manufacturer. Some parameters are expressed in time units (e.g. minutes of rumination per hour or during 24 hours) and thus their values seem more comprehensible whereas for example activity is often expressed as a dimensionless value, which cannot be related to any known scale or unit. With the intention of using these data for breeding or other purposes, which involve the use of data across many farms, standardization or comprehensive definition of those parameters or traits presents an important issue. Even though models may be able to correct for some of the variation this may still not be enough to harmonize these measurements.

Much experience with these data lies within research organisations and while there is more research needed this does not necessarily always require new data. Revising already existing data from new perspectives is one very important task, which may also need more resources in terms of manpower and financing of research activities. However, it is very difficult to get this kind of research funded because it may lack novelty and other important grant criteria. Moreover, not many people stay in this field of research for a longer period of time to continuously work on these topics.

### Sensor data in research

To define normal variation, either phenotypic or genetic, data has to be explored in a neutral way. Although researchers' intention is to be objective in their work, underlying values, mindsets, or presumptions may bias this neutrality. Moreover, data science and domain knowledge need to be combined to yield best results. Domain knowledge is needed before any modelling as well as afterwards and results may have little value for application without it. This may also comprise the documentation of domain knowledge in a way, which is interpretable by an algorithm, so it can feed into the modelling process and make the 'black box' of this process more comprehensive.

Usually, ICAR member organisations do not have the capacity for undertaking this research by themselves, which makes the involvement of research organisations and universities even more important. This requires an open dialogue between all the involved parties, to ensure that developed traits are relevant for implementation and economically interesting. When it comes to traits and trait definition finding a gold standard for reference is a key element. Revising scientific literature on different aspects of the trait in question and relating it to the sensor measurements is a starting point. Furthermore, using reference herds with a lot of detailed phenotypes and sensor data and subsequently validating the results at larger scale may present a good strategy. In case of traits with a low frequency it is helpful to increase the data set and the number of herds, if this is possible. The challenges relating to data quality may best be addressed in a collaborative effort, which may comprise an open documentation of data cleaning and editing when publishing results. If researchers share their experiences with various types of sensor data, follow-up or other research may benefit a lot and may be able to start later in the process instead of 'reinventing the wheel'. Combining these efforts may also help in terms of research funding.

However, none of this research will be possible without access to sensor data and in particular to sensor data from commercial farms. Issues around data ownership suggest that the whole setting is very complex and there are different interests at stake. ICAR as an umbrella organisation is expected to take the initiative to speak on behalf of its members and try to negotiate with sensor manufacturers. Communicating a clear purpose to manufacturers may help in these negotiations.

One of the most obvious positive impacts on animal health and welfare may lie in the potential to early detect cows with potential health issues, which allows for prompt intervention. Even if farmers are very attentive and monitor animal health closely, sensors may detect problems before they are visible to the farmer. Early detection and intervention allows for the chance of reducing suffering of the animal as well as costs due to diseases. Especially farms with large herds may profit from these alerts so they can filter out animals, which may need treatment and pay more attention to those specifically.

From a research perspective these sensor data may help us to understand more about the normal behaviour of cows by exploring (normal) variations due to parity, breeds, age, etc. or diurnal patterns. Similar to lactations curves, we may deduce patterns of rumination or activity over a whole lactation period. Understanding these patterns and knowing when for example rumination time may be higher or lower is important to differentiate physiological from pathological states. If these relationships are well understood, data-driven assessments can be used beyond herd or farm level to assess and report health and welfare across farms, farm types, regions, or climatic areas. When it comes to welfare assessment, these data may help to monitor welfare continuously instead of just at certain points in time. Benchmarking tools together with extension services may function as a tool for health and welfare improvement on farms.

In terms of breeding, these data can be used to define proxies for health traits, which may be closer to the animal's physiology. This could enable genetic selection for more resilient animals or animals, which are more robust towards certain diseases.

The overall impression was that sensor data will be highly important for the dairy sector in the future. Farm management can be improved along with the opportunity for objective monitoring of animal welfare. However, the usefulness of sensor technology for the farmer must be priority, otherwise they will stop using it. Furthermore, the farmer as a mutual client presents an important link between the sensor company and any third party using the data and developing applications for farmers. At this point however, the information from these technologies is not yet ready for the development of routine applications and organisations should be careful not to promise solutions too soon. Or, as one of the interview partners put it: "You can only ring the bell once. And if that bell isn't positive, you cannot undo it."

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## Use of sensor data for (breeding for) animal health and welfare

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## Future perspectives for sensor data in the dairy sector

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## Descriptive evaluation of camera-based lameness detection technology paired with artificial intelligence in dairy cattle

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### Abstract

The aim of this study was to explore whether autonomous camera-based (AUTO) mobility scores could detect first lameness occurrence earlier in cows, by assessing the association between average weekly autonomous camera-based (AUTO) mobility scores and cows with a lesion for the first time. The AUTO scores data were collected from 2,982 cows in a single farm from April to December 2022, including cow ID, mobility score (0 to 100), and observation date and time. Historical farm hoof lesion data were collected from 2,204 cows and used to determine cow lesion history and date of lesion diagnosis (LD). To remove the confounding impact of chronicity, the study focused on cows with no history of lameness and categorized them into two categories: those with a first-time LD (LESION) and those seen by a hoof trimmer without an LD (TRIM). These categories were compared based on when the trimming occurred: within seven days of dry off (DOT) or at a random time based on farm staff observation. Individual AUTO scores were summarized into moving average weekly scores. All weekly AUTO scores were reported as median [IQR]. Comparisons were made for the LESION cows by lesion types. The lesion types for DOT (n = 60) were 3.3% toe ulcer (TOE), 1.7% white line disease (WLD), and 1.7% sole ulcer (SU), while the remaining had no reported lesion (93%; TRIM). For RT (n = 239), 63% were TRIM, 17% digital dermatitis (DD), 7.5% SU, 7.1% WLD, 4.2% foot rot (FR), and 4.2% TOE. Four weeks prior to RT, LESION had a similar median score (37.6 [18.3]) to TRIM (38.5 [13.7]). One week prior to RT, LESION had a higher median score (41.1 [17.5]) compared to TRIM (39.2 [15.5]). For DOT, four weeks prior, LESION had a higher median score (59.2 [2.1]) than TRIM (40.0 [9.9]), and this pattern persisted through 1 week prior. FR had the highest score (47.3 [22.9]) four weeks earlier, followed by SU (42.8 [19.0]), WLD (41.2 [13.5]), and DD (35.0 [14.1]). One week prior, these scores were increased for FR (57.1 [11.5]), SU (44.5 [12.4]), WLD (44.3 [26.8]), and DD (39.5 [10.6]). The results suggest that AUTO scores may have the potential to detect some lesions earlier. However, there is variation between cows and weeks that presents a challenge yet to be addressed.

*Keywords: lameness detection, artificial intelligence, dairy cattle.*

## Introduction

Lameness is a common problem in dairy cows worldwide, with incidence rates in North America ranging from 10% to 55% (Keyserlingk *et al.*, 2012; Cook *et al.*, 2016; Adams *et al.*, 2017). Lameness can have a significant impact on cow productivity, health, and welfare, accounting for 10 to 20% of all involuntary culling (Green *et al.*, 2002; Cha *et al.*, 2010). Several studies have investigated the prevalence, risk factors, and impact of lameness in dairy cows. Adams *et al.* (2017) estimated that the prevalence of lameness in dairy herds in the United States was 18.8%. Cha *et al.* (2010) found that lameness was a major factor in culling dairy cows in Quebec, with 25% of cows being culled due to lameness. Cook *et al.* (2016) reported that lameness can reduce milk production by up to 10%. Green *et al.* (2002) found that lameness can increase the risk of mastitis and other health problems in dairy cows. Keyserlingk *et al.* (2012) found that lameness can reduce cow welfare by increasing stress and pain. Mostert *et al.* (2018) identified that lameness can have a significant economic impact on dairy farms, increasing calving interval, antibiotic usage and GHG emissions.

There are a number of things that can be done to improve dairy hoof health. The FARM program is a lameness prevention program that was developed by the US National Milk Producers Federation. The program requires that less than 5% of lactating cows be scored as severely lame. The FARM program includes several management practices that can help to prevent lameness, such as: providing cows with adequate bedding, using smooth flooring, providing regular hoof care and monitoring cow lameness. Genetic selection can also be used to improve dairy hoof health by selecting animals with good hoof health traits. These traits can be identified by using a variety of methods, such as ultrasound, hoof scoring, etc. and combined with pedigree and genomic data. Dairy farmers can help to improve the hoof health of their herds if they are able to identify and select those animals with more favorable hoof health genetics; however, there are several challenges that need to be addressed in order to make genetic selection effective. One challenge is the low heritability of hoof health traits. Heritability is a measure of how much of the phenotypic variation in a trait is due to genetics. The heritability of hoof health traits is estimated to be less than 1% when using producer-recorded incidence data. This makes it difficult to select for bulls with good hoof health traits. Another challenge is the consistency of reporting. Hoof health data is often not reported consistently across herds. This makes it difficult to compare the hoof health of different herds and to identify bulls with good hoof health traits. Despite these challenges, genetic selection is a feasible approach to improving dairy hoof health. By addressing the challenges of low heritability and inconsistent reporting, it is possible to identify bulls with favorable hoof health traits and to improve the hoof health of dairy herds. In 2017, the ICAR (International Committee for Animal Recording) updated its hoof lesion definitions to allow for more accurate recording of hoof health data. This update will help to improve the consistency of reporting. By addressing the challenges of low heritability and inconsistent reporting, it is possible to use genetic selection to improve dairy hoof health.

Mobility scoring: there are numerous different scales used to score mobility, including continuous scales (0 to 1, 0 to 10, 0 to 100) and ordinal scales (2 levels up to 13 levels with ½ point increments). The choice of scale is often based on the specific purpose of the scoring system. For example, a continuous scale may be used to track changes in mobility over time, while an ordinal scale may be used to make comparisons between different animals. One challenge with mobility scoring is that there is a lack of consistency in how the scales are used. For example, the same scale may be used differently by different people or in different settings. This can make it difficult to compare scores across different studies or to make accurate assessments of mobility. Another challenge with mobility scoring is that there is a potential for bias. For example, the person scoring the mobility may be influenced by their own personal experiences. This can lead to inaccurate assessments of mobility. Despite these challenges, mobility scoring can be a useful tool for assessing and monitoring mobility. By using a consistent

and unbiased scoring system, it is possible to obtain accurate and reliable information about mobility.

Hoof trimmer data can be used to collect information on specific lesions, such as sole ulcers, white line disease, and hoof cracks. This information can be used to improve the accuracy of mobility scoring and to identify cows that are at risk of lameness. The heritability of hoof lesions ranges from 1 to 14% (linear scale); 6 to 39% (threshold scale) depending on the lesion. This means that a portion of the variation in hoof lesions is due to genetics. By using hoof trimmer data to collect information on specific lesions, it is possible to identify bulls more or less susceptible to these hoof lesions. In 2015, Dhakal *et al.* found that the heritability of sole ulcers was 14%. This means that about 14% of the variation in sole ulcers is due to genetics. In 2018, Heringstad *et al.* found that the heritability of white line disease was 39%, indicating that about 39% of the variation in white line disease is due to genetics. The use of hoof-trimming records is recommended for maximum genetic gain (Heringstad *et al.*, 2018). This is because hoof-trimming records provide a more accurate assessment of hoof health than other methods, such as visual inspection. Hoof-trimming records with documentation of specific lesions can serve as a source of more accurate phenotypes to be used for genetic evaluation in order to identify those animals with favorable hoof health traits. These data are more labor-intensive and time-consuming to acquire, however.

### Hoof trimmer data

The Council of Dairy Cattle Breeding (CDCB) and the University of Minnesota (UMN) are collaborating to develop a data pipeline that captures mobility and hoof health phenotypes. This data pipeline will be used to provide genetic evaluations for hoof health, provide hoof health management tools for dairy farms, and enhance the capacity of hoof trimmers. The data pipeline will be developed using a variety of methods, among others, hoof trimmer records and mobility scores obtained with a video analytic platform. The data pipeline will be used to develop genetic evaluations for hoof health, which will be used to identify animals with good hoof health traits. The data pipeline can also be utilized as a hoof health management tool for dairy farms by helping dairy farmers identify cows that are at risk of lameness and preventing lameness from occurring. The data pipeline will also be used to enhance the capacity of hoof trimmers, who will be trained to use standardized methods to identify and treat hoof lesions. The development of this data pipeline will have several benefits, including improved hoof health in dairy cows, increased productivity in dairy herds, reduced culling of lame cows, and reduced costs associated with lameness.

This study had the following objectives:

- Describe how automatically derived scores lead up to the first diagnosis of a hoof lesion.
- Describe how consistent same-day scores are. The consistency of same-day scores is important because it allows for accurate tracking of the progress of an abnormal mobility and

### Objectives

- Can a camera system potentially be used to detect mobility problems or hoof lesions earlier to prevent them from becoming more serious and to improve the hoof health of dairy cows?

## Methods

### Hoof Trimming data

Hoof trimmers were recruited by Dr. Gerard Cramer, at the University of Minnesota to participate in the project. Hoof trimmers received specific training on how to identify and report hoof lesions according to ICAR standards. Hoof trimmer data was collected from one pilot herd in Iowa from 2017/06/16 to 2022/11/30 (ongoing). The data was merged with on-farm software data based on cow ID and calving date. The data collected from the hoof trimmers included the following information: cow ID, trimming date, presence and type of lesions and treatments administered. Historical farm hoof lesion data were collected from 2,204 cows and used to determine cow lesion history and date of lesion diagnosis. To remove the confounding impact of chronicity, the study focused on cows with no history of lameness and categorized them into two categories: those with a first-time lesion diagnosis (LESION) and those seen by a hoof trimmer without a lesion diagnosis (TRIM). Lesions diagnosed included sole ulcer (SU), digital dermatitis (DD), foot rot (FR), white line disease (WLD), toe ulcer (TOE) and unknown. These categories were compared based on when the trimming occurred: within seven days of dry off (DOT) or at a random time (RT) based on farm staff observation and recommendation for trimming.

### Video analytics platform (CattleEye) data

A video analytics platform was used to monitor locomotion in cows and identify those that may require further checking/intervention. The platform included a mobility scoring module that analyzed video footage of cows walking through a standard 2D security camera mounted over the exit of a milking parlor. The data was analyzed by a computer algorithm trained to identify cows that were walking abnormally, these cows were then flagged for further checking. The data collected by CattleEye contained Cow ID, Date, Time, and mobility score (1-100; AUTO). The video analytics platform scoring performance has been validated and performs as well as a human mobility score estimator (Anagnostopoulos *et al.*, 2022). The study data presented herein was collected in one farm from April 16, 2022 to December 29, 2022. Individual AUTO scores were summarized into moving average weekly scores. All weekly AUTO scores were reported as median [IQR].

## Results

Comparisons were made for the LESION cows by lesion types. The lesion types for DOT (n = 60) were 93% TRIM, 3.3% toe ulcer (TOE), 1.7% white line disease (WLD), and 1.7% sole ulcer (SU). For RT (n = 239), 63% were TRIM, 17% digital dermatitis (DD), 7.5% SU, 7.1% WLD, 4.2% foot rot (FR), and 4.2% TOE. Four weeks prior to RT, LESION had a similar median score (37.6 [18.3]) to TRIM (38.5 [13.7]). One week prior to RT, LESION had a higher median score (41.1 [17.5]) compared to TRIM (39.2 [15.5]). For DOT, four weeks prior, LESION had a higher median score (59.2 [2.1]) than TRIM (40.0 [9.9]), and this pattern persisted through 1 week prior. FR had the highest score (47.3 [22.9]) four weeks earlier, followed by SU (42.8 [19.0]), WLD (41.2 [13.5]), and DD (35.0 [14.1]). One week prior, these scores were increased for FR (57.1 [11.5]), SU (44.5 [12.4]), WLD (44.3 [26.8]), and DD (39.5 [10.6]).

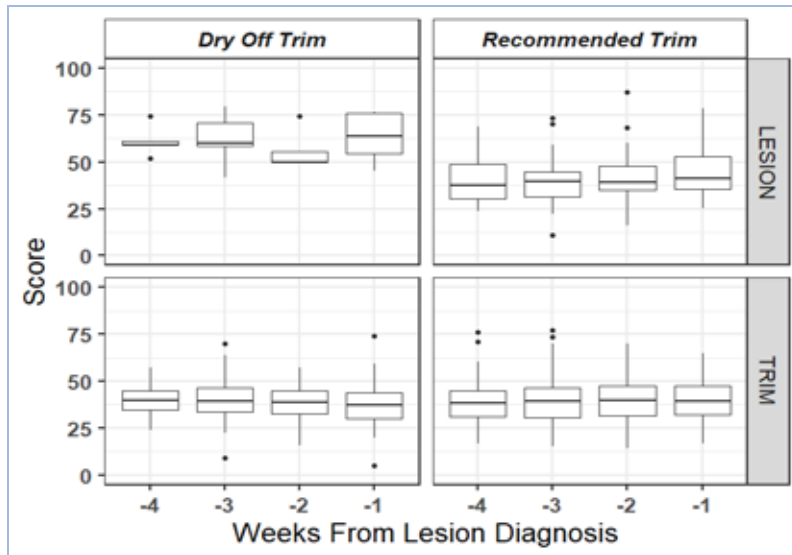


Figure 1. Median [IQR] of video analytics platform mobility scores by trimming type, lesion diagnosis and weeks before lesion diagnosis.

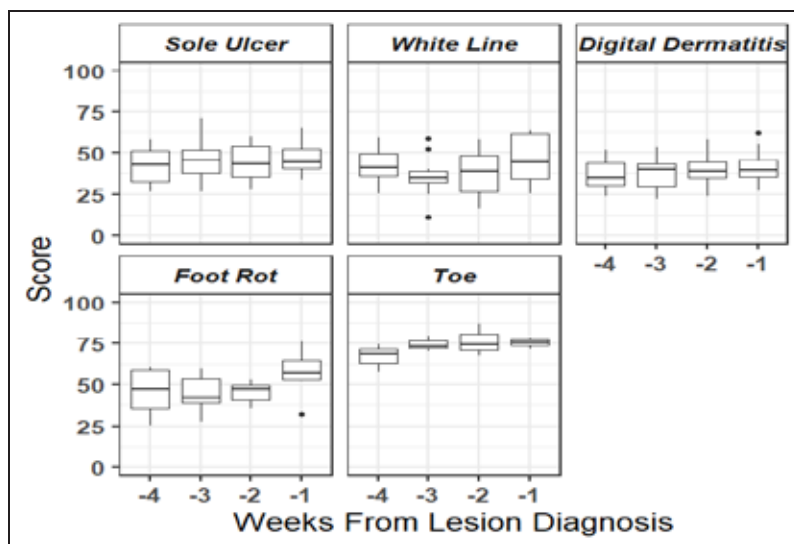


Figure 2. Median [IQR] of video analytics platform mobility scores by lesion diagnosis and weeks before lesion diagnosis.

## Conclusions

The results suggest that AUTO scores may have the potential to detect some lesions earlier. However, there is variation between cows and weeks that presents a challenge yet to be addressed.

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## Assessing the impact of body condition score dynamics from dry-off to calving on the incidence of early lactation disease in Holstein cows

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Automated systems generating body condition scores (BCS) through image technology enable daily assessments of body energy reserves of dairy cows in an efficient non-stressful approach and generate objective information. The availability of high-frequency BCS data allows for the analysis of specific points of interest and could result in quick adjustments of management if necessary. The objective of this study was to evaluate the association between the dynamics of BCS from dry-off to calving and early lactation disease in a population of high-producing Holstein cows. A retrospective observational study was completed using data collected from 12,042 lactations in 7,626 Holstein cows calving between April 2019 and January 2022 in a commercial dairy operation located in Colorado, USA. Scores generated by BCS cameras (DeLaval International AB, Tumba, Sweden), at 0.1 point intervals, at dry-off (BCSdry) and calving (BCScalv) were selected for the analysis and subsequently categorized into quartiles (Q1 = lowest BCS), separately for primiparous and multiparous cows. The change in BCS from dry-off to calving was calculated as  $BCScalv - BCSdry$  and assigned into quartile categories considering Q1 as the 25% of cows with greatest loss. Cows were classified as healthy (HLT; no health event) or affected by at least one health disorder within 60 days postpartum (SCK). Health disorders included reproductive (retained fetal membranes, metritis, and pyometra), metabolic (clinical hypocalcemia, subclinical ketosis, and left displaced abomasum), and other conditions (lameness, clinical mastitis, digestive problem, injury, and pneumonia). Mean (SE) BCSdry for HLT vs. SCK were 3.38 (0.004) vs. 3.42 (0.004) ( $P < 0.0001$ ), while BCScalv for HLT vs. SCK were 3.30 (0.003) vs. 3.33 (0.003) ( $P < 0.0001$ ). Mean BCS differences between dry-off and calving for HLT vs. SCK were -0.088 (0.004) vs. -0.11 (0.005) ( $P = 0.0008$ ). The logistic regression analyses indicated that the odds (95% CI) of disease were smaller in the lower BCSdry categories relative to cows in the highest BCS category (Q4): Q1 = 0.78 (0.65-0.94); Q2 = 0.75 (0.62-0.90); Q3 = 0.79 (0.65-0.96). On the contrary, BCScalv category was not associated with early lactation disease ( $P = 0.48$ ). Reductions in BCS from dry-off to calving were associated with subsequent disease, as cows losing more BCS (Q1 and Q2) had greater odds of disease compared to cows gaining BCS (Q4): Q1 = 1.32 (1.11-1.58) and Q2 = 1.35 (1.14-1.61). Overall, BCS at dry-off and greater loss of BCS between dry-off and calving had a significant impact on occurrence of early lactation disease.

### Abstract

*Keywords: body condition, automated, health .*

## Introduction

Body condition scores (BCS) are an indirect measure of the level of subcutaneous fat in dairy cattle (Ferguson *et al.*, 1994). While BCS at a time point are an indication of energy status, BCS gain or loss, and rate of change are considered as a proxy for the evaluation of energy balance (Roche *et al.*, 2007; 2009). Although BC scoring is an unexpensive tool for monitoring cows' energy dynamics, only one third of the US dairy farms implemented formal BCS into their management practices (Hady *et al.*, 1994; Bewley *et al.*, 2010). A likely explanation for the limited use of this assessment is the time consuming and subjective nature of visual or tactile evaluations (Edmondson *et al.*, 1989; Leroy *et al.*, 2005).

The advent of automated body condition scoring systems has allowed for the use of data originated at multiple and precise time points, with scores that are not affected by inter and intra evaluator variation (Borchers and Bewley, 2015). For example, recent studies using this technology have explored the potential of daily BCS in the prediction of subsequent fertility and health outcomes (Pinedo *et al.*, 2022; 2022a).

Related to the application of BCS in management decisions, the level of energy reserves of the cow at dry-off and at calving, as well as the changes occurring during the dry period, are of special interest in the prediction of the cow performance and health during the subsequent lactation. The magnitude in the change in BCS ( $\Delta$ BCS) following dry-off has been established as a relevant factor impacting subsequent fertility, health, and survival (Carvalho *et al.*, 2014; Chebel *et al.*, 2018) and the association between overconditioning at dry-off and lesser DMI and time feeding has been recently reported (Daros *et al.*, 2021). Moreover, low BCS at calving has been associated with decreased milk yield and reduced likelihood of pregnancy, whereas overconditioning at calving was associated with greater probability of postpartum metabolic diseases (Roche *et al.*, 2009).

Although the interrelationship between inadequate BCS at dry-off and calving and the occurrence of metabolic imbalances and early lactation diseases has been reported (Roche *et al.*, 2009; Chebel *et al.*, 2018; Stevenson *et al.*, 2020), the lack of consistent BC scoring in large cow populations under similar management has limited the potential for conclusive results when exploring these associations. The availability of daily BCS originated from automated camera systems in a large number of cows under the same productive system provides opportunity for precise assessment of the impact of BCS during the dry period on subsequent cow health.

We hypothesized that the dynamics of BCS during the dry period would have a significant impact on cow health during the early stages of the subsequent lactation. Therefore, the objective of this study was to evaluate the association between the dynamics of BCS from dry-off to calving and early lactation disease in a population of high-producing Holstein cows.

## Material and methods

### Study design and study population

This retrospective observational study included information collected from 7,626 Holstein cows calving between April 2019 and January 2022 in a commercial dairy operation located in Colorado, USA.

Data collection started at dry-off and continued until 60 days postpartum or culling. Cow demographic, reproductive, and health data were extracted from on-farm software (Dairy Comp 305; Valley Ag Software, Tulare, CA). Daily milk yield and BCS were extracted from DelPro Farm Manager software. The dataset included cow ID, date of calving, lactation number, calving-related and disease events, daily milk yield for the first 60 days in milk (DIM), and daily BCS.



Scores were generated by an automatic BCS system using DeLaval BCS cameras (DeLaval International AB, Tumba, Sweden) previously validated by Mullins *et al.* (2019) that were mounted on the sorting-gate at each exit ( $n = 2$ ) of the milking parlor. As the cow passed under the mounted camera, a continuous video (30 FPS, 32,000 captured reference points) was taken and a 3D image from the video was automatically created and saved by the BCS camera software (Mullins *et al.*, 2019; Pinedo *et al.*, 2022). In a secondary step, the saved 3D images were processed through an algorithm and analyzed to locate the key physical characteristics (pins, tail head ligaments, sacral ligaments, short ribs, and hooks) of the cow to calculate the automated BCS, viewable in DelPro Farm Manager. The proprietary algorithm used the BCS scoring scale proposed by earlier studies, modified to report BCS in 0.1-point increments (Ferguson *et al.*, 1994).

All automated BCS data were recorded in and downloaded from DelPro Farm Manager and scores generated by BCS cameras at dry-off (BCS<sub>dry</sub>) and calving (BCS<sub>calv</sub>) were selected and subsequently categorized into quartiles (Q1 = lowest BCS). The change in BCS from dry-off to calving ( $\Delta$ BCS) was calculated as BCS<sub>calv</sub> – BCS<sub>dry</sub> and assigned into quartile categories considering Q1 as the 25% of cows with greatest loss.

Calving-related events and diseases were obtained from farm records stored in on-farm software. Only health events diagnosed up to 60 days postpartum were considered in the analyses. Cows were classified as healthy (HLT; no health event) or affected by at least one health disorder within 60 days postpartum (SCK). Health disorders included reproductive (retained fetal membranes, metritis, and pyometra), metabolic (clinical hypocalcemia, subclinical ketosis, and left displaced abomasum), and other conditions (lameness, clinical mastitis, digestive problem, injury, and pneumonia). Calvings were grouped by season (spring, summer, fall, or winter). Finally, a milk yield category was added as a covariable in the models using the quartile distribution of the average daily milk yield in the first 60 DIM obtained from DelPro Farm Manager.

Descriptive statistics were calculated using the PROC UNIVARIATE in SAS 9.4 (SAS institute Inc., Cary, NC). Initial univariable models using only BCS<sub>calv</sub>, BCS<sub>dry</sub> and  $\Delta$ BCS as explanatory variables were followed by multivariable models that considered calving season, and milk yield up to 60 DIM as covariables. Least square means for BCS by health status category were calculated and compared using ANOVA (PROC GLM).

Odds ratios (OR) for occurrence of disease were estimated for the explanatory variables of interest using PROC GLIMMIX. For all outcome variables, significant predictors were selected at P-value  $<0.05$ ; interaction terms and controlling variables remained in the models at P-value  $\leq 0.10$ .

The recent development of BCS automated systems and their implementation in commercial farms has allowed for daily assessment of large populations of dairy cows. The availability of high frequency data provides detailed information on the dynamics of BCS, which permits a more precise evaluation of potential factors affecting BCS and facilitates the analysis of the effect of BCS on performance variables.

The current analysis included 7,626 multiparous cows. Overall, the distribution of calvings across seasons was spring 23.3%, summer 32.3 %, fall 28.2%, and winter

## Body condition scoring and BCS categorization

## Statistical analyses

## Results and discussion

Table 1. Descriptive statistics for body condition scores during the period of interest. Unless stated, least square means (SE) are presented.

Parameter	Healthy	Sick	P-value
BCS at dry-off	3.38 (0.004)	3.42 (0.004)	<0.0001
BCS at calving	3.30 (0.003)	3.33 (0.003)	<0.0001
BCS change	-0.088 (0.004)	-0.11 (0.005)	0.0008

16.2%. Average (SE) milk yield for the first 60 DIM was 44.9 (0.1) kg. A summary for BCS at dry-off and calving by health status is presented in Table 1.

Overall, BCS values in this study were similar to those presented in a recent report that indicated that BCS at dry-off and calving were 3.43 and 3.40 in cows housed in a large commercial dairy farm in Indiana (Truman *et al.*, 2022). In the same study, multiparous cows lost 0.03 BCS points from dry-off to calving.

The logistic regression analyses identified some significant associations between BCS and health. The analyses indicated that the odds (95% CI) of disease were smaller in the lower BCS<sub>dry</sub> categories relative to cows in the highest BCS category (Q4): Q1 = 0.78 (0.65-0.94); Q2 = 0.75 (0.62-0.90); Q3 = 0.79 (0.65-0.96). On the contrary, BCS<sub>calv</sub> category was not associated with early lactation disease (P = 0.48).

When the change of BCS occurring during the dry period was analysed, reductions in BCS from dry-off to calving were associated with subsequent disease, as cows losing more BCS (Q1 and Q2) had greater odds of disease compared to cows gaining BCS (Q4): Q1 = 1.32 (1.11-1.58) and Q2 = 1.35 (1.14-1.61).

The interrelationship between loss in BCS and occurrence of disease is complex and establishing precise cause and effect associations is challenging. Nonetheless, previous studies have reported the associations among BCS variables and health, with partial agreement with our findings (Carvalho *et al.*, 2014). For example, in a recent study by Chebel *et al.* (2018), loss of BCS during the dry period was associated with increased occurrence of health disorders and worsened performance in Holstein cows. In an earlier report, Contreras *et al.* (2004) indicated that cows with BCS  $\leq 3$  at dry off gained BCS during the dry period and were less likely to have retained fetal membranes compared with cows with greater BCS at dry off.

Interestingly, the results from the current study align with those reported recently by our group in similar analyses focused on BCS changes in lactating cows (Pinedo *et al.*, 2022; 2022a). In these studies, cows with larger loss in BCS from calving to 21, and 56 days postpartum had worsened health and performance than cows maintaining or gaining body condition during these periods.

## Conclusions

The strength of our study is the availability of consistent BCS for a for a large number of cows under the same dairy operation. Overall, the associations between BCS dynamics and subsequent health were moderate and more evident at dry-off and for the  $\Delta$ BCS from dry-off to calving. It is anticipated that changes in BCS during early lactation may be more impactful on cow health.

Automatic BCS is a useful tool to monitor and manage energetic status and energy balance. Individual cow or group BCS profiles should be considered when monitoring herd health. The implications for cow health and welfare deserve further exploration.

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## Towards new breeding tools in a context of climate change: Fertility results of the RUMIGEN project on new phenotypes for heat tolerance traits

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RUMIGEN is a project financially supported by the EU that aims to develop breeding programs capable of managing the trade-offs between efficient production and resilience to extreme climate conditions. Previous results on heat tolerance indicators derived from milk recording data and meteorological information showed differences of magnitude of the effects and heat load threshold for heat stress between countries. In this study, we estimated the effect of heat load on fertility in three countries by analysing simultaneously fertility data registered from 2010 through 2020 in national reproductive recording systems and meteorological information from the closest to farm weather stations. More specifically, fertility data were defined as the success or failure in first insemination (CR) from first lactation Holstein cows in France (N=4,450,637), Spain (N=471,793) and The Netherlands (N=417,548) and from first lactation Montbéliarde cows (N=835,751) in France. The heat load was measured from the average of a temperature and relative humidity index (THI) in the day of the record and the seven days post AI. In all countries, the effect of heat load on CR was estimated using animal mixed models including a class effect for THI values together with other fixed and random effects used in national fertility evaluations. Heat stress thresholds and slopes of decay in CR after the threshold were estimated from THI effect solutions using segmented regression models, assuming a fixed number of break-points. Considering a single heat stress threshold (value after which a considerable reduction in CR is observed) showed that this threshold is around 70 for THI in all countries. Slopes of decay in CR did show substantial differences across countries, ranging from 0.79 points per degree of THI in Holstein cows in Spain to 2.25 points per degree of THI in Holstein cows in France.

Keywords: heat tolerance, breeding criteria, dairy cattle.

### Abstract

## Introduction

Climate change is spurring demands from farmers to provide tools to adapt animal production to increasing temperatures. Developing new breeding strategies to help ruminants to adapt to climatic changes is one of the goals of the Rumigen project (<https://rumigen.eu/>), financed by the EU. A first step in breeding programmes is to find measures for the desired selection objectives. In this context, using already available information in breeding schemes, such as productive or reproductive performance of animals to produce heat tolerance indicators is appealing, since no additional cost would be required for the introduction of this new selection objective. Joining performance recording with meteorological information around the date of recording to estimate the response of animals to changes in heat load was initially proposed by Misztal *et al.* (1999) and later developed in many populations. Previous to implementation of genetic evaluations of individual heat tolerance, knowing the overall response to heat load in the target population is needed to quantify the heat stress impact and the characterisation of heat stress (HS) thresholds and productive loss. In a previous contribution within the Rumigen project, Mattalia *et al.* (2022) evaluated differences in response to increased heat loads for production and udder health traits in a range of dairy cattle populations across Europe. Different patterns across countries and breeds were found, probably associated with differing climatic characteristics and production systems. Reproductive performance is also a highly important trait for animal production which is negatively affected by HS (Hansen, 2009). In this study, our goal was to compare patterns of response in reproductive performance to increasing heat loads across dairy cattle populations in Europe as a previous step to establish heat tolerance phenotypes for selection.

## Material and methods

Historical data from years 2010 through 2020 including artificial insemination (AI) results were provided by breeder associations (France Génétique Elevage for Holstein France = HOL-FRA, and for Montbeliarde = MON-FRA, CRV for Holstein Netherlands = HOL-NLD and CONAFE for Holstein Spain = HOL-SPA). Conception rate (CR) for each insemination was coded as success or failure using country rules for routine genetic evaluations of this trait. After edits for abnormal data and selecting records from first inseminations in first lactation, 4,450,637/ 417,548/471,793/835,751 records were available for HOL-FRA/HOL-NLD/HOL-SPA/MON-FRA, respectively.

Meteorological data to match with AI dates were provided by the national meteorological agencies (Météo-France (Safran database) for France, the Koninklijk Nederlands Meteorologisch Instituut (KNMI) for The Netherlands and National Meteorological Agency (AEMET) for Spain). Daily average and relative humidity were the meteorological variables used to compute a combined temperature and humidity index (THI) according to the formula by NRC (1971):

$$THI = (1.8 \cdot T + 32) - (0.55 - 0.0055 \cdot RH) \cdot (1.8 \cdot T - 26)$$

with T being the average daily temperature (degrees Celsius) and RH the average daily relative humidity (in percentage).

According to previous results (not shown), the average of THI values for the day of AI and the seven days after AI was used to define the heat load used in the subsequent models to analyse its effect on fertility.

The effect of THI on CR in each population was obtained by using the following statistical model:

$$y = X_1 \beta_1 + X_2 \beta_2 + Z_1 a + e \quad [1]$$

where  $y$ ,  $hl$ ,  $\beta$ ,  $a$  and  $e$  are the vectors of phenotypes (CR at first insemination in first lactation), heat load (class for average THI for days 0 and subsequent seven days after AI), other environmental effects, cow additive genetic and the random residuals, respectively, and  $X1$ ,  $X2$ ,  $Z1$  and  $Z2$  are the corresponding incidence matrices.

Other environmental effects included in all countries were the herd-year of calving effect (random effect in Spain), class of interval from calving to first insemination, and age at calving, and, day of the week (France and The Netherlands), service sire (France and The Netherlands), sexed semen class (France and The Netherlands), season of calving (France), year-month of calving (The Netherlands) and age of service sire (The Netherlands).

Variance components and the different effects were estimated with software commonly used in animal breeding.

From estimated THI effects (one per THI unit) in equation [1], a smoothed curve was fitted using a cubic polynomial. Change points (CP) and slopes of decay (slp) after the CP were estimated using the R package “Segmented” (Muggeo, 2008) in order to provide values for HS threshold and fertility loss associated with HS.

Figure 1 shows the estimated THI effects in equation [1] and the polynomial smoothed fit used to estimate change points and slopes of decay under HS.

## Results and discussion

Figure 1 shows that the response to increasing values of THI corresponds to the classical response in the broken line model defined by Misztal (1999), with a thermoneutral region where no response to increases in heat load is observed followed by a HS region where the negative impact of HS can be observed. For the different populations, different HS thresholds for THI and slopes of decay were observed, although to a smaller extent to differences in the pattern of HS response in production traits observed in Mattalia *et al.* (2022) for the same populations.

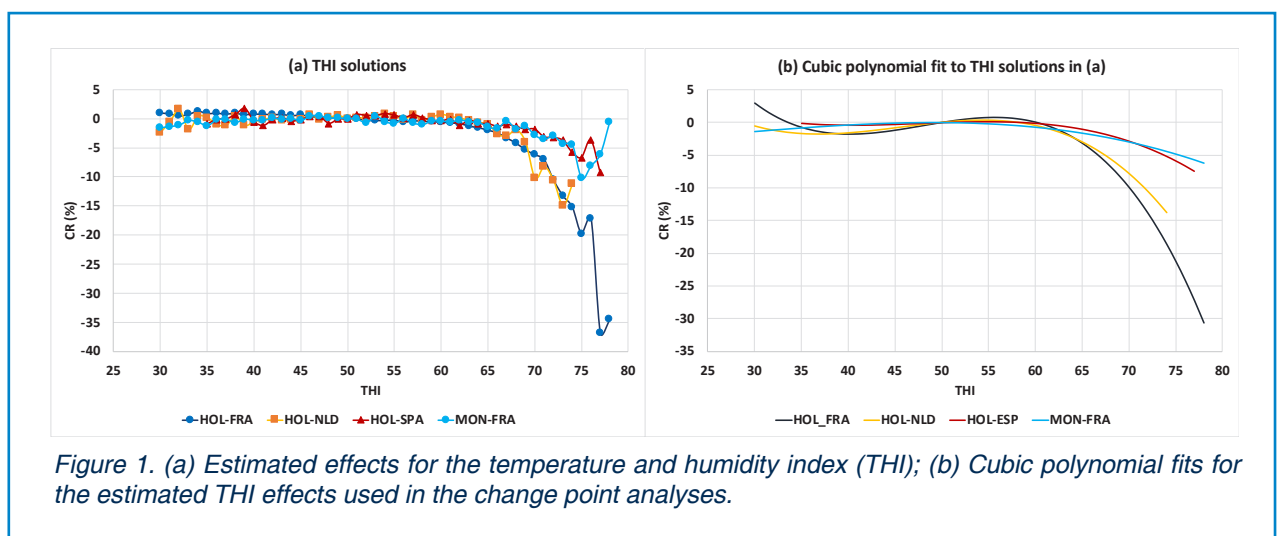


Figure 1. (a) Estimated effects for the temperature and humidity index (THI); (b) Cubic polynomial fits for the estimated THI effects used in the change point analyses.

Table 1. Estimated change point and subsequent slopes of decay obtained from the response curve of conception rate (CR) to the temperature and humidity index (THI).

Breed-Country	Change point (THI)	Slope (%CR/unit THI)
HOL-FRA	63	-1,29
HOL-NLD	62	-1,04
HOL-SPA	64	-0,51
MON-FRA	61	-0,48

Table 1 presents the estimated change points and subsequent slopes of decay under heat stress for each population. Estimated change points were similar across populations, with values ranging from 61 to 64 for HS thresholds. Our estimates of HS thresholds are similar to that reported by Gernand *et al.* (2019) for pregnancies per AI in Germany and Biffani *et al.* (2016) for non-return rate (NRR) in Italy, and smaller to the estimated thresholds found also for NRR by Ravagnolo and Misztal (2002) in the USA and Santana *et al.* (2017) in Brasil. The estimated HS thresholds were higher/similar/smaller for CR than the values obtained for production traits for the French (HOL, MON)/Dutch (HOL)/Spanish (HOL) populations participating in this study, respectively (Mattalia *et al.*, 2022).

Loss in CR due to HS, depicted by the slopes of decay, was substantially larger for HOL-FRA and HOL-NLD (around 1% decrease per THI unit above the threshold) than in HOL-SPA and MON-FRA (around 0.5%) (Figure 1; Table 1). Acclimation of cows to chronic HS during summer and heat abatement in the barns in the Spanish population might explain these results. In the case of MON-FRA, lower productive levels than Holstein cows might result in less compromised energy balance and better fertility under HS.

## Conclusion

This study provided base results for the development of breeding schemes that include heat tolerance as a selection objective in terms of modelling and quantification of heat stress response on fertility in European dairy cattle populations. The pattern of response agrees well with broken line models defined by a HS threshold and a subsequent slope of decay. HS thresholds (61-64 THI degrees) were similar across dairy populations but decays were substantially different, with a smaller impact for the population in Southern areas with chronic HS in summer and in barn heat mitigation devices and for a less intensively selected breed than Holstein cattle.

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## Genetic analysis of lactation consistency using daily milk weights in U.S. Holsteins

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The ability of a dairy cow to perform similarly across time is an interesting trait to include in dairy cattle breeding programs aimed at improving dairy cow resilience. Consistency, defined as the quality of performing as expected each day of the lactation, could be highly associated with resilience, defined as animal's ability to maintain health and performance in the presence of environmental challenges, including pathogens, heat waves, and nutritional changes. A total of 51,415,022 daily milk weights collected from 2018 to 2023 were provided for 255,191 multiparous Holstein cows milked three times daily in conventional parlor systems on farms in 32 states by Dairy Records Management Systems (Raleigh NC).

The temporal variance (TempVar) of milk yield from 5 to 305 days postpartum was computed as the log-transformed variance of daily deviations between observed and expected individual milk weights. Lower values of TempVar imply smaller day-to-day deviations from expectations, indicating consistent performance, whereas larger values indicate inconsistent performance. Expected values were obtained using three nonparametric regression models:

1. LOESS regression with a 0.75 span;
2. polynomial quantile regression using the median (0.5), and
3. polynomial quantile regression using a 0.7 quantile.

The statistical model included age at first calving and herd-year-season as fixed effects and cow as a random effect. Heritability estimates (standard errors) of consistency ranged between 0.227 (0.011) and 0.237 (0.011), demonstrating that cows are genetically predisposed to display consistent or inconsistent performance. Correlations among TempVar traits were high (0.99), indicating that the model used to calculate consistency does not alter the ranking of Predicted Transmitting Abilities (PTAs). Genetic correlations between TempVar phenotypes and milk PTAs were 0.57, while longevity traits included Productive Life (-0.38) and Livability (-0.48). Note that as lower TempVar values are indicative of more consistent animals, negative genetic correlations with longevity traits are desirable. Our results show that cows with inconsistent milk production have lower Productive Life and Livability PTAs, meaning they have a shorter productive lifespan. Correlations between PTAs for log variance of daily milk yield and PTAs for early postpartum health traits ranged from -0.41 to -0.08. Given that health traits are derived from disease resistance measurements, this indicates that more consistent cows tended to have fewer health problems. Overall, our findings suggest

### Abstract

that lactation consistency can be used to select animals that maintain expected milk production performance throughout the lactation.

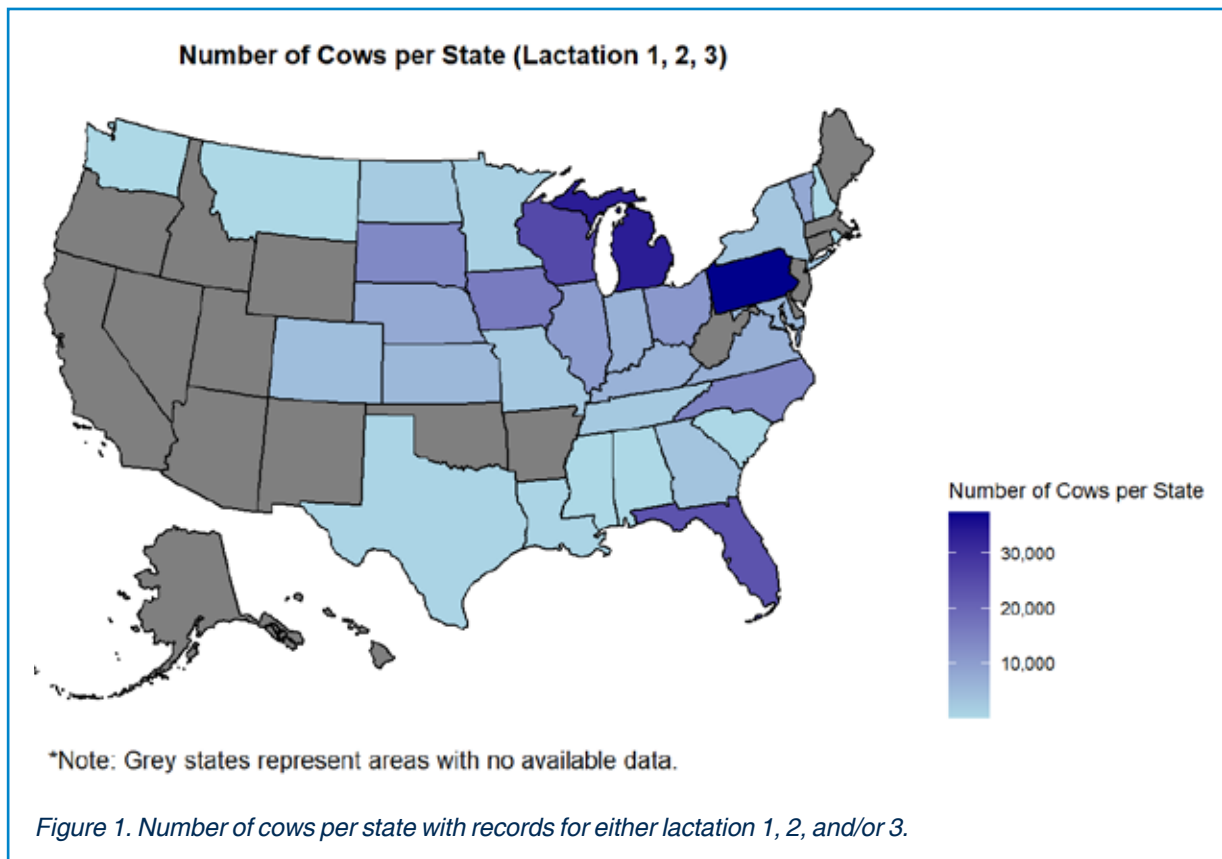
*Keywords: consistency, resilience, non-parametric modelling, daily milk weights.*

## Introduction

The dairy industry has made enormous gains in production efficiency through improvements in genetics, nutrition, and management. Historically, genetic selection indexes emphasized increased milk production per cow, and more recently focused on improving fertility, disease resistance and feed efficiency (VanRaden *et al.*, 2021). Modern intensive farming systems have prioritized the average performance of an animal in optimal conditions, whilst ignoring the animal's ability to perform in variable or suboptimal conditions like extreme weather events, labour shortages and disease outbreaks. Resiliency is a measure of an animal's capacity to bounce back to normal functioning or maintain specific functions in the face of such environmental disturbances (Scheffer *et al.*, 2018). Resilience has been shown to be heritable in different species using high frequency daily observations such as daily milk weights (Poppe *et al.*, 2020), daily egg production (Bedere *et al.*, 2022) and daily feed intake (Putz *et al.*, 2019). Our hypothesis is that consistency is an economically important indicator of resilience. Consistency is defined as a level of performance that does not vary greatly in quality over time. The aim of this study was to calculate TempVar phenotypes using the variance of daily milk weights routinely collected on dairy farms throughout the U.S. We investigated three different methods to model individual cows' lactation curves. Genetic parameters and heritabilities of all three TempVar phenotypes, along with three different lactation stages were calculated for first parity Holstein cows. Secondly, genetic parameters, heritabilities and estimated genetic correlations among sire PTAs were calculated using a bivariate repeatability model for Holstein cows with parity 1, 2 or 3. Finally, a multi trait model was used to calculate genetic correlations between three TempVar phenotypes and economically relevant trait among different parities.

## Material and methods

Data were provided by Dairy Records Management Systems (Raleigh, NC) and were extracted from PCDART on farm management software. Data were appended to a database built using the RSQLite package in R (R Project for Statistical Computing, Vienna, Austria; version 3.6.0). Individual milk weights are stored for up to 100 days while daily milk weights are stored for up to 300 days on the farm, so historical data are limited, and it was necessary to upload data from participating herds monthly and aggregate these data over time to build our SQLite research database. Data were limited to cows milked 3 times per day from 2018 to 2023 and estimated daily milk weights corresponding to days with missing values were removed. Cows milked by automatic milking systems (AMS) were excluded from the analysis. Outliers were identified and removed after decomposing the seasonal trend of the lactation curve with the Multiple Seasonal Trend decomposition method using the function `tsclean` from the `forecast` package in R (Hyndman *et al.*, 2023). `Tsclean` is a robust method to identify outliers in a univariate time series analysis using a modified Z score, in which outliers are identified based on their distance from the median (Hyndman *et al.*, 2023). Herds were required to participate in Dairy Herd Improvement (DHI) milk recording and recorded breed of cow was restricted to Holstein. After summing individual milk weights, a total of 51,415,022 daily milk weight phenotypes recorded between 5 and 305 days in milk (DIM) for 32 U.S. states remained for our analysis of milk yield TempVar (Figure 1).



The first step was to fit lactation curves using daily milk weights. Loess (span 0.75), quantile regression (0.5) and quantile regression (0.7) with a 4th order polynomial were the nonparametric methods used to model expected lactation curves. Loess is a non-parametric modelling technique to model the relationship between variables. The `quantreg` package in R (version 3.6.0) (Koenker, 2020), along with the `poly` function, was used for quantile regression analysis. Cows were required to have at least 100 aggregated daily milk weights within a lactation period to model a lactation curve. Outliers were identified and removed using the `tsclean` function from the `forecast` package in R. `Tsclean` is a robust method to identify outliers in a univariate time series analysis by using a modified Z score. The modified Z score calculates the deviation of each observation from the median, and outliers are identified based on their distance from the median relative to the median absolute deviation.

*Fitting lactation curves using daily milk weights*

TempVar phenotypes were calculated in two steps. First, by measuring the deviations between a cow's actual daily milk weights and her expected daily milk weights across the trajectory of her lactation, with expected values provided by the three lactation curve models described above. In the second step, the variances of these daily deviations were calculated. However, due to skewness of the distribution of variances across individual cows and lactations, a log transformation was applied to variances to derive

*Calculating temporal variance phenotypes*

three TempVar phenotypes, namely LnVar\_loess, LnVar\_qr05, and LnVar\_qr07, for each method and each cow in step 2, as follows:

$$\text{daily deviation}_{ijk} = [\text{yield}_{ijk} - \widehat{\text{yield}}_{ijk}]$$

$$\text{TempVar}_{ij} = \log\{\text{Var}[\text{daily deviation}_{ijk}]\}$$

where  $i$  is the individual cow,  $j$  indicates parity and  $k$  represents DIM between 5 and 305. Thus, a consistent cow is defined by low temporal variation in actual versus predicted daily milk production throughout the lactation, and an inconsistent cow is defined by high temporal variation in actual versus predicted daily milk yield throughout the lactation.

### Univariate analysis of temporal variance of milk yield in first lactation

Our initial analysis, which was restricted to first parity cows, included 20,787,272 daily milk weights from 102,216 cows in 213 herds in 30 states. Variance components and genetic parameter estimates were obtained using the AIREMLF90 software (Aguilar *et al.*, 2018). TempVar phenotypes were analyzed using the following model:

$$y_{ijkl} = \text{AFC}_i + \text{HYS}_{j+} + a_k + e_{ijkl},$$

where  $y_{ijkl}$  is the TempVar phenotype,  $\text{AFC}_i$  is the fixed effect of age at first calving (6 levels; <=22, 23-24, 25-26, 27-28, 29-30, 30+),  $\text{HYS}_{j+}$  is the fixed effect of herd-year-season of calving (2,347 levels, with a minimum of 5 observations per level),  $a_k$  is the random effect of cow with 102,216 levels distributed as  $\mathbf{a} \sim \mathbf{N}(0, \mathbf{A}\sigma_a^2)$ , and  $e_{ijkl}$  is the random residual effect distributed as  $\mathbf{e} \sim \mathbf{N}(0, \mathbf{I}\sigma_e^2)$ .

### Univariate analysis of temporal variance of milk yield by stage of lactation

After fitting individual lactation curves for first lactation cows using LOESS and polynomial quantile regression, three different lactation stages based on DIM were considered to reflect early, mid, and late lactation. Early lactation ranged from 5 to 50 DIM, mid lactation ranged from 51 to 200 DIM, and late lactation ranged from 201 to 305 DIM. Individual cows were required to have daily milk weights spanning at least 50% of the period to be included in the analysis. In other words, at least 22 daily milk weights were required in early lactation, 75 daily milk weights were required in mid lactation, and 52 daily milk weights were required in late lactation. Consequently, a specific first parity cow could be included in the analysis for one, two, or the three periods. After edits, 66,297 cows were used to estimate genetic parameters in early lactation, 85,445 cows in mid lactation, and 71,673 cows in late lactation. Variance components and heritability estimates for LnVar\_loess, LnVar\_qr05, and LnVar\_qr07 were calculated within each lactation period using a univariate model, and relationships across periods were assessed using Pearson's correlations of sire PTAs in different periods.

### Analyses of temporal variance of milk yield across lactations

The edits described previously were subsequently applied to daily milk yield data of second and third parity cows to assess relationships in the TempVar of milk yield across parities. Cows were not required to have records in all three lactations because, given the structure of our database, the number of cows with records in multiple parities was limited. In the dataset, there were 36,589 cows with records in both parity 1 and 2. Additionally, 25,702 cows had records in both parity 2 and parity 3. Furthermore,

5,496 cows had records in both parity 1 and parity 3. Finally, there were 4,904 cows with records in all three parities (parity 1, parity 2, and parity 3). We implemented a repeatability model, as well as two bivariate models (first and second lactation, second and third lactation), using a total of 51,415,022 daily milk weights records from 106,033 first parity cows, 89,315 second parity cows and 59,843 third parity cows. These data represented 222 herds from 32 U.S. states (Figure 2). Variance components and genetic parameter estimates were obtained using the AIREMLF90 software (Aguilar *et al.*, 2018). Fixed effects included parity (3 levels), age at calving (18 levels; <=22, 23-24, 25-26, 27-28, 29-30, or 30+ months for first parity; <=35, 36-37, 38-39, 40-41, 42-43, or 44+ months for second parity, and <=47, 48-49, 50-51, 52-53, 54-55, or 56+ months for third parity), and herd-year-season (2,856 levels with ≥ 5 records per level). Cow was fitted as a random effect using up to five generations of pedigree data.

Repeatability model:

$$y_{ijklmn} = \text{Parity}_i + CA_j + HYS_k + a_l + pe_m + e_{ijklmn},$$

where  $y_{ijklmn}$  is the TempVar phenotype,  $\text{Parity}_i$  is the fixed effect lactation number with 3 levels,  $CA_j$  is the fixed effect of calving age with 18 levels,  $HYS_k$  is the fixed effect of herd calving year season with 2,856 levels,  $a_l$  is the random effect of cow with 255,191 levels distributed as  $\mathbf{a} \sim N(0, \mathbf{A}\sigma_a^2)$ ,  $pe_m$  is the random permanent environmental effect distributed as  $\mathbf{pe} \sim N(0, \mathbf{I}\sigma_{pe}^2)$ , and  $e_{ijklmn}$  is the random residual effect distributed as  $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$ .

Multiple trait model:

$$y_{ijkl} = CA_{i+} + HYS_{j+} + a_k + e_{ijk},$$

where all model terms are as described previously. The assumptions of both bivariate analyses, which were carried out using first and second lactation TempVar phenotypes or second and third lactation TempVar phenotypes, were as follows:

$$\begin{bmatrix} a_1 \\ a_2 \end{bmatrix} \sim N \left[ \begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_{a1}^2 & \sigma_{a12} \\ \sigma_{a12} & \sigma_{a2}^2 \end{pmatrix} \otimes A \right]$$

$$\begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \sim N \left[ \begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \sigma_{e1}^2 & \sigma_{e12} \\ \sigma_{e12} & \sigma_{e2}^2 \end{pmatrix} \otimes I \right]$$

where  $a_i$  is the additive genetic effects for trait  $i$ ,  $\sigma_{ai}^2$  is the additive genetic variance of trait  $i$ ,  $\sigma_{aij}$  is the additive genetic covariance between trait  $i$  and  $j$ ,  $e_i$  is the residual effect for trait  $i$ ,  $\sigma_{ei}^2$  is the residual variance of trait  $i$ , and  $\sigma_{eij}$  is the residual covariance between trait  $i$  and  $j$ .

Sires with ≥ 10 daughters with TempVar phenotypes (repeatability model, n=2,572) were used to calculate approximate genetic correlations between milk yield TempVar and other economically relevant traits using the Calo's method (Calo *et al.*, 1973; Blanchard *et al.*, 1983). PTAs from economically relevant traits evaluated by the Council on Dairy Cattle Breeding (Bowie, MD) were extracted from the April 2023 genetic evaluation. The approximate genetic correlations were calculated as follows:

**Correlations between temporal variance of milk yield and other economically relevant traits**

$$\hat{r}_{i,j} = \frac{\sqrt{\sum(Rel_i) \sum(Rel_j)}}{\sum(Rel_i Rel_j)} r_{i,j},$$

where  $\hat{r}_{i,j}$  = approximate genetic correlation between traits i and j;  $\sum Rel_i$  and  $\sum Rel_j$  = the sum of reliabilities of traits i and j;  $Rel_i$  and  $Rel_j$  = reliabilities of traits i and j; and  $r_{i,j}$  = Pearson correlation between PTA for traits i and j.

## Results and discussion

Heritabilities (se) of TempVar phenotypes were moderate and ranged from 0.227 (0.011) to 0.237 (0.011) (Table 1). These heritabilities indicate two important concepts – that we can select for consistent milk performance genetically, and the non-parametric methods used to model lactation curves have little impact on our trait definition. We found differences in heritabilities among lactation stages (Table 2). In early lactation, the estimated heritabilities of TempVar phenotypes ranged from 0.129 (0.010) - 0.154 (0.011), in mid lactation heritabilities ranged from 0.190 (0.011) to 0.197(0.011) and in late lactation from 0.159 (0.011) to 0.164 (0.011). Across all three TempVar phenotypes,

*Table 1. Variance components and heritability estimates (SE)<sup>1</sup> for temporal variance (TempVar) of daily milk yield in first parity Holstein cows over the entire lactation. TempVar phenotypes were calculated as log-transformed variances of daily deviations from lactation curves predicted with LOESS regression (0.75 span parameter) or polynomial quantile regression (0.5 or 0.7 quantile).*

Method	$\sigma_a^2$	$\sigma_e^2$	$h^2$
LnVar_loess	0.050 (0.002)	0.162 (0.001)	0.237 (0.011)
LnVar_qr05	0.052 (0.002)	0.171 (0.002)	0.231 (0.011)
LnVar_qr07	0.052 (0.002)	0.176 (0.002)	0.227 (0.011)

<sup>1</sup>  $\sigma_a^2$  = additive genetic variance;  $\sigma_e^2$  = residual variance;  $h^2$  = heritability.

*Table 2. Phenotypic means, standard deviations (SD), variance components and heritability estimates (SE)<sup>1</sup> for the temporal variance (TempVar) of daily milk yield in first parity Holstein cows at 3 different lactation stages. TempVar phenotypes were calculated as log-transformed variances of daily deviations from lactation curves predicted with LOESS regression (0.75 span parameter) or polynomial quantile regression (0.5 or 0.7 quantile).*

Method	Lactation			$\sigma_a^2$	$\sigma_e^2$	$h^2$
	Stage	Mean	SD			
LnVar_loess	5 to 50	3.95	0.73	0.046 (0.003)	0.256 (0.003)	0.154 (0.011)
	51 to 200	3.53	0.76	0.046 (0.002)	0.187 (0.002)	0.197 (0.011)
	201 to 305	3.42	0.77	0.043 (0.003)	0.221 (0.002)	0.164 (0.011)
LnVar_qr05	5 to 50	3.72	0.80	0.049 (0.003)	0.312 (0.003)	0.136 (0.010)
	51 to 200	3.56	0.77	0.047 (0.002)	0.196 (0.002)	0.194 (0.011)
	201 to 305	3.43	0.78	0.043 (0.003)	0.225 (0.002)	0.161 (0.011)
LnVar_qr07	5 to 50	3.75	0.81	0.048 (0.003)	0.326 (0.003)	0.129 (0.010)
	51 to 200	3.56	0.77	0.047 (0.002)	0.200 (0.002)	0.190 (0.011)
	201 to 305	3.44	0.78	0.043 (0.003)	0.230 (0.002)	0.159 (0.011)

<sup>1</sup>  $\sigma_a^2$  = additive genetic variance;  $\sigma_e^2$  = residual variance;  $h^2$  = heritability.



estimates of the additive genetic variance were highest during the period from 5 to 50 DIM. This is an interesting and promising result, as it seems to indicate that genetic differences in milk yield consistency are expressed more fully under challenging conditions, albeit with the complication of greater residual variances. Mulder *et al.* (2013) previously indicated that greater genetic variation in resilience would be observed when an animal is exposed to environmental challenges. Increased variation in daily milk production during this period may reflect the challenges of decreased voluntary feed intake, coupled with the physiological demands of rapid increases in energy requirements for milk production (White, 2015). Previous authors have described challenges such as negative energy balance (Collard *et al.*, 2000), hyperketonemia (Duffield *et al.*, 2009), and resumption of ovarian cyclicity (Gaillard *et al.*, 2016) during this period, all of which can contribute to an increase in the environmental variance. Heritabilities were lowest for LnVar\_qr07 across all three lactation stages. This is most likely caused by fitting the 0.7 quantile, which reflects the animals potential milk production and could be more informative for calculating resilience indicators (Poppe *et al.*, 2020)

The assumption of the repeatability animal model is that the genetic correlation between records is equal to 1, indicating that TempVar traits are genetically identical across parities. In table 4, genetic correlations among different parities for each TempVar phenotype are shown. Genetic correlations (se) ranged from 0.963 (0.010) to 0.999 (0.003) which indicated that consistency is the same trait regardless of parity. Repeatability (se) ranged from 0.331 (0.003) to 0.341 (0.003) indicating that the genetic influence on consistent performance is relatively stable and repeatable over time, suggesting that selection for that trait is likely to be effective (Table 3). Because first lactation cows are immature and still growing, many traits are genetically or physiologically distinct between primiparous and multiparous cows. Therefore, we decided to estimate heritability, repeatability, and genetic correlations of TempVar phenotypes in first, second, and third parities. Heritability estimates were slightly lower when calculated using the repeatability model, but relatively few cows had TempVar phenotypes for multiple parities, because daily milk yield records from cows that calved prior to initiation of our research database were unavailable. Genetic correlations among TempVar phenotypes were >0.95 between parities (Table 4), indicating that TempVar phenotypes are genetically quite similar throughout the cow's life, and suggesting that we should consider milk yield consistency as the same trait across lactations.

*Table 3. Variance components and heritability estimates (SE)<sup>1</sup> for the temporal variance (TempVar) of daily milk yield using a repeatability model applied to full lactation data of cows with records in lactation 1, 2 and/or 3. TempVar phenotypes were calculated as log-transformed variances of daily deviations from lactation curves predicted with LOESS regression (0.75 span parameter) or polynomial quantile regression (0.5 or 0.7 quantile).*

Method	$\sigma_a^2$	$\sigma_{pe}^2$	$\sigma_e^2$	$h^2$	$r^2$
LnVar_loess	0.052 (0.001)	0.026 (0.001)	0.151 (0.001)	0.227 (0.006)	0.341 (0.003)
LnVar_qr05	0.053 (0.001)	0.026 (0.001)	0.158 (0.001)	0.222 (0.006)	0.339 (0.003)
LnVar_qr07	0.053 (0.001)	0.028 (0.001)	0.164 (0.001)	0.216 (0.006)	0.331 (0.003)

<sup>1</sup>  $\sigma_a^2$  = additive genetic variance;  $\sigma_{pe}^2$  = permanent environmental variance;  $\sigma_e^2$  = residual variance;  $h^2$  = heritability;  $r^2$  = repeatability.

*Table 4. Variance components and heritability estimates (SE) using a multiple-trait model applied to full lactation data of cows with records in lactation 1, 2 and/or 3. Phenotypes representing the temporal variance in daily milk yield were calculated as log-transformed variances of daily deviations from lactation curves predicted with LOESS regression (0.75 span parameter) or polynomial quantile regression (0.5 or 0.7 quantile).*

Lactation	No. of cows	Method		
		LnVar_loess	LnVar_qr05	LnVar_qr07
1 and 2	195,348	0.976 (0.006)	0.977 (0.006)	0.978 (0.006)
1 and 3	165,876	0.963 (0.010)	0.963 (0.010)	0.964 (0.010)
2 and 3	149,158	0.998 (0.003)	0.999 (0.003)	0.999 (0.003)

*Table 5. Correlations estimated using the Calo's method between sire PTAs for temporal variance (TempVar) of daily milk yield and other economically relevant traits. PTAs for TempVar were obtained for sires with  $\geq 10$  daughters using a repeatability model applied to full lactation data of cows with records in lactation 1, 2 and/or 3. PTAs for production, longevity, fertility, health, and efficiency were retrieved from the April 2023 National Genetic Evaluation generated by the Council on Dairy Cattle Breeding. TempVar phenotypes were calculated as log-transformed variances of daily deviations from lactation curves predicted with LOESS regression (0.75 span parameter) or polynomial quantile regression (0.5 or 0.7 quantile).*

Trait	Method		
	LnVar_loess	LnVar_qr05	LnVar_qr07
Milk (lb)	0.57	0.57	0.57
Fat (lb)	0.11	0.11	0.11
Protein (lb)	0.39	0.39	0.39
Productive Life (months)	-0.38	-0.38	-0.38
Livability	-0.48	-0.48	-0.48
Heifer Livability	-0.04	-0.04	-0.04
Daughter Pregnancy Rate (%)	-0.42	-0.42	-0.42
Heifer Conception Rate (%)	-0.20	-0.20	-0.20
Cow Conception Rate (%)	-0.41	-0.41	-0.41
Gestation Length	-0.02	-0.02	-0.02
Early First Calving	0.06	0.05	0.05
Somatic Cell Score	0.26	0.27	0.27
Milk Fever	-0.08	-0.08	-0.08
Displaced Abomasum	-0.25	-0.25	-0.25
Ketosis	-0.28	-0.28	-0.29
Mastitis	-0.41	-0.42	-0.42
Metritis	-0.21	-0.20	-0.20
Retained Placenta	-0.10	-0.10	-0.10
Residual Feed Intake	0.05	0.05	0.05
Feed Saved	-0.05	-0.04	-0.04

Estimated genetic correlations showed favourable relationships between TempVar phenotypes and several traits included in the U.S. Net Merit selection index. Interestingly, the correlation between TempVar phenotypes and milk was 0.57 indicating that as milk production increases, TempVar phenotypes increase. This reflects the scaling relationship between mean and variance where we expect milk yield and TempVar PTAs to increase simultaneously. Interestingly, for all health-related traits, the estimated genetic correlations among sire PTAs were negative. Specifically, for displaced abomasum, ketosis, mastitis, and metritis we found moderate correlations ranging from -0.21 to -0.42. Correlations between TempVar phenotypes and somatic cell score (SCS) ranged from 0.26 to 0.27, which is also favourable as a higher SCS indicates higher levels of mastitis (Table 5). This is logical, because consistent cows will tend to have fewer disease events, fewer visits to the hospital pen, and fewer management interventions that may cause fluctuations in daily milk yield. The strongest correlations observed in this study were between milk yield TempVar and mastitis, presumably because mastitis is a common disease that causes large decreases in milk production (Liang *et al.*, 2017; Seegers *et al.*, 2003). Overall, lower temporal variance (consistent performance) was associated with superior health, longevity, and fertility. It should be noted that, while we required a minimum of 100 daily milk weights to compute TempVar phenotypes in the present study, we recognize that fragile cows with health or fertility problems may get culled at a higher rate than resilient cows, and we should therefore recognize that improvements in generalized resilience will likely come from a selection index containing PTAs for milk yield TempVar, longevity, and early postpartum health disorders. Correlations of TempVar traits with feed efficiency were near zero, suggesting that selection for lower residual feed intake and greater feed saved will increase farm profitability (Parker Gaddis *et al.*, 2021) with no adverse impacts on resilience.

This study aimed to examine the genetics of milk yield consistency within and between lactations of U.S. Holstein cows. Our findings suggest that TempVar is moderately heritable, which may allow selection to focus on cows with smaller fluctuations in daily milk yields throughout lactation than their contemporaries. TempVar phenotypes appear to be robust to the choice of lactation curve models, and genetic rankings seem to be consistent across lactations. Cows displaying superior milk yield consistency tend to be genetically superior for productive life, female fertility, and resistance to early postpartum health disorders relative to their inconsistent contemporaries. Definition of consistency phenotypes and characterization of their genetic basis is an important initial step in developing resilience indicators that will allow selection for consistent performance in unpredictable conditions. Improving resilience will lead to improvements in dairy farm profitability, reduce animal health and welfare risks associated with management and weather disturbances, and improve the social and environmental sustainability of dairy farming.

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## Conclusions

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## Use of vocalizations to detect pain and discomfort in dairy cows at dry-off

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Identifying and characterizing the acoustic taxonomy of cows' vocalizations might be useful to detect welfare problems such as pain. Drying-off is recognized as a painful and stressful event due to abrupt cessation of milking and consequently udder engorgement, change of pen and re-grouping, and change of diet. The aim of the study was to analyze vocalizations of dairy cows to determine their acoustic characteristics at dry-off and contrast them with pain-related behaviors and mechanical nociceptive thresholds (MNTs). An environmental microphone was placed above a pen with six cows at the beginning of the dry-off process. The cows had milk production of  $13.24 \pm 7.35$  liters<sup>-day</sup> at dry-off (day 0). The audio recording lasted five days uninterruptedly (101.5 hours recorded). A behavioral pain score, built with direct observations of pain-related behaviors (cow's attention, ear position, facial expression, back position, head position, tail position, limb posture, and lying position; from a score of 0 or painless to a score 13 or severe pain), was used/observed twice daily for 5 days. Additionally, MNTs were measured in newtons (N) using a hand-held algometer to assess the pain due to udder engorgement. Statistical analyses were performed with a Glimmix model. Two kinds of vocalizations were identified based on listening and spectrogram analyses using the Audacity® software:

1. "High vocalization", short and with an ascendant fundamental frequency ( $f_0$ ) with repetitions; and
2. "Low vocalization", longer and with a lower  $f_0$ .

Other spectral characteristics such as spectral bandwidth, centroid, flatness, and roll-off were computed for the two types of vocalizations. Significant differences were found for the duration,  $f_0$ , and spectral bandwidth between *High* and *Low* vocalizations. *High* vocalizations average count per cow was higher on the day+1 ( $6.41 \pm 10.81$ ) and day+3 ( $4.41 \pm 6.62$ ) after dry-off compared to other days studied (day 0:  $0 \pm 0.00$ ; day+3:  $1.00 \pm 2.37$ ; day+4:  $1.08 \pm 2.35$ ;  $P < 0.05$ ). The total number of vocalizations decreased over the five days ( $P < 0.05$ ). The pain score was higher on day+2 ( $1.91 \pm 1.31$ ) and day+3 ( $1.58 \pm 1.16$ ) compared to other days studied (day0:  $0.50 \pm 0.54$ ; day+1:  $0.75 \pm 0.62$ ; day+4:  $0.66 \pm 0.77$ ;  $P < 0.05$ ). The MNTs values were different across the five days assessed ( $P < 0.05$ ). Day+2 had the lowest MNTs measurement ( $23.35 \pm 0.18$  N), and day+4 presented the highest MNTs measurement ( $24.60 \pm 0.18$  N). In the current study, the contemporaneity of *High* vocalizations, pain scores, and MNTs might elucidate the possibility that vocalizations are related to the expression of pain and/or discomfort produced by the dry-off. These results may help understand dairy cows' welfare based

### Abstract

on their vocalizations. Vocalizations show a big potential to assist farmers in detecting welfare problems and facilitate rapid interventions to mitigate them.

*Keywords: dairy cattle, vocalizations, pain, animal welfare monitoring.*

## Introduction

The implementation of technologies to monitor animal production conditions is called Precision Livestock Farming (PLF). PLF aims to provide the farmer with information about the animal gathered in a continuous fashion to facilitate the decision-making process, increasing the efficiency of the production system (Guarino *et al.*, 2017). Additionally, cattle stakeholders and society have demanded the use of monitoring methodologies that do not affect the physical integrity of the animals (Gołębiewska *et al.*, 2018). In this context, animal vocalizations provide the opportunity to detect reliable information about the animals' welfare without animal manipulation.

In dairy cow production, some husbandry practices might produce discomfort, one of them is the dry-off. During the dry-off, cows are ceased to be milked, moved to the dried cows' pen, and their diet changes to a lower-calorie diet. All these changes might alter animal welfare status, as cows need to get adapted to their new management routine and environment. In addition, the irruption of the milking routine causes udder engorgement, as its milk production continues for some days, causing discomfort and udder pain (Bertulat *et al.*, 2013; Silanikove *et al.*, 2013, Mainau *et al.*, 2015).

The aim of the study was to analyze the vocalizations of dairy cows to determine their acoustic characteristics at dry-off and contrast them with pain-related behaviors and mechanical nociceptive thresholds (MNTs). The goal is to monitor the level of discomfort experienced by the cows during the dry-off process. If successful, the results of this study could validate the use of vocalizations as a non-invasive method for monitoring the welfare of dairy cows at drying off.

## Material and methods

### Animals and farm management

Six Holstein-Friesian cows (mean  $\pm$  SD/SE; 44.9  $\pm$  8.39 months old, 1.5  $\pm$  0.54 parities) were housed in an open-sided barn (15 m x 23 m). The pen had straw bedding at the resting zone and concrete flooring for the feeder and drinker areas. The cows were fed a TMR diet (76% silage, 12% straw, 12% grain) three times a day at a feeder with headlocks, and *ad libitum* access to water. One day before dry-off, the cows had a mean milk production of 13.24  $\pm$  7.35 liters/day. On the day of dry-off (day 0), the cows were milked for the last time at 11:30h, spray-marked for individual identification, and then moved to the dry-off pen along with thirteen other dry cows.

## Experimental design

### Vocalizations recording and acoustic analyses

Vocalizations were recorded using an environmental microphone (XM1800S, Behringer, Germany) placed above the pen and attached to a digital recorder (Zoom H5; Zoom, Madrid, Spain). The study started on the dry-off day (day 0) and lasted for five consecutive days. Two hours of direct observations were done twice a day (09:00h-11:00h and 15:00h-17:00h) to register the vocalizations from the cows. The observer was positioned at an elevated location on the side of the pen, approximately 1.50 meters above ground level. On day 0, only one observation was performed since the cows entered the dry-off pen until 11:30h. Overall, 101.5 hours of audio were



recorded, but only the vocalizations registered during the observation hours were considered for acoustic analysis.

The detected vocalizations were processed through Audacity audio editing software (Audacity® 3.1 version, 2021). The recorded audio was segmented into 15-minute tracks and converted into the spectral domain including frequencies between 100 and 1200 hertz (Hz), allowing the vocalizations to be aurally identified. Additionally, vocalizations were analyzed to extract acoustic features such as duration, fundamental frequency ( $f_0$ ), and spectral features such as bandwidth, centroid, flatness, and roll-off (McFee *et al.*, 2015, Yamamoto *et al.*, 2019). Vocalizations were categorized and clustered based on their acoustic taxonomy.

The same observer assessed individual pain scores twice daily (11:30h and 17:00h) during the recording period. The score was built from nine behaviors scored from 0 to 1-2, and integrated into a total pain score, from a score of 0 (painless) to a maximum of 13 (severe pain), calculated by summing up all behavioral scores. The behaviors assessed were attention toward the surroundings, head position, ear position, facial expression, back position, lying position, tail position, and limp posture (Gleerup *et al.*, 2015, de Boyer des Roches *et al.*, 2015).

#### *Pain score assessment*

A pressure algometer (Digital Force Gauge ZMF-100; Boshi Electronic Instrument, Japan) equipped with a pointless pressure pad was used to measure the mechanical nociceptive thresholds (MNTs) three times per day (11:30h, 14:00h, and 17:00h). The algometer measured in newtons (N) the pressure applied to the cows' udders at a constant rate of 5 N/s while placed on the cauda-ventral side of the rear quarters with the cow standing and locked at the feeder. Two measurements per quarter were taken at 60 seconds intervals. The maximal peak force applicable was set at 24.6 N (Giovannini *et al.*, 2017), further than that, it was registered as a lack of reaction (Krug *et al.*, 2018). Whenever the algometer and pain score measures were scheduled at the same time, the pain score was performed first to avoid affecting the cows' behavior. Each cow's base level was the measurement taken on day 1 after the last milking at the beginning of the study.

#### *Udder engorgement / Udder pain threshold*

All statistical analyses were performed with the statistical package SAS (version 9.4, SAS Institute Inc., Cary, NC). The number of cows' vocalizations was analyzed with a Proc Glimmix after a logarithmic transformation. The acoustic features (duration,  $f_0$ , and spectral features) were analyzed with a non-parametric test through a Proc Npar1way. The pain score and MNTs were analyzed using a Proc Genmod with day, hour and its interaction as the fixed effects of the model. Results are presented as the average values of the variables (mean  $\pm$  SD/ES). Significant differences were declared at  $P \leq 0.05$  whereas near-significant trends were considered at  $0.05 < P \leq 0.10$ .

#### *Statistical analysis*

## Results

### Vocalizations detection and characterization

Two main groups of vocalizations were aurally identified from the detected vocalizations: “High” vocalizations and “Low” vocalizations (Figure 1). The comparison of the acoustic features of both vocalizations is reported in Table 1.

### Number of vocalizations

High vocalization count was significantly different across the days ( $P=0.0455$ ), being the day+1 the one with the highest count ( $6.41 \pm 10.81$ ). On the other hand, Low vocalization did not have differences across the days ( $P=0.1643$ ).

### Pain score assessment

There were significant differences among the total pain scores among the days ( $P<.0001$ ). Higher pain scores were reported on day+2 and day+3 ( $1.91 \pm 1.31$  and  $1.58 \pm 1.16$ , respectively) compared to day0 ( $0.50 \pm 0.54$ ), day+1 ( $0.75 \pm 0.62$ ) and day+4 ( $0.66 \pm 0.77$ ).

### Udder engorgement / Udder pain threshold

The engorgement of the udder of the cows was different among the five days ( $P<.0001$ ). Higher engorgement was recorded on day+2 ( $23.35 \pm 0.18$  N). On day+1 the cows

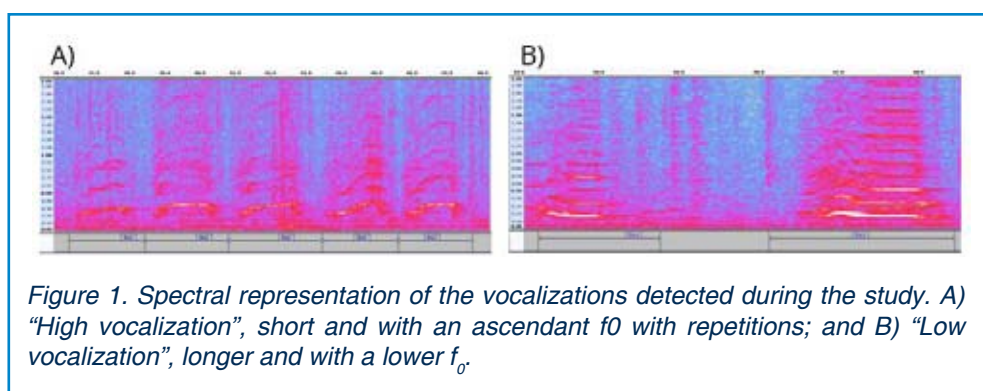


Figure 1. Spectral representation of the vocalizations detected during the study. A) “High vocalization”, short and with an ascendant  $f_0$  with repetitions; and B) “Low vocalization”, longer and with a lower  $f_0$ .

Table 1. Acoustic features measured from the “high” and “low” vocalizations recorded during the five consecutive days after dry-off.

	High		Low		P-value
	Mean	SD	Mean	SD	
Duration	1.72	0.538	2.60	1.012	<.0001
$f_0$	212.16	55.393	106.59	65.144	<.0001
Spectral centroid	2993.38	827.598	3536.51	975.151	<.0001
Spectral bandwidth	3888.41	654.359	4796.66	892.374	<.0001
Spectral flatness	0.009	0.009	0.014	0.013	<.0001
Spectral roll-off	5982.06	1832.040	7632.90	2979.760	<.0001

presented high sensibility to the MNTs measurement ( $23.91 \pm 0.18$  N). On days 0, +3, and +3 the MNTs measurements were  $24.53 \pm 0.22$  N,  $24.20 \pm 0.18$  N, and  $24.60 \pm 0.18$  N, respectively.

Vocalizations have the potential to provide information about animal welfare in a continuous and less invasive manner. *High* and *Low* vocalizations identified in the present study, have been previously reported in the literature as possible cues of cows to express discomfort (Briefer, 2012, de la Torre *et al.*, 2015). *High* vocalization count was higher on days+1 and +2, and pain behavioral score and udder pain threshold pointed out that the discomfort of the cows was higher on day+2. Thus, *High* vocalizations might be a way to express pain and discomfort in dairy cows at dry-off. Other authors have associated vocalizations with discomfort. For instance, Coetzee *et al.* (2010) counted the number of vocalizations during the castration in calves as a pain indicator.

Vocalizations have shown to be a feasible tool to monitor animals without disturbing their physical integrity or daily routine, particularly at dry-off., in the assessment of stressful husbandry practices such as the dry-off in the dairy systems. However, further research is needed to validate the preliminary results of this study and elucidate how the incidence can change in each animal over time.

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## Discussion

## Conclusions

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## Practical Lessons from Data Hub Implementation

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The International Dairy Data Exchange Network (iDDEN) was developed to optimise data exchange between dairy herds, dairy data organisations, farm service providers, dairy equipment manufacturers and on-farm software organisations. The implementation of iDDEN's data exchange hub by dairy data organisations and equipment manufacturers provides practical lessons on solving the drivers, barriers and challenges of innovation uptake and change in this area.

The drivers of more streamlined data exchange are from organisations seeking operational efficiencies and lower operating costs (including reduced manual data entry and transfer), standardisation of interfaces and animal data, access to data that is currently inaccessible or difficult to retrieve, and a consistent data transfer mechanism for the increasing number of devices and sensors on-farm.

Barriers and challenges to optimising data exchange and uptake that iDDEN has addressed are a combination of technical, regulatory, and organisational elements.

A standardised approach and the use of open standards means that technical difficulties and obstacles to implementation are relatively minor compared to these other factors.

Regulatory barriers are usually due to confusion about data use regulations and oversight and having to translate and understand legal jargon, especially across different countries. These hurdles can be overcome via good communication and the use of standardised, simple data use agreements.

Organisational barriers include a desire to 'control' data, a lack of a data management strategy, or an unclear business case on the value of data sharing. iDDEN has worked with both technical teams and senior management to ensure there is not a disconnect in the organisation about the importance of data exchange and it is seen as a business imperative.

*Keywords: data exchange, data transfer, innovation, standardisation.*

The International Dairy Data Exchange Network (iDDEN) was developed to optimise data exchange between dairy herds, dairy data organisations, farm service providers, dairy equipment manufacturers and on-farm software organisations.

iDDEN is the largest international dairy data partnership, bringing together farmer-owned organizations and national databases across thirteen countries representing approximately 200,000 dairy herds, 20 million dairy cows in total and 13 million milk recorded dairy cows.

### Introduction

iDDEN is owned and governed by a consortium of farmer-controlled member organizations from different countries providing dairy data services in Australia, Austria, Belgium, Canada, Denmark, Germany, Iceland, Finland, Luxembourg, Norway, Sweden, The Netherlands, and the United States. The seven iDDEN foundation shareholders are CRV, DataGene, Lactanet, National Dairy Herd Information Association (NDHIA), NCDX (Nordic countries), RDV, and vit.

The iDDEN hub enables two-way data exchange between farm management system software located on-farm and cloud-based farm management system solutions with milk recording and other industry organisations databases located around the world.

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## Technical barriers and challenges

Data hub implementations involve integrating data from various sources and systems. This can result in complexity and challenges associated with data integration, such as data format differences, data quality issues, and data synchronization problems. It underscores the need for robust and standardised data integration strategies and tools.

Data hub implementations often involve an iterative process of development and refinement. Many organizations realize the importance of adopting an agile approach, enabling them to iterate and test the data integration before going into the full 'production' environment. Flexibility, adaptability, and continuous 'real world' testing become essential for addressing technical barriers and challenges with implementing a data exchange.

A data hub implementation highlights the importance of establishing centralized data governance processes and frameworks. It is evident that consistent data definitions, standards, and policies are crucial for ensuring data quality, integrity, and security across the organization and its data exchange partners.

Specific technical barriers and challenges to data exchange encountered to date include dairy data organisations, farm service providers, dairy equipment manufacturers and on-farm software organisations operating across different countries, languages, and data providers (using different data definitions). The key technical barrier however is a lack of people or resources allocated to the data hub implementation and integration.

## Solutions and practical lessons learned

A standardised approach and the use of open standards means that technical difficulties and obstacles to implementation are relatively minor compared to these other factors. Key approaches have been:

- Standardize as much as possible – for example, using Open Standards and International Committee for Animal Recording (ICAR) Animal Data Exchange (ADE) data definitions. iDDEN has implemented the ICAR ADE data message standards, and these common standards and guidelines make data interchange easier and more effective.
- Provide a high level of technical support, including an information pack for new users, biweekly coordination meetings during the integration phase, and technical discussions via a dedicated Slack channel.
- Provide support tools such as a Translation Tool to support companies for different languages and markets and an Admin Tool to monitor the day-to-day operation of the data exchange.
- Engage a professional service partner; in iDDEN's case, Mtech (Finland), an experienced agricultural software service provider.
- Ensure a critical size of the organisation to finance the necessary technical infrastructure and to influence or set data standards.

Data hub implementations necessitate handling data from multiple sources. Organizations appreciate the criticality of robust technical and organisational measures to protect data security and ensure compliance with regulations. Specific regulatory or legal barriers and challenges encountered to date with some of iDDEN's partners include confusion about data use regulations in different countries or jurisdictions, disconnection between technical teams, senior management, and legal representatives (especially around interpreting and understanding legal jargon). However, the key concern raised during discussions on data hub implementation is whether data is stored or not.

## Regulatory and legal barriers and challenges

The iDDEN solution is designed to ensure that no data is stored within the system other than temporarily to deal with technical interruptions and the use of log files to help customers monitor their own data exchange.

## Solutions and practical lessons learned

iDDEN uses standardised, simple data use agreements and authentication approaches to ensure that farmers retain control of their data. iDDEN also has an international approach in dealing with regulatory and legal matters, especially as many current and potential partners most are global or operate across several countries.

Implementing a data hub often requires a cultural shift within the organization towards a data-driven mindset. iDDEN's experience shows that organizations with successful data exchange implementations have senior executive sponsorship, technical buy-in, and a "data sharing" culture at all levels. Collaboration and communication become vital to achieving data-driven objectives.

## Organisational barriers and challenges

Companies that are unclear on the value of a data exchange usually have no data management strategy or want to 'control' data (both their 'own' and even that from other sources). Often, in these cases, data exchange is seen as a "technical function"

instead of a business priority and there is a disconnect between the technical teams and senior management (the “decision makers”). As a result, there is limited focus and budget allocated to data exchange.

## Solutions and practical lessons learned

A key lesson from data hub implementations is the focus on deriving tangible business value. Successful organizations align data initiatives with strategic objectives and prioritize use cases that deliver significant outcomes. The key lessons in this area have been the importance of ongoing engagement and communication at multiple levels with partner organisations. Other key factors to overcome organisational barriers include simplifying the data exchange process as much as possible, having ownership and governance of iDDEN by a consortium of farmer-controlled member organisations from around the world, and implementing an Advisory Committee comprising iDDEN shareholders and strategic partners.

## Conclusions

Overall, implementing a data hub has provided practical insights into various aspects of data management, governance, integration, security, and culture. These lessons have guided iDDEN and its partner organizations in building robust data ecosystems that support informed decision-making, innovation, and competitive advantage.

A summary of practical lessons from iDDEN’s data hub implementation:

- Farmers want their service organizations to help them make better use of data.
- The benefits to data exchange are clear – but not all companies or organisations have seen the value (yet).
- Overcoming technical barriers to data exchange is relatively easy, especially when a standardised approach and common data standards are used.
- Legal or regulatory barriers can be overcome by only exchanging (not storing data), authentication, and standard agreements.
- Any organisational barriers are addressed by frequent communication, simplifying data exchange, and building trust.



## Prediction of heat stress status by infrared spectroscopy in dairy sheep

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Advances in high-throughput phenotyping using Fourier-transform infrared spectroscopy (FTIR) offer the opportunity to efficiently measure new traits on a large scale that can be exploited in breeding programs as indicator traits. As new traits, the definition of phenotypes to improve the adaptation of animals to heat stress events is gaining interest within the context of Climate Change. Thus, in this work we evaluate the suitability of using FTIR to predict whether an animal has been exposed to a heat stress event. Milk samples from 305 ewes from the same flock were collected in two seasons: comfort (spring) and summer (hot season). Fourier-transform infrared spectra were collected on the same day as milk sampling and consisted of the transmittance values measured at 1,060 wavenumbers ranging from 5,011 to 925 (cm<sup>-1</sup>). A quality control analysis using principal components analysis in the FTIR spectra was carried out in order to remove outliers. After QC, a PLS-DA analysis was conducted to evaluate if we were able to discriminate between both conditions, comfort and heat stress. The results showed a high discrimination capacity between ewes under comfort and ewes under heat stress. The variability observed within the group of samples analyzed under comfort was significantly lower than that observed for the group of samples analyzed under heat stress. This would indicate that while exposure to heat stress events produces physiological changes in the animal that are reflected in the composition of the milk, these changes are not the same in all animals. A detailed study of the regions of the spectra in which large variability among individuals was observed could provide insight into the metabolic pathways involved in the heat stress response. Also, the results open the possibility of considering the use of infrared spectroscopy as a breeding tool. For this, a more detailed study of the individual variability of spectra under heat stress would be necessary.

### Abstract

*Keywords: mir spectra, heat stress, climate adaptation, breeding, sheep*

## Introduction

Exposure to HS events has negative consequences on milk production - both in quantity and quality – fertility, health and wellbeing (Baumgard *et al.* 2016). In response to the Climate Change (CC) challenge, breeding programs have been presented as very useful tools to improve the adaptation of animals to those negative consequences of CC that cannot be mitigated (Carabaño *et al.* 2017; Pryce *et al.* 2020). For this purpose, however, it is necessary to define phenotypes easy to measure on a large scale to characterize the thermotolerance of animals. In this regard, advances in high-throughput phenotyping using Fourier-transform infrared spectroscopy (FTIR) offer the opportunity to efficiently measure new traits on a large scale that can be exploited in breeding programs as indicator traits (Hammami *et al.* 2015). Mid-infrared (MIR) methodology is routinely used in quality assessment of milk samples. Focusing on adaptation to CC, FTIR spectra data could be used to the identification of animals suffering from HS and characterization of their thermotolerance as a way to improve adaptation to HS within breeding programs. With this background, the present work was designed as a preliminary study to examine the suitability of the use of FTIR spectra to discriminate between milks samples collected under comfort or heat stress (HS) and examine if they could be as thermotolerance indicator in dairy sheep.

## Materials and methods

A total of 232 ewes belonging to a flock of the national association of breeders of the Manchega dairy sheep (AGRAMA) were sampled during two periods: (i) comfort season: April, May and September; and (ii) hot season: June to August. At each visit, individual milk quantity was recorded, and a milk sample was collected and sent to the laboratory to perform the quality analyses. At the lab, mid-infrared spectra were obtained individual milks samples over the spectral range of 4000 to 900  $\text{cm}^{-1}$  using a Milk-Scan equipped with a Fourier transform infrared interferometer (FOSS electric A/S, Hillerod, Denmark).

In a first step, principal components analysis of the FTIR spectra data was carried out and the presence of outlier spectra was explored by calculating the Mahalanobis distance, so those spectra outside de range of  $\pm 3$  standard deviations were removed from the data base. Milks samples were assigned to 2 groups based of the collection date: comfort (samples collected in April, May or September) and HS (samples collected in June, July or August) groups. Figure 1 shows average FTIR spectra for milk samples collected under comfort and HS and their differences. A partial least squares – discriminant analysis (PLSDA) was conducted to see whether it is possible to discriminate between samples collected under HS from those collected in comfort.

## Results and discussion

Figure 1 shows average FTIR spectra for milk samples collected under comfort (top) and heat stress (centre), and the differences between both (bottom; comfort – heat stress). Differences were mainly in two regions of the spectra: around 1500  $\text{cm}^{-1}$  and from 3500 to 3000  $\text{cm}^{-1}$ . Some studies have reported that at these wavelength, carbon-oxygen and nitrogen-hydrogen bonds from fatty acids and proteins absorb being these regions used to predict fat and protein contents of milk (Nicolau *et al.* 2010). However, as this was an untargeted study, we do not have information to identify which milk components are responsible for the differences between samples collected under HS or Comfort conditions. Subsequent targeted studies will seek to deepen the study of the milk components that change due to exposure to heat stress through the use of metabolomics.

As we indicate above, the aim of this preliminary study was to see if FTIR spectra data was useful to identify when and animal was under HS or not. Figure 2 shows the

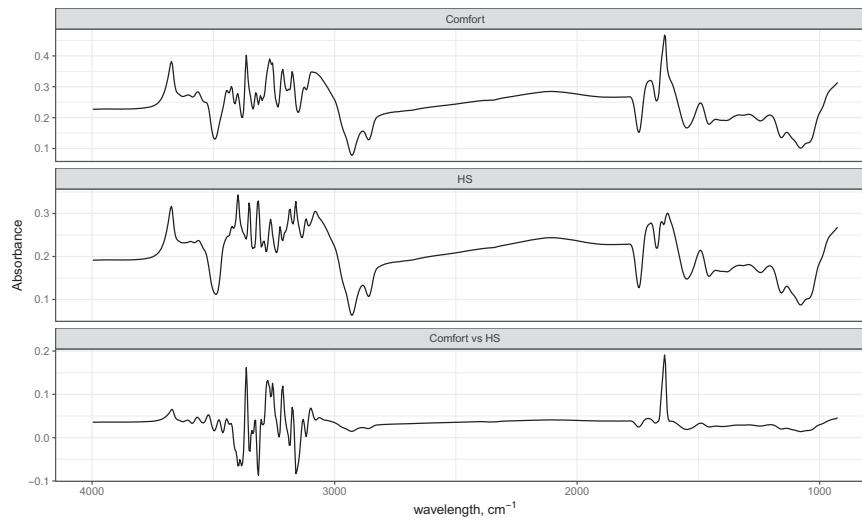


Figure 1. Average FTIR spectra of ewe milk samples collected under comfort and HS conditions and their differences

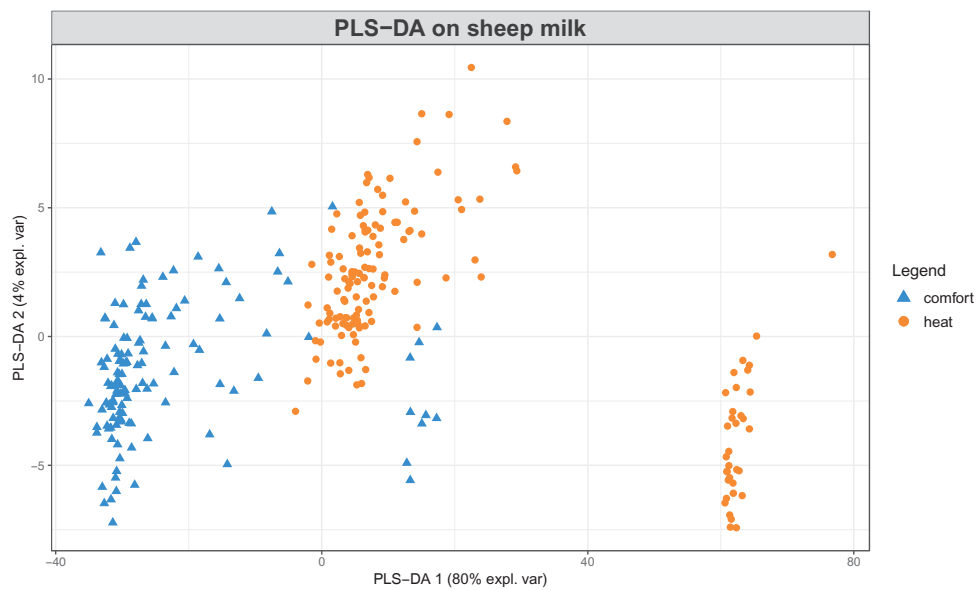


Figure 2. Classification of ewe milk samples of ewes based on PLS-DA outcomes using FTIR spectra as predictor. Milk samples collected under comfort (blue triangles) and HS (orange circles) are represented.

how milk samples were classified based on PLSDA outcomes using FTIR spectra as predictor. In general, the PLSDA procedure was able to discriminate between samples collected under comfort from those collected under HS. For the latter, two groups were clearly differentiated. Although not indicated in this paper, as was not an objective in this preliminary study, these two subgroups of milk samples under HS represent primiparous (the group of orange circles close to the group of samples collected under comfort) and multiparous (the group of orange circles further away from the group of samples collected under comfort) ewes. The same two groups existed for the milk samples collected under comfort, but no differences were observed between them.

## Conclusions

The objective of this preliminary work was to examine whether FTIR spectra data from milk samples could be used to identify if an animal had suffered from heat stress and its suitability as a HS phenotype to be used in breeding programs in order to select for animals more adapted to heat stress. Some regions of the spectra have clearly differed between samples collected under comfort or HS conditions, although it has not been possible to identify the milk components to which these differences are due. Predictions of status of the samples (comfort vs. HS) by PLSDA methodology appear promising to discriminate between both types of samples.

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## NEU.rind - Digital farm assistant for assessing sustainability, efficiency and environmental impact on the dairy farm

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In order to reduce the environmental impact of Austrian cattle farming and to improve sustainability, farm-specific recommendations for action are essential. However, these can only be provided if meaningful key figures and information on the potential of farm-specific measures are known for individual farms. Representative and comparable key figures with benchmarking are essential for this. In collaboration with representatives from research, farmer representatives, recording organisations, consultancies, dairy processing and marketing organisations, needs and requirements were elaborated. We use as much pre-collected data as possible to assess highly important sustainability aspects on a dairy farm-specific but internationally comparable level by aiming at reducing the workload for data recording. These data come from the central cattle database and interfaces to other official and relevant data, e.g. farms' land use (from the Integrated Administration and Control System, IACS) or economic parameters. Existing data are supplemented with on-farm primary data to calculate eight aggregated indicators based on life cycle assessment methods in the environmental dimension. Indicators cover global warming, food-feed-competition, ammonia emissions, cumulative energy demand, biodiversity aspects and are complemented by animal health aspects and economic key figures. Functional units are kg milk, hectare and farm. Sensitivity analyses have been conducted to assess the most important data and changes in accuracy due to minimised additional farm data records. Currently, we use a prototype for data collection to analyse 200 dairy farms representing different environmental conditions and production systems. Based on that information, we will elaborate a final user-friendly version of the digital farm assistant for routine use.

### Abstract

*Keywords: greenhouse gas emissions, carbon footprint, online tool.*

## Introduction

Emissions from cattle farming are under critical discussion and there is a need for an increase in sustainability, especially regarding greenhouse gas (GHG) emissions (Twine *et al.*, 2019, Leip *et al.*, 2021). Cattle farming is in the area of conflict between food security and securing ecosystem services while maintaining sustainable and competitive agricultural production (Mottet *et al.*, 2018). Consumers expect transparency and high standards in production like sustainable products with low environmental footprint, good animal health and welfare, but also favourable pricing (Schiano *et al.*, 2020).

In order to reduce the environmental impact of Austrian cattle farming and to improve sustainability, farm-specific recommendations for action are essential, which sufficiently take into account the complexity of milk production (Schils *et al.*, 2007). These can only be provided if meaningful key figures and information on the potential of possible measures are known for the individual farm (Robling *et al.*, 2023). Representative and comparable key figures with benchmarking are essential for this. FarmLife is an already existing LCA tool in Austria in this area, which is very precise but not broadly used, as it requires a time effort of around two days for farmers to complete data entry (Herndl *et al.*, 2016). To establish key figures and enable benchmarking, a broader use is necessary. This shall be achieved by developing a user-friendly simplified but scientifically sound tool with minimized effort for data entry.

## NEU.rind digital farm assistant

The EIP AGRI Project NEU.rind aims to develop a digital farm assistant for assessing sustainability, efficiency and environmental impact on Austrian dairy farms. The goal is to generate a broad data basis and provide current facts and key figures for representative Austrian farms. A benchmarking with farm comparisons for the estimation of improvement potentials and recommendations for farm-specific measures for improvements are the goal of NEU.rind.

In the first step, the needs and requirements for such a tool were elaborated in collaboration with representatives from research, farmer representatives, recording organisations, consultancies, dairy processing and marketing organisations. Important aspects are, that the application is user-friendly with little effort for additional data collection for the farmers and with meaningful and easily understandable key figures for the practice. Broad use can only be expected if these requirements are met.

## Selection of indicators and methods used by the NEU.rind tool

While some of the standard impact categories in the Product Environmental Footprint Category Rules (PEFCR) or in the Eco-Score do not have a high relevancy in dairy production, the important issue of biodiversity is not covered by this life cycle assessment (LCA)-based assessment (EDA 2018, Curran *et al.*, 2016). Moreover, the Eco-Score does not consider all dimensions of sustainability, but only product-related assessments on efficiency in ecology. In addition, some of the PEFCR-LCA impact indicators have large uncertainty ranges (ADEME and INRAE, 2020). Given the large problem areas highlighted in the planetary boundaries (Steffen *et al.*, 2015) and the dairy-specific issues, stakeholders within our project defined a new set of indicators. Calculation and definitions consider international standards like IDF (2023) and ICAR (2023). Furthermore, most of the indicators in the NEU.rind-tool link impacts of dairy farming on two functional units, per kg milk and per hectare of farmland, e.g. for global warming. These indicators are primarily intended to provide valid and practical results to farmers, which are also used for on-farm recommendations (Table 1).

*Table 1. The final set of indicators in the NEU.rind-tool as defined by the stakeholders within the NEU.rind-consortium. Impacts of dairy farming on two functional units, per kg milk and per hectare of farmland; the second biodiversity indicator is based on farm-level, animal health aspects are based on herd level.*

Indicator	Per kg milk <sup>1</sup>	Per ha utilized area or per farm	
Global warming	kg CO <sub>2</sub> -eq	kg CO <sub>2</sub> -eq	LCA <sup>2</sup>
Human edible feed conversion efficiency / Protein production	heFCE factor	kg CP / ha	SUS <sup>3</sup>
Biodiversity	Potentially disappeared fractions of species	% HN VF <sup>4</sup> ; rare / endangered crops / breeds	LCA <sup>2</sup> / SUS <sup>3</sup>
Fossil energy demand	MJ	GJ	LCA <sup>2</sup>
Ammonia emissions	kg NH <sub>3</sub>	kg NH <sub>3</sub>	LCA <sup>2</sup>
Nitrate emissions	kg NO <sub>3</sub>	kg NO <sub>3</sub>	LCA <sup>2</sup>
Animal health aspects	Scores	Scores	SUS <sup>3</sup>
Contribution margin	€	€	SUS <sup>3</sup>

<sup>1</sup>Consideration of co-products, allocation

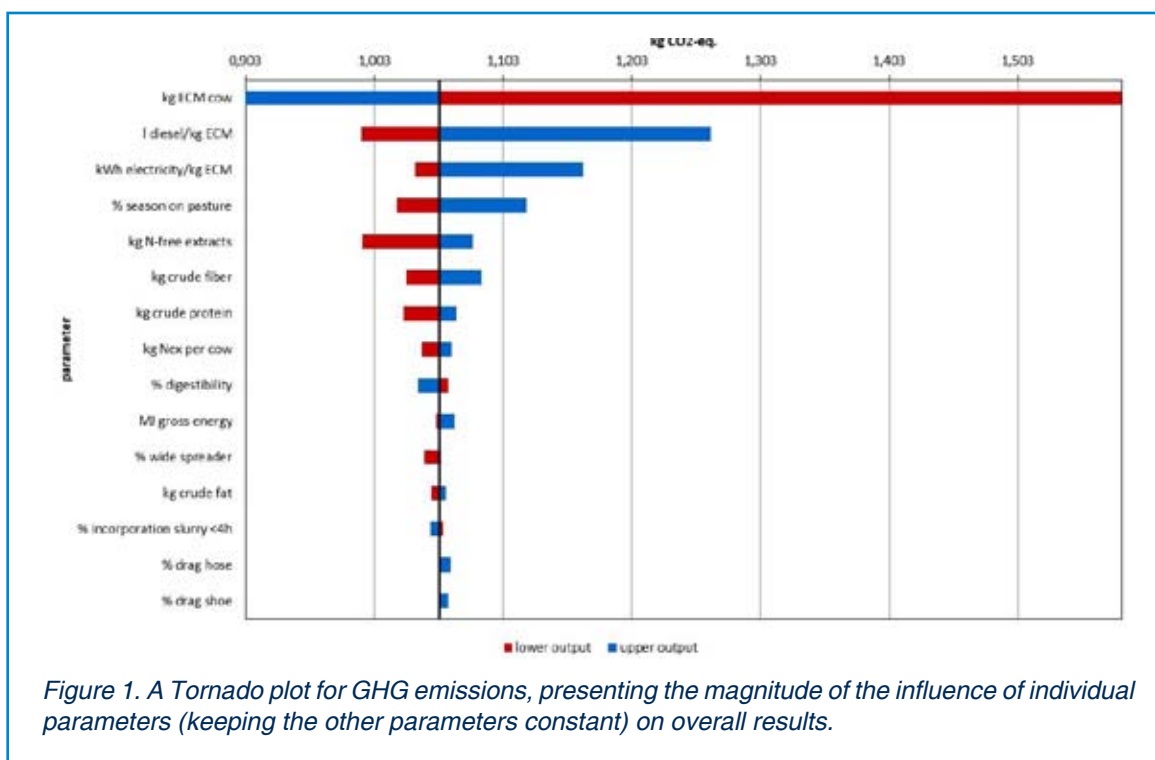
<sup>2</sup>Typical LCA-based methods, which are evaluated over the life cycle from cradle to farm-gate

<sup>3</sup>Supplementing sustainability indicators

<sup>4</sup>High nature value farmland

GHG emissions are also reported on a single basis (CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O) for on-farm recommendation, and in CO<sub>2</sub>-eq (GWP<sub>100</sub> and GTP<sub>100</sub>; IPCC, 2021). The human-edible feed conversion efficiency (heFCE) is calculated for protein, including the protein quality (DIAAS; see Ertl *et al.*, 2015, 2016) in addition to the amount of protein, which is produced on a hectare-basis. Firstly, biodiversity conservation is assessed according to the potentially disappeared fraction of species method developed by Chaudhary and Brooks (2018), taking into account aspects such as feed conversion efficiency and imported feed. Secondly, for another perspective on biodiversity, the on-farm status is evaluated by the proportion of high nature value farmland (% HN VF) and whether rare or endangered livestock breeds and crop varieties are used. Fossil energy consumption is inter alia an important driver for GHG emissions and also a finite resource and thus assessed within the NEU.rind-tool. Moreover, ammonia and nitrate emissions are evaluated for both functional units. The same applies to farms' economic results as gross margins. Animal health aspects are assessed based on recorded data on animal or herd level. A specific goal with regard to user-friendliness is, that the defined indicators and evaluation methods are based as far as possible on data already collected (Central Cattle Database, CCD; IACS) and require as little additional data as possible to be collected on the farms.

Sensitivity analyses by Tornado plots (Figure 1) and by diverse runs of the LCA method with default data have been conducted to assess the most important farm data and changes of accuracy due to reduced farm-specific data records. For example, in the sensitivity analysis for GHG emissions, the kg energy corrected milk per cow and year, feeding and fossil energy parameters have the greatest influence on results and are therefore most important for data collection (Figure 1).



### Data demand for the NEU.rind tool

For the NEU.rind tool, data on farm characteristics, land use, animals, housing, manure management, milk yield, milk quantities, diet composition, feeding, energy consumption, buildings and machinery and on farm management are used. Data integration is a central point in order to generate a simple tool with reduced data entry effort, but also meaningful results. Already existing data are used to keep the time required for data recording to a minimum. For those data that nevertheless need to be collected additionally, a user-friendly solution, the NEU.rind tool, was developed.

Some data are well available from already existing sources, like animal and housing data from the CCD. Other data that are already partly available are: data on feeding (also in the CCD for farms using the CCD tool “efficiency check”) and on land management from IACS (the Ministry of Agriculture) or economic data by the Federal Institute of Agricultural Economics (BAAF). In general, economic data should be collected specifically on-farm. Already available data are collected through online interfaces (Figure 2).

### Data collection with app prototype

Data is collected online with an Oracle APEX App in 40 acquisition steps with more than 170 individual parameters as well as several other parameter complexes (e.g. ration composition and feeding period, degree of mechanisation and work processes). For each parameter there is an input field, a detailed description of the parameter and, if available, a default value from already available data sources and information on the origin of the default value.

Data entry is mainly done by employees of the milk recording associations and by farmers themselves under supervision by recording associations. Data collection on farms started in 2023 and is planned to be carried out on 200 farms that are selected to cover different production conditions and systems as base for representative and comparable figures. Evaluation routines are gradually added in the APEX application.



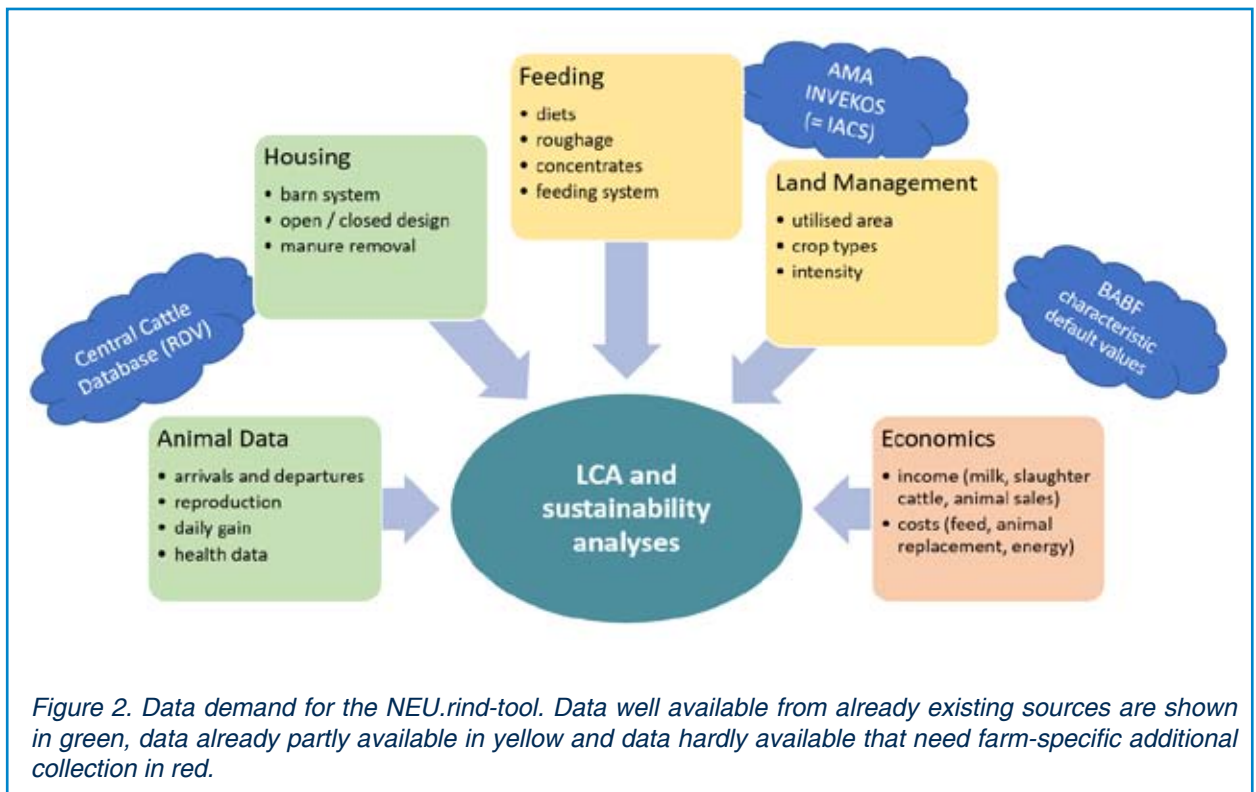


Figure 2. Data demand for the NEU.rind-tool. Data well available from already existing sources are shown in green, data already partly available in yellow and data hardly available that need farm-specific additional collection in red.

The NEU.rind app can be continuously developed and adapted based on practical experience (evolutionary prototyping) by implementing the app as an APEX application. In addition to the possibility of data collection, analysis and result feedback as well as graphical preparation will also be developed in APEX as the project progresses.

Since ongoing adjustments to the calculation methods are to be expected even after the project has been completed in order to always correspond to the current state of knowledge and international standards, all recordings and analyses are always assigned to the current version of the analysis routines at the time of recording. This means that analyses that have already been carried out remain reproducible at any time. This is the basis for a possible annual certification of farms based on the NEU.rind tool in the future.

Farm-specific results show the status quo for environmental impacts and supplementing sustainability aspects as presented above and measures for improvement. On the basis of first representative results, a system for farm comparisons (benchmarking) will be developed to further illustrate the strengths and weaknesses of the individual farms (within groups of comparable farms) and show improvement potentials. Additionally, recommendations for farm optimization can be provided on the basis of the results. If the system is broadly used in practice, current key figures on the sustainability of Austrian dairy farms will be extracted and used for the information of consumers, agricultural policy, etc.

## Results, discussion and conclusion

Internationally, different tools to assess sustainability are in use. The approach used in NEU.rind is comparable to tools like ANCA in the Netherlands (De Haan, 2021), or Arla's Climate Check tool (Arla, 2022). Approaches used by ICAR (ICAR, 2023) with the list of the parameters to describe sustainability, or Lactanet's Sustainability Index (Warner *et al.*, 2022), are based on data routinely recorded by milk recording organizations. However, this is not comparable to a direct and comprehensive sustainability analysis, as such indices do not assess a carbon footprint (kg CO<sub>2</sub>-eq) or other quantitative environmental impacts of a LCA. For a comprehensive analysis, it is inevitable to include input parameters that dominate LCA results, such as the feed composition and the feed production including the origin of the feed (with inter alia parameters on yields in relation to the efforts of cultivation and fertilizer use, emissions from land use and land use changes, transport distances, etc.). These are not included in routinely recorded input parameters and therefore their significance regarding overall sustainability is limited. Moreover, dairy production (agriculture in general) is connected to many social and economic aspects of a local community, including farmers, farm workers, and consumers. Regarding resource use it is for instance important to evaluate human edible feed conversion efficiency and (net-) protein production. All of these important sustainability aspects can be assessed using quantitative methods, such as those listed in Robling *et al.* (2023). Of course, this needs a lot of farm-specific data, which can be collected efficiently by using data from the CCD, IACS, farm questionnaires and supplemented by economic default data.

In conclusion, our NEU.rind- sustainability analysis is an up-to-date tool that meets the requirements of dairy LCAs (IDF, 2022) and addresses further economic and social sustainability aspects.

The assessment of sustainability, efficiency and environmental impact on Austrian dairy farms based on routine data (CCD, IACS data and interfaces to other official and relevant data) is possible, but some additional manual data collection is needed. The working time requirement is approximately 1 to 2 hours per farm per year.

Oracle APEX is a suitable tool to develop such a web application using a prototype approach with continuous further development. A user-friendly data recording and recommendations for improvement are essential for broad use. Participation of the relevant stakeholders in the development ensures acceptance and practicability.

## Acknowledgement

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## The Sustainability Index: A new tool to breed for reduced greenhouse gas emissions intensity in Australian dairy cattle

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The Australian dairy industry has set a target to reduce greenhouse gas (GHG) emissions intensity by 30% by 2030 compared to the 2015 level. At the animal level, apart from nutritional modifications and other management practices, selecting animals which emit less GHG can be a cost-effective and long-term strategy. Given the world's demand for protein is increasing, selecting for animals with lower GHG emissions per unit of production (aka emissions intensity) is a realistic approach that addresses the key issue of emissions reduction while maintaining farm productivity. In August 2022, DataGene released the Sustainability Index which can be used by dairy farmers to select bulls and cows with lower environmental footprints. The index was built based on the existing Balanced Performance Index (BPI) but placed greater emphasis on production, survival, health and feed efficiency. Compared to BPI, the weightings for protein, fat, survival, mastitis resistance and feed efficiency are increased by 2.6, 1.4, 2.8, 1.3 and 3.8-fold; respectively. It is expected that with the use of the Sustainability Index, emissions intensity will be reduced by 6.3%, 7.3% and 4.4% in Holstein, Jersey and Red breeds by 2050 compared to the current level; respectively. By comparison, the corresponding values for BPI were 5.0%, 6.2% and 4.1%; respectively. However, the trade-off in BPI when using the Sustainability Index will be \$1.50, \$1.05 and \$0.27 per year for Holstein, Jersey and Red breeds; respectively. The Sustainability Index is published on DataVat and the Good Bulls App.

### Abstract

*Keywords: emissions intensity, sustainability, selection index.*

Improving environmental sustainability through reduced greenhouse gas (GHG) emissions is a global priority. In Australia, the agriculture sector produces 67 Mt carbon dioxide equivalents (CO<sub>2</sub>-e), accounting for 13% of the country's total emissions in 2020 (Australian Government Climate Change Authority 2021). The dairy sector accounts for 12.5% of agriculture emissions, or about 2% of national emissions (Dairy Australia 2021). Emissions intensity per cow and per kg of fat protein corrected milk were estimated 6.9 ± 1.46 t CO<sub>2</sub>-e and 1.04 kg CO<sub>2</sub>-e, respectively (Christie *et al.*, 2011) Although the carbon footprint of Australian dairying is one of the lowest internationally (Mazzetto *et al.*, 2022), there is still scope for further reduction. The Australian dairy industry has made a commitment to minimising its environmental footprint, including reducing GHG emission intensity by 30% by 2030 across the whole industry compared to the 2015 level (Dairy Australia 2020).

### Introduction

Enteric methane accounts for about 57% of emissions on an average Australian dairy farm (Dairy Australia 2021). While recognising that management and dietary solutions can be used to reduce enteric methane, selective breeding can provide a complementary solution which is cost-effective, permanent, and cumulative (de Haas *et al.*, 2021; Manzanilla-Pech *et al.*, 2021), while potentially benefiting both emissions intensity and total emissions. One solution for the latter is to produce a breeding value for enteric methane, which requires a large number of records and good quality methane phenotypes or predictors on individual animals. In Australia, the current number of records on methane data is still limited, which impedes the implementation of genomic selection for the trait (Richardson *et al.*, 2021b). While the long-term goal is to have a breeding value for methane, increased emphasis on improved milk production, survival, fertility and feed efficiency could be a short-term approach (Løvendahl *et al.*, 2018) as breeding values for these traits are readily available in the national genetic evaluation. Richardson *et al.* (2021b) used this approach and developed a GHG subindex of milk yield, fat yield, protein yield, survival and feed saved with an accuracy of ~0.50. The subindex was expressed in CO<sub>2</sub>-e gross emissions per cow.

There are two philosophical approaches that can be taken to reduce greenhouse gas emissions associated with livestock production. Broadly these are targeting reductions in emissions either with or without consideration of the animal's productive output.

The first approach is to target a reduction in gross agricultural emissions from the livestock sector. When considering this approach, geneticists tend to focus on measuring the total GHG output per animal per day and apply downward selection pressure to this measure. One consequence of this is that because more productive, higher milk yielding animals tend to eat more feed than lower producing animals, and because GHG output is tightly linked to feed intake (Pickering *et al.*, 2013), selection for reduced gross per animal GHG emissions is likely to be highly antagonistic to the current selection direction for productive performance. Selection on any index predicting GHG output per animal per day, will penalise high milk producing animals.

A second approach is to focus on emissions intensity in the breeding goal. Emissions intensity measures gross GHG output per animal per day against their productive output. High milk producing animals and farming systems tend to have lower emissions intensity than lower producing animals and systems because their proportional superiority in milk yield is only partly offset by the higher GHG output associated with the feed required for higher milk production (Gerber *et al.*, 2011; Pryce and Bell 2017). This perspective is consistent with the dilution of maintenance principal, whereby high yielding animals have only slightly higher feed requirements for maintenance and/or for rearing their replacements while the extra feed required for milk production is proportionally offset by the additional milk production resulting in a net gain in overall efficiency. This approach aligns well with farmers' objectives, that is to maximise profit through efficiency, increased cow fertility and longevity (Lovett *et al.*, 2006; Waghorn and Hegarty 2011; Richardson *et al.*, 2021a). Most of the trait changes being driven by current Australian dairy indexes (i.e. the Balanced Performance Index or BPI, and to a more modest extent the Health Weighted Index or HWI) are already improving emissions intensity, as they are simultaneously improving milk yield and fertility, which are both favourably associated with emissions intensity.

In this study, we aimed to:

1. Quantify improvement in emissions intensity made since the implementation of the BPI,
2. Describe the development and implementation of the Sustainability Index which can be used as a tool to reduce emissions intensity in Australian dairy cattle, and
3. Predict the effect of alternative indexes on emissions and economics.

This study will utilise kg of CO<sub>2</sub>-e per kg of protein equivalent (kg CO<sub>2</sub>-e/kg prot-e) as a measure of expressing emissions intensity rather than kg of CO<sub>2</sub>-e per kg of fat protein corrected milk or kg of CO<sub>2</sub>-e per kg of energy corrected milk. This choice is based on protein's higher economic value (\$6.76/kg) compared to fat (\$2.08/kg) in the Australian context.

For this analysis, the impacts of variations of a 'sustainability index' were estimated and compared to the existing BPI and HWI indexes. Three possible sustainability selection indexes were considered. These sustainability indexes were developed by including three variations of a GHG subindex at three carbon values within the BPI to produce the Sustainability Index, based on methodology adapted from Richardson *et al.* (2022). The three carbon prices were \$500/kg CO<sub>2</sub>-e, \$1000/kg CO<sub>2</sub>-e and extreme high or infinite. Acknowledging that these prices are high in the current Australian context, the index we aimed to develop, however, is a desired gains index and high assumed carbon prices are necessary to invoke meaningful change. Initial consultations with Australian farmers indicated their willingness to sacrifice economic gains to reduce environmental impacts, which allows the adjustment of BPI for this purpose, similar to the way the HWI was developed with major emphasis on health and fertility (Axford *et al.*, 2021).

As the traits included in the GHG subindex are currently included in the breeding objective, the additional emphasis received by each trait within the GHG subindex was applied to its economic weight to present the total relative emphasis of each trait, as opposed to the emphasis of a subindex. Richardson *et al.* (2021a) used methodology adapted from Amer *et al.* (2018) to calculate coefficients that express the kg of CO<sub>2</sub>-e associated with a unit change in index traits. These coefficients were used as weights and applied to Australian breeding values (ABVs) commonly used in selection and most strongly associated with emissions to derive three possible subindexes aimed to rank the environmental impact of individual animals based on their genetic merit. The environmental and economic impact of the three index scenarios were measured and compared to the two current national indexes.

Genotypes for 5,499 registered bulls (n=4,382 registered Holstein, n=734 registered Jersey, n=383 registered Red Breeds including Aussie Red, Ayrshire, Illawarra and Dairy Shorthorn) used in this study were provided by DataGene Ltd., with processing and genotyping methods being consistent with the national genetic evaluation dataset. Bulls were born between 2010 and 2015. The ABVs used in this analysis included milk yield, protein yield, fat yield, survival and feed saved, as well as other traits of interest such as heat tolerance and liveweight and were accessed from the August 2021 official genetic evaluation run.

Emission intensity coefficients were previously calculated by Richardson *et al.* (2021a) based on the approach used by Amer *et al.* (2018) and adapted to calculate the effect of a unit change in milk yield, fat yield, protein yield, feed saved, and survival traits on CO<sub>2</sub>-e emissions per kg kilogram of protein equivalents (Table 6). Protein equivalents are a weighted aggregate of the product outputs from protein yield, fat yield, and milk yield weighted on the component value ratio relative to protein. Briefly, this method estimates the change in total emissions and product output caused by a 1 unit change in each index trait, resulting from either a direct emissions trait (GHG yield), changes in herd structure (fewer replacements), or the dilution effects of higher yields (milk

## Material and methods

### Genetic bull data

### Calculating relative weights

production) and proliferation (more offspring/dam). As fertility is a primary reason for culling, the environmental impact of fertility is largely accounted for by the survival ABV, with minimal additional effects applying to extended lactations observed in seasonal calving systems (Richardson *et al.*, 2021a; Workie *et al.*, 2021). Therefore, the survival GHG coefficient is considered in the index, with no coefficient directly for fertility. The model was used in the current study to dynamically represent an Australian dairy herd and assess effects of changes in traits.

### Developing the sustainability index

Multiple variations of an environmentally focused national selection index were previously developed using gross GHG coefficients as described by Richardson *et al.* (2022). However, these indexes only explored the application of developing a GHG subindex targeting gross emissions. The variations of sustainability index investigated in this paper were developed using the methodology described in Richardson *et al.* (2022), adapted to generate intensity coefficients. Briefly, the component traits used in the development of the index are the same as those in the BPI, namely milk yield, fat yield, protein yield, survival, fertility, somatic cell count, mastitis resistance, temperament, mammary system, udder depth, overall type, pin set and feed saved (Axford *et al.*, 2021). Emissions intensity coefficients/values (IV) were estimated that describe the change in enteric methane per unit of output attributed to traits currently under selection in Australian dairy cattle (expressed in kg carbon dioxide equivalents per kg protein-equivalents). Since these IV coefficients were estimated to be independent, they can be used as weights within an index to place non-economic emphasis on traits with environmental impact. The calculated IV coefficients were applied to existing ABVs shown to have an independent effect on enteric methane emissions and used to develop a GHG subindex. As the GHG subindex contains traits already include in the breeding objective, the additional weight of each trait within the GHG subindex was directly applied to the trait within the sustainability subindex. The investigated index scenarios were as follows:

$$SI_j = \sum (EW_n + (IV_n * CP_j)) * ABV_n$$

Where  $SI_j$  is the sustainability index calculated using  $j^{th}$  carbon price,  $EW_n$  is the economic weight of the  $n^{th}$  trait (milk yield, fat yield, protein yield, survival, fertility, somatic cell count, mastitis resistance, temperament, mammary system, udder depth, overall type, pin set and feed saved),  $IV_n$  is the emissions intensity coefficient (kg CO<sub>2</sub>-e/kg protein-e changed in 1 cow per unit change in the trait ABV) for the  $n^{th}$  trait (milk yield, fat yield, protein yield, survival and feed saved),  $ABV_n$  is the Australian Breeding Value for the  $n^{th}$  trait (milk yield, fat yield, protein yield, survival, fertility, somatic cell count, mastitis resistance, temperament, mammary system, udder depth, overall type, pin set and feed saved), and  $CP_i$  is the  $j^{th}$  carbon price (AUD\$500, AUD\$1000 and extreme or infinite/tonne CO<sub>2</sub>-e).

### Relative emphasis

The relative emphasis of each trait and subindexes for every variant of the BPI was calculated using the approach of Zhang and Amer (2021), which accounts for the accuracy of the ABVs as well as the (favourable or antagonistic) relationships between traits in contrast to traditional approaches that are often a simple multiplication of the relative contribution of each trait's economic value (converted to absolute value)



by its genetic standard deviation. Here, we applied the method of Zhang and Amer (2021) using correlations between the ABVs. The resulting trait emphasis values more accurately present the true selection pressure each trait receives within the given index.

Pearson correlation coefficients among all indexes and ABVs were calculated. The method for computing correlations in the presence of missing values that was used is 'pairwise.complete.obs' in R (R Core Team 2022). In this methods, each correlation can be based on a different number of observations as all complete pairs of observations on two ABVs/indexes are used to calculate the correlation between these ABVs/indexes. Pearson correlation coefficients between ABVs and indexes used the same number of observations, as indexes are only calculated for bulls without any missing values for ABVs in the breeding goal.

### Correlations between traits

The response of a trait ( $R$ ) to a particular index  $j$  was calculated using the following formula:

### Estimating trait responses

$$R = \frac{\rho(ABV, Index_j) \times SD(ABV)}{SD(Index_ref)} \times \Delta(Index_ref)$$

Where  $\rho(ABV, Index_j)$  denotes the correlation between the trait ABV and  $Index_j$  (i.e. SI, BPI and HWI),  $SD(ABV)$  and  $SD(Index_ref)$  are the standard deviation of each ABV and selected  $SD(Index_ref)$  (BPI and HWI) respectively,  $\Delta(Index_ref)$  is the amount of unit change in the  $Index_ref$ , used as a baseline to compare responses across traits and indexes.

Geneflow modelling was used to assess the economic and environmental impact of implementing the three variations of the sustainability index in the national breeding objective. The geneflow model utilises selection index theory combined with capital budgeting methodologies to quantify the industry level impacts of genetic selection for reduced GHG, and conventional production traits (i.e., milk yield, fat yield, protein yield) on key national metrics of GHG emissions in Australian dairy cattle. The model was used to quantify any trade-offs required between increasing genetic gain in traditional production traits versus GHG mitigation. Scott *et al.* (2021) reported that the annual rate of genetic gain in BPI since 2013 ranged between 0.11 and 0.22 genetic SD per year for Holstein cows and bulls, respectively. Consequently, it was assumed that a 1-SD improvement in BPI (AUD\$84.06; Axford *et al.* (2021)) would be achieved over around 10 years of selection. The responses in BPI units achieved by selection on each of the considered indexes, as well as the total CO<sub>2</sub>-e reduction achieved by selection for each index, are presented.

### Environmental and economic response

## Results

Historic changes in Australian dairy industry emissions intensity (kg CO<sub>2</sub>-e/kg prot-e) from 2015 to 2022 have resulted in improvements of 1.3%, 1.4% and 0.8% in Holstein, Jersey and Red Breeds, respectively (Table 1). This is equivalent to the reduction of 0.25, 0.27 and 0.15 kg CO<sub>2</sub>-e/kg prot-e in the three breeds, respectively. That is, most of the trait changes being driven by the BPI, and to a more modest extent the HWI, are already improving emissions intensity. This is because they are simultaneously improving production and survival, which are both favourably associated with emissions intensity.

The future changes to be expected in emissions intensity with deployment of the new indexes considered, particularly by 2030 are modest. It is important to note that the trajectory of genetic change between now and 2026 has already been set by historic selection decision. Figure 1 shows the improvements made by each of the indexes for Holstein, Jersey and Red Breeds. Among the indexes, HWI showed the least improvement with a reduction of 5.39%, 6.74% and 4.94% in emissions intensity in Holstein, Jersey and Red Breeds, respectively, by 2050 compared to the 2015 levels. The SI\_extreme index showed the most improvement with a percent reduction in emissions intensity were 8.02%, 9.20% and 5.59% in the three breeds, respectively.

We predicted future changes in emissions intensity using three indexes with different carbon pricing, namely SI\_500, SI\_1000 and SI\_extreme, which had the carbon price of AUD 500/t CO<sub>2</sub>-e, AUD 1000/t CO<sub>2</sub>-e, and AUD infinite/t CO<sub>2</sub>-e, respectively, applied as a weight to the carbon emissions associated with subindex component traits (milk yield, fat yield, protein yield, survival and feed saved). When considering the changes in BPI (AUD per cow) over time for different indexes, SI\_extreme resulted in the largest loss in profit relative to selection based on BPI with a BPI loss of 18.12%, 12.80% and 6.16% by 2050 in Holstein, Jersey and Red breeds, respectively (Figure 2). That is a reduction of AUD 90.5, 55.5 and 16.1 per cow per year in the three breeds. SI\_500 showed the least sacrifice in BPI with a reduction of AUD 12.5 (2.5%), AUD 8.8 (2.0%) and AUD 2.4 (0.9%) per cow in Holstein, Jersey and Red breeds, respectively, but achieved the least gain in emissions intensity. When comparing SI\_1000 and SI\_extreme, the gain in emissions intensity made by SI\_extreme was moderate (e.g., 8.02% vs. 7.64% in Holstein) but the relative negative impact on BPI gain was much higher (e.g. AUD 90.5 vs. AUD 27.5 per cow).

For these reasons, SI\_1000 was chosen as the Sustainability Index (SI), which was predicted to result in a reduction of emissions intensity by 7.64%, 8.96% and 5.52% in Holstein, Jersey and Red breeds, respectively, by 2050 relative to the 2015 level. Using SI\_1000 also resulted in expected slower gain in BPI compared to the use BPI itself by AUD 27.5 (6.87%), AUD 19.0 (5.49%) and AUD 5.1 (2.42%) per cow in Holstein, Jersey and Red breeds, respectively, in the same time period. That is equivalent to AUD 0.79, AUD 0.54 and AUD 0.15 per cow per year. From this point forward, SI refers to the index SI\_1000 as it was chosen by industry for implementation.

Table 1. Changes in emissions intensity (kg CO<sub>2</sub>-e/kg prot-e) from 2015 to 2022 as a result of implementation of the Balanced Performance Index (BPI)

Breed	Unit gain (kg CO <sub>2</sub> -e/kg prot-e)	Percent change (%)
Holstein	-0.25	-1.3
Jersey	-0.27	-1.4
Red breeds	-0.15	-0.8

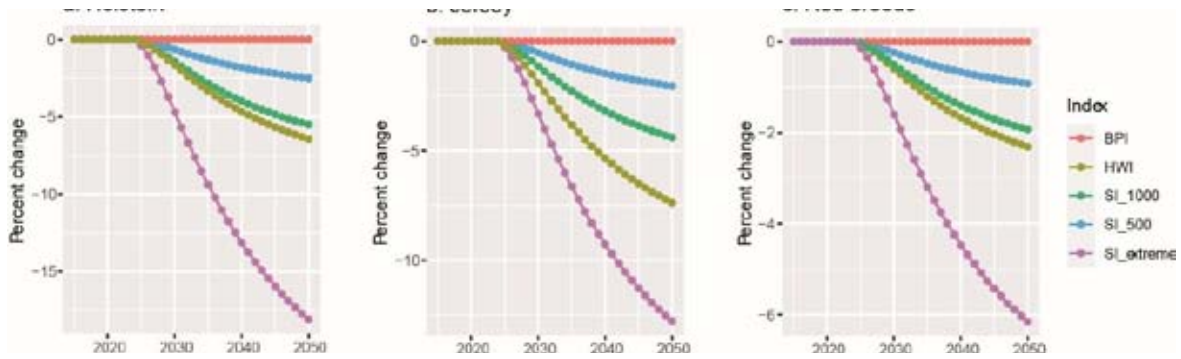


Figure 1. Percent change in emissions intensity (kg CO<sub>2</sub>-e/kg prot-e) in a) Holstein, b) Jersey and c) Red Breeds with different indexes (BPI = Balanced Performance Index, HWI = Health Weighted Index, SI<sub>500</sub>, SI<sub>1000</sub>, SI<sub>extreme</sub> = Sustainability Index with carbon price per tonne CO<sub>2</sub>-e = AUD 500, 1000 and infinite, respectively).

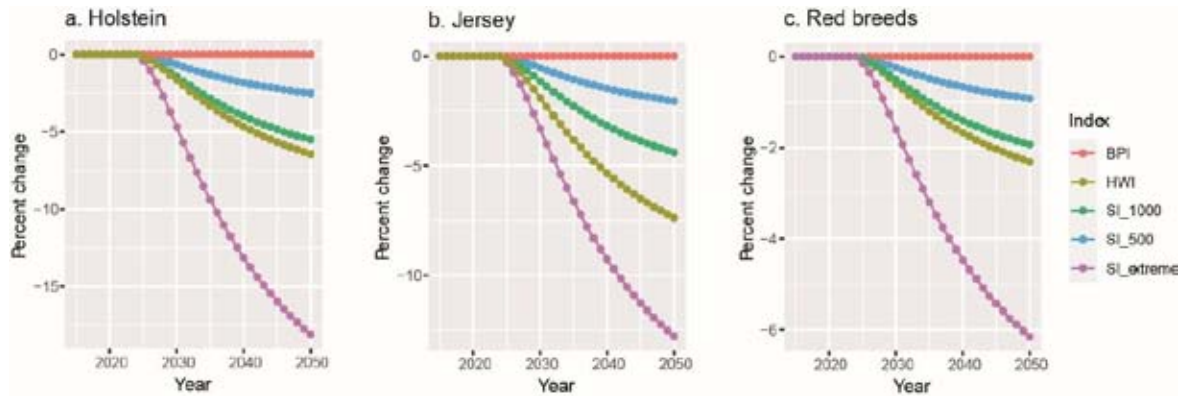


Figure 2. Percent change in units of Balanced Performance Index in a) Holstein, b) Jersey and c) Red Breeds with different indexes (BPI = Balanced Performance Index, HWI = Health Weighted Index, SI<sub>500</sub>, SI<sub>1000</sub>, SI<sub>extreme</sub> = Sustainability Index with carbon price per tonne CO<sub>2</sub>-e = AUD 500, 1000 and infinite, respectively).

Table 2. Economic weights of traits included in the Sustainability Index (SI). Economic weights of the same traits for the Balanced Performance Index (BPI) the Health Weighted Index (HWI) are also included for comparative purposes.

Trait	Holstein		Jersey		Red Breeds		HHWI*
	SI	BPI*	SI	BPI*	SI	BPI*	
Protein yield	17.49	6.76	17.49	6.76	17.49	6.76	4.36
Fat yield	2.82	2.08	2.82	2.08	2.82	2.08	1.35
Milk yield	-0.11	-0.11	-0.11	-0.11	-0.11	-0.11	-0.07
Survival	20.21	7.20	20.21	7.20	20.21	7.20	7.20
Daughter fertility	6.94	6.94	6.94	6.94	6.94	6.94	14.11
Somatic cell count	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Mastitis resistance	8.70	6.75	8.70	6.75	8.70	6.75	6.75
Milking speed	5.02	5.02	5.02	5.02	5.02	5.02	5.02
Temperament	3.60	3.60	3.60	3.60	3.60	3.60	3.60
Mammary system	2.76	2.76	2.76	2.76	2.76	2.76	3.59
Udder depth	0.82	0.82	0.82	0.82	0.82	0.82	0.00
Overall type	1.36	1.36	1.36	1.36	1.36	1.36	1.36
Pin set	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Feed saved	0.7227	0.1927	0.5300	0	0.7227	0.1927	0.3853

\*Axford et al. (2021). Economic weights for HWI component traits are the same in three breeds.

Economic weights for index traits calculated for the SI are summarised in Table 2. Compared to BPI, more emphasis was placed on protein yield, survival, mastitis resistance and feed saved. Compared to BPI, the weightings for protein, fat, survival, mastitis resistance and feed efficiency are increased by 2.6, 1.4, 2.8, 1.3 and 3.8-fold; respectively.

Correlations between the August 2022 breeding values between SI, BPI and HWI for bulls born in 1990 or later are presented in Table 3. Correlations with SI were higher for BPI than for HWI (0.96 vs 0.89 in Holstein, 0.95 vs 0.86 in Jersey and 0.94 vs 0.82 in Red breeds). This means although there was some level of reranking, majority of bulls which are ranked highly in BPI also have high SI breeding values.

Table 3. Correlations between August 2022 breeding values of the Sustainability Index (SI) and the Balanced Performance Index (BPI) and the Health Weighted Index (HWI) for a) Holstein, b) Jersey and c) Red bulls born in 1990 or later.

	SI	BPI	HWI
Holstein			
SI	1		
BPI	0.96	1	
HWI	0.89	0.96	1
Jersey			
SI	1		
BPI	0.95	1	
HWI	0.86	0.93	1
Red bulls			
SI	1		
BPI	0.94	1	
HWI	0.82	0.92	1

Table 4. Relative emphasis (%) of traits within the Sustainability Index (SI) in Holstein, Jersey and Red bulls. Relative emphasis of the same traits for the Balanced Performance Index (BPI) and the Health Weighted Index (HWI) are also included for comparative purposes.

Trait	Holstein			Jersey			Red Breeds		
	SI	BPI*	HWI*	SI	BPI*	HWI*	SI	BPI*	HWI*
Protein yield	32.3	20.1	12.6	39.9	24.3	15.4	55.6	25.1	16.4
Fat yield	11.0	9.3	5.9	7.9	9.8	6.3	10.4	9.7	6.4
Milk yield	8.2	14.9	9.2	9.9	16.7	10.5	8.4	13.8	8.9
Survival	11.8	8.4	8.2	13.7	8.8	8.7	7.6	5.1	5.1
Daughter fertility	9.3	13.2	26.0	4.9	9.9	19.8	4.2	0.0	0.0
Somatic cell count	2.4	5.5	5.3	2.8	5.6	5.5	2.5	11.0	22.5
Mastitis resistance	5.5	8.0	7.7	5.8	7.4	7.3	2.4	6.2	6.3
Milking speed	1.7	4.0	3.8	1.7	3.1	3.1	0.7	7.0	7.1
Temperament	1.2	2.3	2.3	1.7	3.0	3.0	0.9	5.2	5.3
Mammary system	2.9	4.3	5.4	3.5	6.0	7.6	1.7	2.8	2.8
Udder depth	0.6	1.2	0.0	0.7	1.5	0.0	0.3	4.4	5.8
Overall type	1.3	2.0	1.9	1.7	2.7	2.6	0.9	1.4	0.0
Pin set	1.0	1.4	1.3	0.7	1.3	1.3	0.4	2.0	2.1
Feed saved	11.7	5.3	10.4	5.0	0.0	9.1	3.9	5.0	10.1

\*Axford et al. (2021)

Table 5. Predicted responses to selection (SD unit response to 1 SD change in the Sustainability Index (SI), the Balanced Performance Index (BPI) and the Health Weighted Index (HWI)) for Holstein, Jersey and Red bulls.

Trait	Holstein			Jersey			Red Breeds		
	SI	BPI	HWI	SI	BPI	HWI	SI	BPI	HWI
Protein yield	0.68	0.42	0.18	0.83	0.63	0.35	0.91	0.80	0.70
Fat yield	0.50	0.55	0.28	0.64	0.67	0.39	0.68	0.67	0.52
Milk yield	0.32	0.05	-0.07	0.45	0.18	0.07	0.43	0.22	0.12
Survival	0.48	0.50	0.42	0.50	0.48	0.50	0.57	0.54	0.47
Daughter fertility	0.16	0.32	0.63	-0.14	0.01	0.40	0.63	0.71	0.85
Somatic cell count	0.42	0.50	0.45	0.02	0.10	0.22	0.35	0.41	0.45
Mastitis resistance	0.34	0.46	0.49	0.08	0.24	0.33	0.11	0.21	0.26
Milking speed	0.06	0.11	0.06	0.37	0.39	0.28	0.09	0.11	0.11
Temperament	0.25	0.25	0.14	0.46	0.46	0.36	0.13	0.12	0.01
Mammary system	0.16	0.18	0.05	0.40	0.40	0.35	-0.34	-0.37	-0.45
Udder depth	0.14	0.21	0.19	-0.37	-0.27	-0.10	-0.07	0.00	-0.02
Overall type	0.18	0.19	0.05	0.44	0.42	0.29	-0.31	-0.37	-0.51
Pin set	0.03	0.02	0.04	0.37	0.34	0.31	0.28	0.33	0.40
Feed saved	-0.02	-0.05	0.19	-0.17	-0.19	0.11	-0.06	0.03	0.23

Relative emphasis of traits in SI is shown in Table 4. The SI has major emphasis on production (52% in Holstein, 58% in Jersey and 74% in Red breeds), followed by health and fertility (29% in Holstein, 27% in Jersey and 17% in Red breeds), feed saved (12% in Holstein, 5% in Jersey and 4% in Red breeds), type (6% in Holstein, 7% in Jersey and 3% in Red breeds), and workability (3% in Holstein, 3% in Jersey and 2% in Red breeds).

Predicted responses to selection with the SI are summarised in Table 5 for Holstein, Jersey and Red bulls. Responses in other traits were also predicted but not presented in this paper. Compared to BPI, in general SI was predicted to accelerate the rates of reductions in emissions intensity and increase the rate of gain in production. These results confirmed that production traits are closely linked to GHG emissions. Using SI is also expected to reduce gains in mastitis resistance, cell count and fertility in Holstein and Red breeds. In Jersey, selection on SI versus BPI would diminish gains in mastitis resistance and cell count, with slight declines in fertility and udder depth. However, natural genetic variation in the breed populations means that there are many Jersey bulls that have both a high SI and a high fertility ABV or a high SI and a high udder depth ABV to choose from (DataGene 2022).

## Discussion

In this study, we have presented realised historic and predicted future genetic gains in both environmental emissions variables and familiar genetic traits and indexes when selecting Holsteins, Jerseys and Red Breeds for current and novel future industry indexes. The results indicate that the current selection indexes have reduced emissions intensity but have scope for further improvement. Among the potential indexes which were modelled based on the current index BPI with different emphasis on production, fertility, survival, health and feed saved with different carbon prices, SI\_1000 or the Sustainability Index (SI) was implemented as it would lead to a reduction in emissions intensity with minimal sacrifice in profit.

This study is an extension of the work undertaken by Richardson *et al.* (2021a) and Richardson *et al.* (2022). The former estimated the independent effects of traits in the Australian National Breeding Objective on the gross GHG production and GHG intensity. The latter investigated options to reduce GHG emissions in the Australian dairy industry by including environmental component in the national breeding program. Richardson *et al.* (2022) focussed on prediction of changes in gross per-animal GHG production. Selection on a gross emissions index in the Australian dairy context is

*Table 6. Intensity coefficients, defined as the independent change in emissions intensity due to a unit change in each trait, used in the derivation of weights applied to traits within the sustainability indexes.*

Trait	Intensity Coefficients, kg CO <sub>2</sub> -e/ kg prot-e
Protein yield, kg	-0.032
Fat yield, kg	-0.002
Milk yield, L	0.001
Survival, %	-0.029
Feed Saved, kg	-0.002
Mastitis Resistance, %	0.006

<sup>†</sup>Previously calculated by Richardson et al 2021a

expected to favour high fertility but at the same time penalise animals with high milk yield potential. The extent of the swing from milk yield to fertility then depends on how much weight is given to direct economic profit versus achieving gross emissions reductions in the formulation of the index. The Australian dairy industry, however, has set target to reduce emissions intensity (Dairy Australia 2020).

The SI placed more emphasis on protein yield, survival, mastitis resistance and feed saved compared to the BPI. For the Australian dairy situation, the main trait of current commercial interest to farmers which also reduces emissions is fertility. Improving genetic merit for fertility reduces culling of infertile cows, and thereby reduces the number of GHG emitting replacements required on a dairy farm which reduces gross emissions. Selection for reduced emissions intensity swings the balance of selection effort towards milk production and away from fertility. In the SI, the economic weight for fertility remains the same as BPI (6.94) but relative emphasis has reduced from 13.2% to 9.3% in the case of Holstein, largely as a result of the selection emphasis moving to milk production traits.

Expected future changes in emissions intensity through the SI predicted in this study from shifting selection from the BPI to SI are modest when compared to the gains in emissions intensity already being achieved through selection on the BPI. This is partially due to the approach which only uses existing ABVs which reduce feed intake per unit of production and therefore not capturing the variation in GHG emissions per unit of feed consumed among animals (Richardson *et al.*, 2022). Recently, Agriculture and Horticulture Development Board (UK) has implemented its EnviroCow Index which also aims to reduce emissions intensity. It was predicted that EnviroCow reduces emissions intensity over 1% each year when direct and indirect effects due to genetic improvements are taken into account. It is noted that the reductions reported in our study did not include indirect effects. In Ireland, the Economic Breeding Index (EBI) for dairy cattle has recently been updated to include a Carbon Subindex (<https://www.icbf.com/?p=18914>). This subindex penalises traits which increase feed intake and therefore increase gross per cow emissions. This approach targeting a reduction in gross emissions results in more selection emphasis on fertility, and less selection emphasis on milk production.

Richardson *et al.* (2022) reported that a reduction of approximately 21% in emissions intensity can be achieved after 30 years of genetic selection if a residual methane trait is available at the prediction accuracy of 0.54. In the absence of a novel methane trait with adequate reliability for industry implementation, the most practical approach is to take advantage of existing traits as shown in the present study. It provides an alternative that does not require the infrastructure needed for new trait recording. However, to further accelerate reduction of GHG emissions intensity, a large number of records of direct or indirect measures of methane may be required. There are several methods to measure enteric methane for dairy cows. Australia has methane records for ~400 animals measured using the sulfur hexafluoride (SF<sub>6</sub>) tracer method (Deighton *et al.* 2014) which is costly to implement on a large scale. Other systems such as GreenFeed® (Zimmerman and Zimmerman 2012) or 'sniffer' (Garnsworthy *et al.*, 2012) are increasingly being used to collect methane related data, especially the latter can be used on-farm conditions and on a large number of animals. Other proxies for methane could also be used in addition to direct measures of methane, such as milk mid-infrared spectroscopy (Vanlierde *et al.*, 2018; Shadpour *et al.*, 2022), microbiome (Zhang *et al.*, 2020), or volatile fatty acids in ruminal fluids (Williams *et al.*, 2019).

This study focusses on reducing emissions intensity as it is aligned with the current industry goal. However, we also recognise that the goal is to reduce gross emissions when considered at an industry, national or global level. This can be achieved by targeting other aspects of livestock production. Combination of additional measures such as management of diet, adjustment to animal numbers, management of stored

manure, and appropriate use of carbon neutral fertiliser, renewable fuels and energy, will need to be adopted on farms. With more explicit methane records, direct selection for a trait which reduces methane emissions per unit of feed consumed should become possible and be a more effective option.

## Conclusions

The results from the present study indicate that the current Australian selection indexes for dairy cattle have contributed to lower emissions intensity and it is possible to further improve by using a new Sustainability Index although with modest marginal additional reduction. It is predicted that the Sustainability Index will reduce emissions intensity by 7.64%, 8.96% and 5.52% in Holstein, Jersey and Red breeds by 2050 compared to the 2015 level; respectively and the corresponding sacrifice in profit will be AUD 0.79, AUD 0.83, AUD 0.22 per cow per year. The Sustainability Index has been implemented by DataGene since August 2022 and the results on bulls and cows can be accessed on DataVat ([datavat.com.au](http://datavat.com.au)) and Good Bulls App. While the Sustainability Index is a practical and cost-effective approach to breed for the reduction in emissions intensity at this point in time, faster genetic gain can be achieved by selecting directly on methane trait or its proxy. Many countries endeavour to collect methane and related data, an international collaborative effort in sharing these data would be beneficial to all in achieving our common goal.

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## Farm-to-table science: dairy data mining for future resilience

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Adoption of automated milking systems (AMS) and other precision livestock farming (PLF) systems offers access to large, multidimensional data that allow exploration of resilience traits and sustainable farming strategies in real-world scenarios. Since their inception in the Netherlands in the 1990s, AMS have seen increased adoption in the Nordic countries, with around a third of the total milk production collected by robots. The major brands of farm management systems (FMS) in the Nordic region are only configured to report data as a current overview, discarding older information that is vital to studies of the herd's genetics, behaviour, and environment.

### Abstract

In this work, we present the infrastructure for dairy cattle data at the Swedish University of Agricultural Sciences, Gigacow (SLU Gigacow) that collects data from a set of Swedish dairy farms. Each farm's FMS sends nightly reports to SLU Gigacow, where records are harmonised and collected in a central database. Collected records include milking statistics, health events, traffic data, and SNP genotypes for thousands of cows, and are made accessible to researchers through SQL or R queries. SLU Gigacow also integrates data from the Swedish national cow registry, including pedigrees and herd transfers for cows resident at participating farms.

SLU Gigacow's longitudinal observations (first data collected in 2020) link genotype to phenotype and animal welfare with the goal of accelerating pilot studies in dairy science, as well as providing a big dataset from cows in active, commercial settings. The data collection software written in Python 3 (Beaverton, USA) has modules that enable collection from several versions of DeLaval DelPro (Tumba, Sweden), and can be extended to any FMS with a graphical user interface running on most consumer operating systems. After harmonisation to resolve differences in language and FMS versions, data are stored in a database maintained at SLU with SQL Server Integration Services (SSIS) (Microsoft, Redmond, USA). By agreement with Växa Sverige AB (Uppsala, Sweden), participating farmers also get a large number of animals genotyped using the 45k EuroG MD beadchip (Amsterdam, The Netherlands). Currently, the database includes information on over 17,000 cattle, over 3,000,000 milkings, and 2,969 SNP genotypes. The cross-referenced data can be mined for various purposes, including stress responses and resilience traits.

While SLU Gigacow is intended to collect from Swedish farms and support Swedish researchers, it serves as a proof-of-concept that data from diverse sources and systems at dairy farms can be automatically gathered and collated in a researcher-friendly format. We believe that this shows the great utility of farm-to-table statistics

and increased FMS interoperability. SLU Gigacow was constructed essentially without standardised interfaces for dairy data communication. Establishment of such data standards is ongoing within the industry but the development and adoption of standards take time and rely on active participation of multiple actors. The research-driven approach of SLU Gigacow enables more rapid and integrated measurements of many facets of the dairy farm environment, creates new niches for PLF equipment, and opens great new vistas of information to explore for adaptation to changing climates. .

*Keywords: ingemar ohlsson, tomas klingström, dirk-jan de koning, dairy science, big data, resilience.*

## Introduction

Changing climates make resilience a highly desirable target in livestock breeding programs, including for dairy cattle production. Some resilience indicators specific to dairy cattle have been identified (Bengtsson, C., 2022; Kašná, E., 2022), and increasingly sophisticated methods are being applied to find genetic factors implicated in e.g. heat tolerance (Carabaño M.J., 2017; Chen S., 2023). Automated milking systems (AMS) and farm management systems (FMS) integrating a variety of sensors around the cow and the farm provide a great amount of data that can be leveraged to refine resilience studies. In a simple example, daily temperature on a farm and daily milk yield from its resident cattle can be correlated to explore the impact of temperature on productivity. If those cattle are also genotyped, which is done as a routine measure in genomic breeding, varying responses to heat stress can be correlated with genomic features, and novel heat stress tolerance traits can be identified.

Access to data can be a challenge to new data-driven dairy science projects, not least in the case of resilience studies, where location and timing can greatly affect the stresses animals experience. Having an existing database of recent and historical data alleviates the problem of timing, and can reduce the threshold investment necessary for a pilot study. The SLU infrastructure for dairy cattle data at the Swedish University of Agricultural Sciences, Gigacow (SLU Gigacow) aims to provide such a data source. .

## Framework and implementation

### Legal framework

Data collection has been organised based on the EU Code of conduct on agricultural data sharing by contractual agreement and Swedish animal protection law. The farmer is the data originator for all data collected in SLU Gigacow and provides a broad consent for research using data originating from the farm. The farmer also authorises SLU Gigacow to request data from service providers such as Våxa Sverige and the Nordic Cattle Genetic Evaluation (NAV; collaboration including Våxa Sverige) who provide data to SLU Gigacow under separate contracts regulating immaterial property rights and requiring each researchers using SLU Gigacow to sign a researcher consent to comply with the contracts set between SLU Gigacow, the data originator and the data providers.

Data collected directly from the farm or different data providers are kept separately to ensure that researchers do not accidentally use data for which they are unable to fulfil their obligations to a specific data provider. Specifically, in the current implementation, data may originate from participating farms (anything extracted from FMS), from Våxa Sverige (Kokontrollen), or from NAV (SNP genotypes), and research users may be granted specific access to data from any combination of these sources. All unique

identifiers for a farm are pseudonymised and public example datasets are scrambled to provide examples of Gigacow content without the risk of a viewer using third party data to identify a farm. Farmers can request access to data collected from their farms and when data collected for SLU Gigacow is useful for the farmer, such as in the case of genotyping for genomic selection, the data is available for the farmer through their normal service provider.

Currently, all farms providing data to Gigacow use DeLaval DelPro AMS or milking parlors, monitored by DelPro Farm Manager software (versions 5.1-6.13).

### Data collection and storage

The central Gigacow data collection pipeline consists of three blocs of software:

Client scripts	Python 3.10 script running on the FMS client computer at each farm. This script is executed nightly, simulating a user via the PyAutoGUI Python package, to output the past day's milking reports from the FMS. It then attempts to upload these milking reports, plus others scheduled to output from DelPro Farm Manager, to the SLU Gigacow harmonisation server by SFTP connection.
Server scripts	Python 3.10 script running nightly on the SLU collection server. The script processes all data in the farm upload file area. Herd identifiers (in the Swedish system, an integer of max 6 digits; occurring independently, or as part of animal ID) are pseudonymised to an 8-character alphanumeric string. A farm ID-to-pseudonym key is retained for future reference on the collection server. Thus, the same pseudonym can be used downstream to cross-reference animals belonging to the same herd, but the herds and farms are not directly identifiable by third-party users of the data. Data are also harmonised to CSV files with structured file and field names for each data type (milking records, culling records, feed data, etc.).
Database	SQL Server Integration Services storage platform managed by SLU. A set of import scripts takes in the pseudonymised and harmonised CSV intermediates and processes them for storage in SQL tables. End users with SLU intranet credentials can then access this database, either by direct SQL queries or intermediary applications such as the R DBI (R-SIG-DB, 2022) and ODBC (Hester <i>et al.</i> , 2023) packages.

Separate Python scripts also exist for maintenance and updates, as well as pseudonymisation integration of corresponding data from the Swedish national cow registry VÄXA Kokontrollen.

A repository of auxiliary scripts is under active development (<https://github.com/TKlingstrom/Gigacow-tools>), which includes various tools for accessing and manipulating data from the SLU Gigacow database.

All cattle born on the farm since joining SLU Gigacow are genotyped, and as many older animals as possible are genotyped when a farm joins SLU Gigacow. Genotyping is done using the normal commercial process for genomic selection where ear tissue removed when punching and tagging animals' ears is collected for genotyping by chip sequencing. SLU Gigacow covers the cost for the farmer to order genotyping from

### Genotyping

Växa Sverige, which outsources the sequencing work to Eurofins, and sends the data to the Nordic Cattle Genetic Evaluation (NAV) for breeding evaluation. NAV shares the SNP genotype files with SLU Gigacow, where the files are pseudonymised and stored in separate tables in the SQL database.

### Data access

Once stored in the SQL database, collected data can be extracted and viewed with SQL queries, either directly or through intermediary helper programs. Access requires registration with the SLU Department of Animal Breeding and Genetics, Quantitative Genetics division, which maintains records of farmer and researcher consent forms, ensuring that research users reach only the subsets of SLU Gigacow data which they are allowed to use.

Data are primarily organised in views corresponding to the originating FMS export file.

### Results

Data collection from participating farms commenced in 2020. The SLU Gigacow software was iteratively developed as more farms were connected, and currently collects data from DelPro Farm Manager versions 5.1-6.13 controlling fully automated milking systems as well as parlor milking systems. The current client-side scripts will continue to support versions past 5.10.

Support for Lely Time4Cows was planned but put on hold, as this software is supposed to be replaced by Lely Horizon, with major changes expected. As of the time of writing, the SLU Gigacow team has left Lely integration on indefinite hiatus until the participating Lely farms (N = 2) receive software updates and FMS operation can be tested.

Farm Pseudonym	Milking		Traffic		Feed	
	Aggregate	Unique animals <sup>1</sup>	Events	Unique animals <sup>1</sup>	Records	Unique animals <sup>1</sup>
169e580a	- <sup>2</sup>	-	27401	355	706	93
540275a1	315778	232	1008	168	-	-
5b581702	12912	105	-	-	5235	83
5c06d92d	600110	469	-	-	-	-
5f7f33d6	1684	1 <sup>3</sup>	-	-	-	-
752efd72	566127	474	74725	487	925444	1170
a624fb9a	316963	194	128945	276	423617	212
a756bc39	161326	193	505	1 <sup>3</sup>	863452	999
ab18b151	308466	201	25658	161	269083	203
ad0a39f5	923850	482	135299	485	-	-
afdd9a78	46301	68	-	-	25741	69
f454e660	336456	287	38036	232	394661	341
Total	3589973	2706	431577	2165	2907939	3170

<sup>1</sup>Unique animal ID found associated with the given data type.

<sup>2</sup>Available data varies extremely from farm to farm.

<sup>3</sup>Client scripts will attempt to find and upload historical data that is up to one year old, 10 files at a time. Recently added farms take some time to catch up to current records.

culling, feeding, milking, traffic, and health related events. Genotyping has returned SNP data for 2,969 individual cows to date, and updates will be ordered every 3 months.

Some cow identities in the collected data are incomplete. Animal ID numbers used by DelPro may occur without any associated national animal ID, though every animal should have complete data in its birth record. While the number of incomplete identities is small, some patterns of missing data can be found.

An example dataset with scrambled and pseudonymised data is available at [https://tklingstrom.github.io/gigacow\\_exampledata/](https://tklingstrom.github.io/gigacow_exampledata/).

Since connecting the first farms in 2020, the volume and diversity of data collected by SLU Gigacow has grown considerably. Farms using DelPro now upload, at minimum: daily milking reports, culling events, reproduction events (calving, heat, dry-off, etc.), and cow identity information. Additional reports can be attached depending on the data that farmers choose to store in their FMS, such as health events and gate traffic. Progress with Lely has been delayed due to implementation of Lely Horizon coinciding with the planned rollout, highlighting the difficulty of working with the rapid pace of development in the industry.

It should be noted that some forms of data are often absent or unreliable, for reasons that were common between farms. Health events, for example, are frequently recorded in hardcopy and kept in binders, likely in the same office as the FMS client computer. This can, for example, simplify handling of veterinarians' signatures on certain procedures, and may involve forms and record sheets that have been in use much longer than the digital FMS. The main challenge in redirecting hardcopy records to FMS would seem to be making the interfaces simple yet competent enough to match pen and paper.

In addition to information being physically stored, fragmentation of data between different digital ecosystems of data present a challenge for farmers and researchers. In Sweden feeding systems and activity monitors are frequently chosen from other manufacturers than the provider of the milking equipment and the farm management system. Farmers are therefore often forced to consult multiple different applications to inform themselves of the current status of the farm. Identifying key data sources in this digital milieu is therefore an important consideration for further development of SLU Gigacow. Some companies like Nedap make data sharing a competitive advantage and provide an easily accessible API to which farmers can generate and share 'tokens' facilitating data access for advisors or other actors such as SLU Gigacow (<https://api.nedap-bi.com/api/redoc/>). In other cases the fragmentation of data between equipment manufacturers create new business opportunities such as Feedlync (formerly Cowconnect, Asperup, Denmark), which can be installed on feed mixer wagons and supply live feed data to a cloud storage with further integration with advisory systems and other equipment. Mapping these resources and identifying the best way to collect research data from them is therefore a continuous task to expand the capabilities of a dairy data infrastructure like SLU Gigacow. Incomplete cow identities are not unique to DelPro, although some error classes we have identified are shaped by the way data is input and linked in DelPro Farm Manager. With another FMS, you might for example not encounter an integer "animal number" as the primary identifier, and thus the records would be broken in a slightly different way. In the end, broken records derive either from human error during input into the FMS, or due to problems with automated detection of animal tags. It is difficult to conclusively prevent or repair every possible error, but we continue to investigate ways to identify and highlight errors. So far, a part of the design philosophy behind SLU Gigacow has been to present the

## Discussion

data unaltered whenever possible, so we want to avoid editing or removing records unless they specifically damage data consistency. However, near-future versions of SLU Gigacow may implement methods similar to those presented by Hermans *et al.* (2017), where key cattle life events are codified and examined to make sure they occur in logical order, e.g. heat-insemination-calving.

An important component of SLU Gigacow has been the focus on agile development. Each bloc of development has been done in collaboration with researchers able to use the data collected as a “minimum viable product”. This has provided the development team with rapid input from researchers helping to prioritise efforts and make design decisions such as relying on Rstudio (Posit Software, Boston, USA) and the dplyr package (Wickham *et al.*, 2023) as a primary data collection method from the database rather than a programmatically more complex solution relying on OLAP cubes or similar business information management solutions.

SLU Gigacow operates from a farmer-centric view where data sources and sensors useful for farming operations are evaluated and technical solutions for data collection identified. Most commercial sensor developers operating in the region recognise the rights of the farmer as the data originator and owner of data which makes data collection possible even if not always easy due to technical barriers. An ongoing trend with new systems such as Nedap and CowConnect providing open Application Programming Interfaces only requiring a token enables farmers, advisors and researchers to maximise the value of sensor investments by integrating data from multiple sources. In combination with the development of iDDEN as a standard widely supported by major equipment manufacturers not yet providing full APIs this is likely to lead to greatly enhanced data access for livestock researchers. The structure of SLU Gigacow, with data harmonised into a unified format and stored in a versatile SQL database, makes it well adapted to follow data standards that overlap with its available information, including iDDEN. We hope it will convincingly show the promise of open data exchange to enable and empower future livestock research..

## Conclusions

Even with the great data generation potential of modern digitised milk collection systems and other PLF technologies, researchers and developers must be mindful of the most basic errors, like mistyped input and falsely identified cows. It is not possible to ask perfection of either the farmers or their technology, but aggregating diverse data sources, such as with SLU Gigacow, can help detect and correct animal identity errors.

Merging data from the wealth of available sources on a modern PLF-enabled farm has uses beyond simply verifying identities. Connected data sources allow farmers, equipment developers, and researchers to find and observe complex patterns in livestock management. Such connections benefit from, or outright demand that data standards be in place to enable communication system-to-system and system-to-user. Global standards projects like iDDEN represent a unifying force, while the expanding and diversifying market of PLF devices may drive systems apart. Representatives of farming, scientific, and industrial interests should maintain communication to encourage a future PLF market that allows both creativity and diverse niches for system developers, and a good range of systems that work together for the farmers. In that way, we can ensure the resilience of PLF technologies going forward.

The process of developing SLU Gigacow has repeatedly shown that farms are individual, and both FMS and systems like ours that extract data from them must carefully consider



the needs and idiosyncracies of each customer farm. Active cooperation with farmers is necessary to advance agricultural tech and research, and open data standards can only support it. Research can here motivate early use cases for data integration and provide the experience necessary for optimizing new standards and define which data to be shared between systems. We hope SLU Gigacow will serve as a springboard for new researchers in the intersection of PLF and resilience, and illustrate what data sharing can accomplish with a modest effort..

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## Prediction of body condition in Jersey dairy cattle from 3D images using machine learning techniques

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The body condition of a dairy cow is one of the important indicators of the animal's welfare and health status. Maintaining optimal body condition in dairy cows is associated with more functional cows (healthy, fertile, etc.). Currently, the assessment of body condition in dairy cows is performed through manual scoring by trained classifiers, which is labor intensive and limits frequent application on farms. The use of computer-vision shows great potential as a high-throughput method for predicting the body condition score (BCS) of cows. However, despite its promise, no study has investigated the predictive ability of using 3D cameras to assess BCS in Jersey dairy cattle. Data from three commercial farms with 808 individual cows was obtained every second month from December 2021 to August 2022, with a total of 2,253 BCS observations. Body condition scores were scored by two trained classifiers from SEGES (Aarhus, Denmark). The feature data consisted of contours from top-down 3D images, generated when a cow leaves the milking area. The features represent the depth on specific points of the back. When a cow enters the image frame, the spine and circumference are identified, and a 3D cloud of the back is made within the circumference. The features used in this study, were the points on the back where there was a drop from the spine of 3, 5, 10, 15 cm each side. For each of these drops, 100 features were generated from the neck to the tail of the cow. Splitting the training and validation data was carried out as a random split of 7:3 clustered by cows and replicated 10 times. The clustering by cows ensured that cows could not appear in both the training and validation dataset. The H<sub>2</sub>O AutoML algorithm was used to find the best performing classification and regression model. Furthermore, AutoML was used to tune input parameters for the machine learning model. Among classification and regression models, DeepLearning performed best. Additionally, a Partial Least Square (PLS) model was tested with the Proc PLS procedure in SAS software. Validating the classification model, showed accuracies with a weighted mean of 48.1% (range: 45.9-50.7%) on the exact phenotypic class. The accuracy increased to a weighted mean of 93.5% (range: 92.7-95.3 %) by adjusting a 0.5-unit deviation. The results from the regression models showed R<sup>2</sup> and RMSE at 0.67 and 0.31 for PLS and 0.66 and 0.29 for DeepLearning. The validation accuracies were comparable to reports for Holstein cows in the literature. The results indicate that we can predict BCS in Jersey cows with a 3D camera-based system, which potentially could be used to improve management decisions in Jersey dairy herds.

### Abstract

*Keywords: Body condition, 3D-images, Jersey dairy cattle, machine learning.*

## Introduction

Body condition is a widely acknowledged and accepted indicator for dairy cows welfare (Welfare Quality@consortium, 2009). Maintaining good management of dairy cow's body condition is associated with more functional cows (healthy, fertile, etc.). The assessment of a dairy cow's body condition, is currently performed through manual scoring by trained classifiers through a body condition score (**BCS**) (Roche *et al.*, 2009). That is labor intensive and a frequent routine application on commercial farms is limited. Therefore, BCS at multiple times over the lactation is mostly recorded only in nucleus and research farms. From a dairy management perspective, frequent and precise BCS data on commercial dairy herds could improve animal welfare and functionality. In addition, genetic evaluation model for feed efficiency lacks a phenotype for BCS to distinguish between adipose and muscle tissue (Stephansen *et al.*, 2021a). Availability of accurate phenotypes for BCS could potentially improve modelling of feed efficiency especially in early lactation. Research in high-throughput methods to predict daily BCS in commercial farms has been applied with varying accuracy and level of automatization (Qiao *et al.*, 2021). Most studies have used 2D or 3D camera technology to develop machine learning (**ML**) algorithms to predict BCS in Holstein cows (Qiao *et al.*, 2021). However, despite its promise, no study has investigated the predictive ability of using 3D cameras to assess BCS in Jersey dairy cattle. The aim of the study was to establish a reliable prediction of body condition using 3D-images and ML techniques in Danish Jersey cows on commercial farms.

## Material and methods

### Body condition score recording

Three Danish commercial Jersey farms participated in the project, with an annual herd size of 150, 260 and 280 cows. The scoring was performed by two trained classifiers from SEGES (Skejby, Denmark, <https://www.seges.dk/>) every second month from December 2021 to August 2022. The classifiers took rotation to visit the project herds during this recording period and classified all cows in the herds. In total 2,253 BCS phenotypes were recorded on 808 Jersey cows. The cows were scored on a scale from 1 to 9 following ICAR (2022). As most studies (Qiao *et al.*, 2021) and farms use the 1 to 5 scale, the score were transformed to the 1 to 5 scale following Garnsworthy (2006):

$$\text{BCS} = 0.5 \times \text{score} + 0.5 \quad (1)$$

Basic information, such as calving date (December 2020 to August 2022) and lactation data (parity range 1-9, average parity 2.65; days in milk in the range 10-401, average days in milk 142.4 days), were extracted from the Danish Cattle database.

### Feature extraction and quality control

Feature data of the animals within  $\pm 3$  days from the day of BCS scoring were provided by VikingGenetics (Randers, Denmark). Detailed description of the hardware and software used in capturing and processing 3D-images into contour features can be found in Gebreyesus *et al.* (2023), Lassen and Borchersen (2022) and Lassen *et al.* (2023). Briefly, the hardware used was a 3D camera using time-of-flight technology (Microsoft Xbox One Kinect v2), placed in a narrow corridor through which cows leave the milking area. The feature data were generated as contours from top-down 3D camera-images and represent the depth on specific points of the back. The camera is triggered by an electronic identification of the animal. In this case an electronic ear tag. When a cow enters the image frame, the spine and circumference are identified, and a 3D cloud

on the back is made within the circumference. The features used in this study were the points on the back where there was a drop from the spine of 3, 5, 10, 15 cm each side. For each of these drops and the spine, 190 features were generated from the neck to the tail of the cow. In total 950 features across the four contours and spine.

Quality control was undertaken on the feature data using the SAS software version 9.4 (SAS Institute Inc, 2013), to remove outlier values. Features were set missing for values out of the range of mean  $\pm$  3SD. This was done twice by cow and date of evaluation. Hereafter features with a missing rate higher than 25% were discarded. Cows has on average 32.8 pictures (SD of 11.9) per round of classification. Animals with fewer than five pictures per classification round were removed. This resulted in a total of 700 features used as predictors for training the models. We calculated a mean feature per round of scoring to give the most stable prediction of BCS. A mean feature was calculated for all individual features by cow and classification date for the individual features and weighted by

$$\frac{1}{1 + |\text{date of feature} - \text{date of classification}|} \quad (2)$$

The weighting was used to put emphasis on features from the day of classification, assigning more weight to closer days apart between visual classification and image data capture.

Splitting training and validation datasets for model development is commonly done with a 7:3 random split of the data (Rodríguez Alvarez *et al.*, 2019, Yukun *et al.*, 2019). The 7:3 random split was performed using Proc Survey procedure in SAS version 9.4, and clustered by cow ID to ensure individual (cows) only appeared in either the training or validation dataset. Ten replicates of training and validation datasets were created for the model development. The two most extreme BCS classes (1.0 and 5.0) were grouped with the immediate next class due to very low observations (three in each) and to ensure adequate observations were available for the learning step.

#### Data split, learning algorithms and evaluation metrics

We used the *AutoML* algorithm from H<sub>2</sub>O package in R (LeDell *et al.*, 2022) for testing best-performing classification and regression algorithms. We used the first training dataset replicate in the *AutoML*, to test which ML algorithm performed best. The non-default parameters in *AutoML* were set to test maximum 2,000 models for classification or regression and had seed set to 1 and *nfolds* to 10. Common class predictors including classifier, parity number, round of classification and herd were considered across all the ML methods. Predictors were features from 3D-images, which were standardized to a mean of 0 and SD of 1, and Legendre polynomials fitted on weeks of lactation up to 5<sup>th</sup> order. Tuning parameters for the various classification and regression models in the *AutoML* algorithm were optimized based on cross-validation with “logloss” and mean squared error (**MSE**) as optimizing metrics for the classification and regression models, respectively.

The best performing algorithm for both classification and regression were DeepLearning (**DL**) which is a multi-layer feedforward artificial neural network algorithm in H<sub>2</sub>O. In addition, we tested a Partial Least Square (**PLS**) model, as it works well on correlated predictors (James *et al.*, 2013). The PLS model was tested in SAS with the Proc PLS procedure (SAS Institute Inc, 2013) fitting the same features and class variables as in the DL algorithm. The first training and validation dataset was used to fine-tune the PLS model and to define the optimum number of components. The tuning process of PLS showed 20 components were the optimum.

Output from the validation process of classification models were grouped into four individual classes based on confusion matrices between observed and predicted BCS: True Positives (**TP**), True Negative (**TN**), False Positives (**FP**) and False Negatives (**FN**).

Accuracy of classification (**AOC**) defined as (Rodríguez Alvarez *et al.*, 2019):

$$AOC, \% = \frac{(TP + TN)}{(TP + FP + TN + FN)} \times 100 \quad (3)$$

F1-score is a measure that combined the trade-offs of precision and recall and defined as:

$$F1, \% = \left( 2 \times \frac{TP \times FP}{TP + FP} \right) \times 100 \quad (4)$$

Accuracy of classification and F1-score were evaluated for their ability to predict on the exact phenotype and with a 0.50-unit deviation (**DEV**) to account for the human error judgement. For regression models, R-square (**R<sup>2</sup>**) and Root Mean Squared Error (**RMSE**) were used and estimated with the Proc ANOVA procedure in SAS. Another evaluation parameter for the regression methods, was to evaluate the percentage of predicted BCS phenotypes that were equal to the observed phenotype and on the exact phenotype and with a 0.5-unit DEV. This was implemented by rounding the predicted BCS phenotype from a regression model to the nearest 0.5-unit. The percentage of correctly assigned phenotypes were then reported for each class of observed BCS, but also a weighted average based on frequency was reported.

## Results and discussion

The AOC of DL models were 48.1% for the exact phenotype (range 45.9 to 50.7%). With a 0.5-unit DEV the AOC of DL models increased to 93.5 (range 92.7 to 95.3%). Rodríguez Alvarez *et al.* (2019) estimated a lower AOC on the exact phenotype 41.2%, compared to this study. However, they found a higher AOC of 97.4% with a 0.5-unit DEV. Rodríguez Alvarez *et al.* (2019) developed an ensemble model from Convolutional Neural Network (**CNN**) models, trained on 1,661 Holstein cows in Argentina. A study on 512 Chinese Holstein cows by Shi *et al.* (2023) reported an AOC of 49% on the exact phenotype and 96% with a 0.5-unit DEV. Both studies of Rodríguez Alvarez *et al.* (2019) and Shi *et al.* (2023) used complex CNN models, which have high computational requirements, compared to simpler models (regression).

For the F1-score, a tradeoff metric between precision and recall, we found a weighted average of 46% on the exact phenotype (Table 1). A lower level was reported by Rodríguez Alvarez *et al.* (2019) at 38% for the best CNN model. Shi *et al.* (2023) estimated an average F1-score for the exact phenotype at 44%. With a 0.5-unit DEV in this study, the weighted average of the F1-score increased to 91%. That was lower than the 97% in Rodríguez Alvarez *et al.* (2019) and 95% in Shi *et al.* (2023).

The approximated AOC from regression models (Table 2), showed that the choice among the PLS and DL algorithms in this study were limited. On the exact phenotype both regression models performed better on AOC (51.2-52.0%) than the DL classification model (48.1%), but also higher than Rodríguez Alvarez *et al.* (2019) and Shi *et al.* (2023). Allowing a 0.5-unit DEV increased the weighted average of AOC to 95.5 and 96.1% for

**Table 1. Validation results for sensitivity, precision, and F1-score in percentage for DL, using. The parenthesis represents the range among replicates. DL = DeepLearning, BCS = Body Condition Score, Exact = exact score, DEV = deviation, WAvg = weighted average by frequency.**

BCS	F1-Score	
	Exact	0.5-unit DEV
1.5	3 (0-14)	39 (0-100)
2.0	59 (51-63)	98 (97-99)
2.5	55 (52-57)	96 (95-97)
3.0	36 (27-43)	94 (93-97)
3.5	42 (32-48)	85 (80-92)
4.0	9 (0-34)	81 (73-91)
4.5	4 (0-25)	13 (0-57)
WAvg	46 (44-49)	91 (89-94)

**Table 2. Validation results from regression models. The parenthesis represents the range among replicates. PLS = Partial Least Square, DL = DeepLearning, BCS = Body Condition Score, R<sup>2</sup> = R-square, RMSE = Root Mean Square Error, Exact = exact score, DEV = deviation, WAvg = weighted average by frequency.**

BCS	PLS		DL	
	Exact	0.5-unit DEV	Exact	0.5-unit DEV
1.5	33 (19-52)	91 (80-100)	16 (6-29)	89 (72-100)
2.0	49 (46-52)	97 (94-99)	50 (45-54)	97 (95-99)
2.5	60 (56-69)	98 (96-99)	67 (61-73)	98 (97-99)
3.0	55 (51-58)	98 (97-100)	52 (44-60)	98 (97-99)
3.5	45 (36-51)	94 (91-99)	41 (34-48)	91 (86-98)
4.0	23 (10-35)	86 (80-96)	23 (7-29)	78 (71-83)
4.5	9 (0-20)	65 (42-78)	9 (0-20)	64 (40-78)
WAvg	51.2	96.1	52.0	95.5
R <sup>2</sup>	0.67 (0.65-0.68)		0.66 (0.64-0.68)	
RMSE	0.31 (0.29-0.33)		0.29 (0.26-0.32)	

DL and PLS respectively. This was higher than the DL classification model (Table 1) and similar level as reported in Rodríguez Alvarez *et al.* (2019) and Shi *et al.* (2023).

The aim was to build a reliable prediction algorithm of BCS using 3D-images and ML techniques in Danish Jersey cows on commercial farms. Among classification and regression models, DL performed best. Additionally, a PLS model was tested. Validating the classification model, showed an accuracy of 48.1% (range: 45.9-50.7%) on the exact phenotype. The accuracy increased to 93.5% (range: 92.7-95.3 %) with a 0.5-unit DEV. The results from the regression models showed R<sup>2</sup> and RMSE at 0.67 and 0.31 for PLS and 0.66 and 0.29 for DL. The approximated AOC for regression models showed for PLS 51.2 and 96.1% and for DL 52.0 and 95.5% on the exact and 0.5-unit DEV, respectively. The results indicate that we can predict BCS in Jersey cows with contour features from a 3D camera-based system in ML models. This can potentially improve management decisions on Jersey dairy herds.

## Conclusions

## Acknowledgements

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## How to optimize feed efficiency by automatic data exchange

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Feeding the dairy cow the right and optimal way is an increasing challenge.

- There is an increasing focus on the overflow on especially nitrogen and phosphorus to the environment.
- The climate challenge in general is setting requirement for decrease the Carbon Footprint
- The genetic improvement increases the requirement for the composition of the nutrients in the diet.

To face these challenges the dairy farmer needs to monitor both the input- and the output side very closely and of course use newest knowledge about cattle nutrition. The input side consist of monitoring the amount of each feedstuff and the quality including content of nutrients. The output side is the performance of the cow meaning the milk production and gain/loss in weight.

In Denmark *SEGES Innovation* has been working on these issues for years together with relevant stakeholders like feed equipment manufacturers, dairies, milk recording and laboratories. For a start we used manual generated data, both on the input and the output side. We have together with other Nordic Countries developed a dynamic feed evaluation system, NorFor, which is used for both optimizing the ration and for evaluating the performed feeding. The challenge has been that it is labor costly to do manually and that the manual monitoring will be a snapshot on the specific day, and not the whole picture with data from each day. The overcome the challenge, there has been focus on getting the data automatic recorded by the feeding equipment and get data automatic transferred.

Today the full data chain is operational. Data from the storage, the laboratory, the feeding equipment, the dairy are transferred automatic to the Central Cattle database in Denmark. In the management system data are used for automatic daily efficiency calculation including parameters on nutrients and different relevant key figures. The farmers and the advisor use the output via different kind of reports to evaluate the feeding for the best of the cow, the production, the climate, and the economy.

*Keywords: Feed efficiency, data exchange, data transfer, innovation, standardisation, automatization.*

### Abstract

## Introduction

Producing milk on a dairy farm is a complex task. You need to control and manage a lot of different factors e.g. genetics, reproduction, health management and feeding. Regarding the feeding you need to optimize the input on order to optimize the output. The output side will mainly be the milk including the content parameters defining the value of the milk. On the input side the farmers need to optimize the feed to the need of the cow and the bacteria in the rumen. This is all very detailed described in different model for feed evaluation and feed optimization, which the farmers and nutritionist advisors are working with. The benefit has for a long time been economy and over the last 5-10 years we see some others values to increase the focus on the optimized feeding of the dairy cow f. ex. decrease of nitrogen and phosphor to the environment and latest reduce the methane emission from the dairy cow. All in all, it just gives even more incentive to look for more improvement in the feed management.

## Optimize the feed management

### *The feed management consist of the following steps*

Optimize the feed ration for the cow or the group of cows to be fed.

- Feed the ration including the right amount.
- Follow up on the feeding be measuring the fed amount of feedstuff and the correctness of the mixture.
- Calculate the feed efficiency and the different nutrient parameters to see how the fit with the plan.
- Adjust next day's ration according to the calculated result.

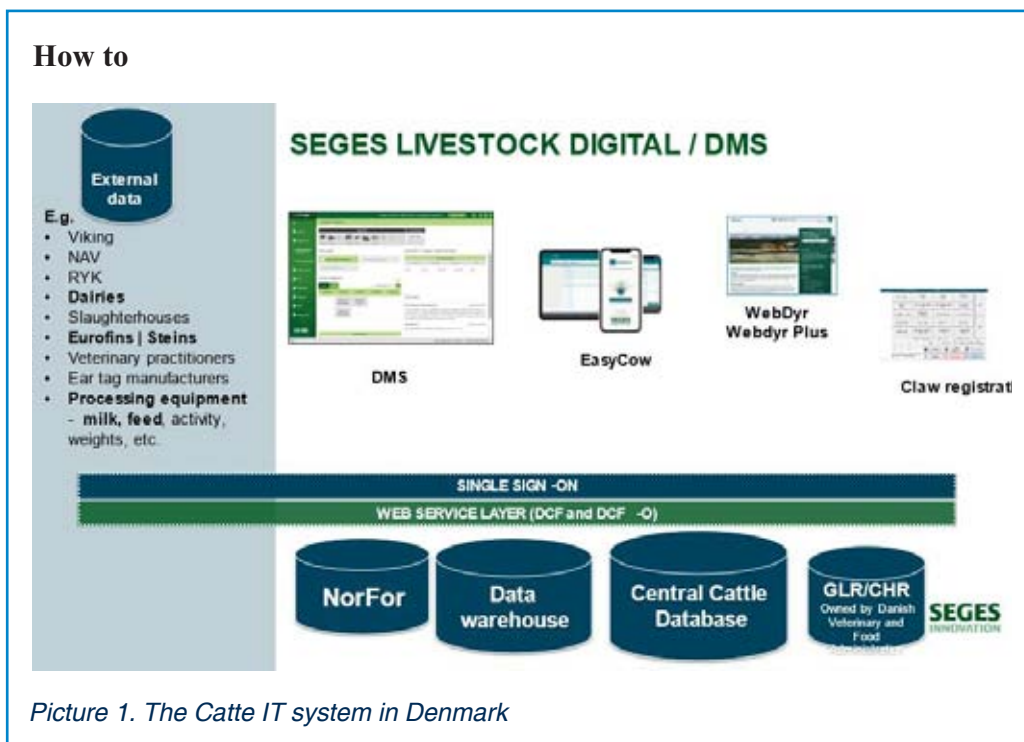
At SEGES Innovation we have been working with the whole feeding management for decades and our experiences are that is one our more points are not done sufficient the feed management will not be optimal with the consequence that the farmer lose money the animal do not function optimal, the loos of nutrient to environment is increase og the climate impact are challenged. The reason for this is not lack of will og knowledge, but lack of automated ways the track the management.

To overcome the described challenge SEGES Innovation in several project has been working on how to digitalize the data flow in or to automate all steps in the feeding management.

The overall picture of the Cattle systems in Denmark are described in picture, where the main parts are:

- The central Cattle database, which hold data – both mandatory movement and medicine data on all cattle in Denmark
- The Data WareHouse (DWH) database, which hold key figures on a lot of production parameters.
- The NorFor IT system, which manage the NorFor biologic system and by this provides the feed evaluation, feedstuff parameters and feed evaluation.
- DMS, Dairy Management system, the management system used by 97% of the Dairy farmers most advisors and veterinarians in Denmark. DMS is used for daily management, feeding, performance check, planning, budgeting etc.

- Connection to external databases like
  - Milk recording
  - Breeding
  - Veterinarians,
  - Laboratories
  - Slaughterhouses
  - On farm equipment like milking, feeding and activity equipment.



In the following we will describe how we have solved the challenges

Data on the cow and the herd are together with data on the feed including automatic transferred feed analysis are send to the NorFor system. The NorFor system returns with and optimized ration. The user might edit the ration to optimize for specific local conditions on the farm and/or in the herd.

*Optimize the feed ration for the cow or the group of cows to be fed*

**Feed the ration including the right amount**

The ration is converted into a recipe for the individual group. The recipe is sent to the feeding equipment, managed by different systems. The 4 most common in Denmark do have this connection.

**Follow up on the feeding by measuring the fed amount of feedstuff and the correctness of the mixture.**

The management system on the feeding equipment returns with the mixed amount including data on what has been loaded in the mixer wagon and how much of the mixture has been fed to specific groups. Data are automatic exchange once per day.

**Calculate the feed efficiency and the different nutrient parameters to see how the fit with the plan.**

Data from the feeding are now together with automatic data on the milk from the Dairy and relevant data from the Cattle database send to the NorFor system and a daily efficient report on the feeding is generated,

The parameters and key figures from the feed evaluation are stored in the DWH to be used in different reports on time series of data – see examples in figure 2 and 3.

**Adjust next day's ration according to the calculated result.**

Based on the results from the reports and which parameters are not optimal, the farmer and/or the advisor can adjust the ration

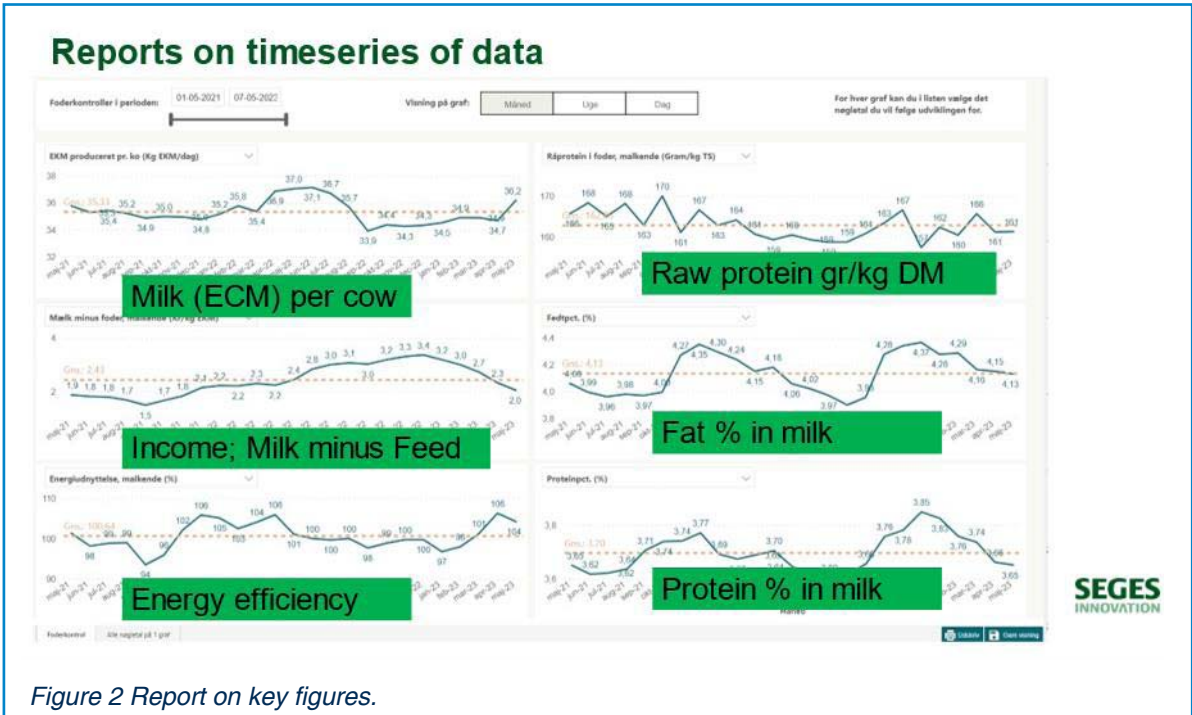


Figure 2 Report on key figures.

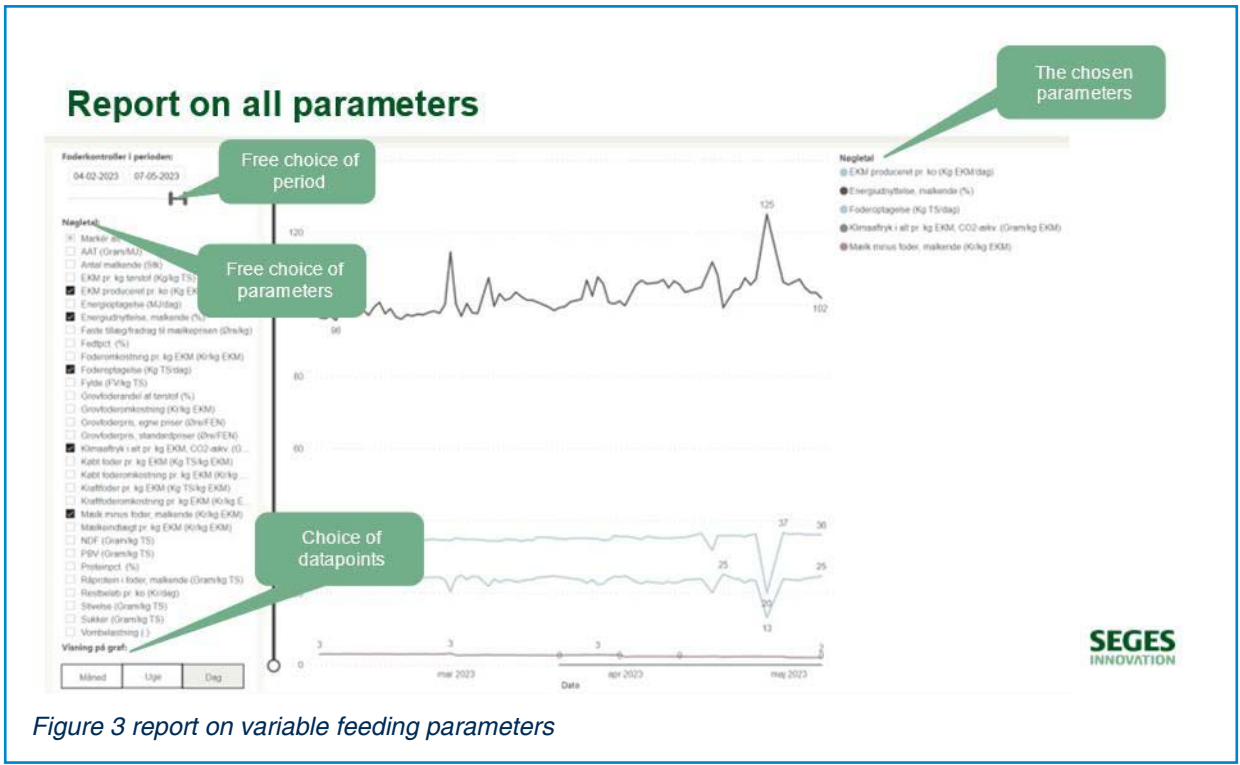


Figure 3 report on variable feeding parameters

- Data connections have been automated.
- Newest knowledge within nutrition have been implemented in the "Data machine"
- Newest technology to present status, benchmark, and development in production.
- Automated data
- Provides the tools for
  - Optimizing the nutrition
  - Optimizing the economy
  - Minimizing the waste of nutrients to the environment
  - Documentation and optimization for Carbon Footprint
- Used by 25% of the herds / 40% of the cows in full scale
- Still a lot of improvement potential
  - More users
  - More details on group/animal level
  - Calibration of equipment
  - Use to days output for regulations in tomorrow's input
  - Improve data generation and atomization in the whole feed chain.

**Summery on how to optimize the feed management**





## System and biological effects on quantitative milking speed phenotypes from inline milk meters

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Milking speed (MS) is actively used by herds with both conventional and automatic milking systems (AMS) in the USA. A genetic evaluation for MS could be of significant economic value, and dairy producers surveyed express enthusiasm for the development of this new trait. The classification system successfully implemented in other countries that evaluate MS is unlikely to be practical in the USA due to larger average herd sizes, and so the use of quantitative measurements of milking speed is being explored. Many farms now have in-line milk meters that can supply the information required to calculate MS, but there is not a strong consensus on the milking system effects and other biological influences on quantitative milking speed phenotypes. A large dataset was assembled comprising ~300 U.S. herds, >230,000 cows, >300,000 lactations, and >40 million observations of individual milkings from January 2022 to February 2023, and representing 6 dairy breeds, 11 different meter manufacturers, and 2X, 3X, and AMS herds. Milking speed was defined as lbs per minute and calculated for every milking in a day for each individual cow. Data quality control involved only using records with durations between 1 and 15 minutes, weights between 1 and 60 lbs, speeds between 1 and 15 lbs per minute, and cows with at least 10 observations. Milking speed varied by breed, lactation number, and milking frequency. Among 2X and 3X herds, MS mirrored the milking curve over the course of a lactation for Holstein and Jersey, which was to be expected given the favorable correlations between MS and milk yield observed in the literature and this dataset ( $R^2 = 0.4-0.6$ ). Trends were less clear for Ayrshire, Brown Swiss, Guernsey, and Milking Shorthorn due to the sparsity of data available for those breeds. The highest variation in MS was observed during early and late lactation, suggesting MS for genetic selection should be measured during a certain window of DIM only. Among Holstein, the speed of those milked by AMS also mirrored the milk production curve, but with substantial differences observed between meter manufacturers. This is likely an artifact of how the data is collected by each manufacturer, such as differing definitions of when milk flow begins, and the total duration of a milking (box time, or amount of time the milking unit is attached), suggesting that meter manufacturer is a major effect that will need to be accounted for in the harmonization of data collected from different systems. The work to characterize other system and biological effects like udder health parameters and milking interval is

### Abstract

ongoing, and will be integral to our efforts to standardize quantitative MS phenotypes and determine their suitability for selection.

*Keywords: milking speed, system effects, biological effects, dairy cow, quantitative phenotype.*

## Introduction

Dairy producers actively use milking speed (**MS**) metrics to guide their management and make economic decisions. While the USA does not yet have evaluations for MS, at least 18 other countries regularly supply this information to their producers. The Milking Speed Evaluations Task Force was appointed in October 2021 by the Council on Dairy Cattle Breeding (**CDCB**) to review the possibility of implementing genetic evaluations for MS in all dairy breeds and to make recommendations to the CDCB Board of Directors on the necessary steps to make this happen. Interbull-participating countries with evaluations for milking speed collect nearly all phenotypes during the first lactation and sometimes from a single classification. In the rare instance that quantitative milk flow rates are available the classifications are discarded, but the availability of these data varies by country and breed. A classification system is unlikely to be practical in the USA with larger average herd sizes and the task force agrees that eliminating the human factor is ideal for both reducing labor costs and the potential biases introduced with subjective scoring. Genetic correlations for MS across participating countries are calculated routinely as part of the Multiple Across Country Evaluation (**MACE**) report for “Workability” traits, and they are quite high for all breeds. This is encouraging because if this much uniformity can be achieved using subjective scores, attempts to integrate and use quantitative data are likely to be successful.

The long-term goal of this work is to provide accurate, low-cost genomic evaluations for MS that can be predicted at birth. Following analysis of preliminary data, the task force concludes that considerable research is required to develop a clear phenotype definition and identify the relevant data types and quality control/assurance measures required to standardize and integrate these data into the existing national evaluation system. Many OEM meters now provide the type of data needed to calculate milking speed but even these quantitative measurements are subject to confounding bias. In addition to the genetic analysis of MS, phenotypic studies are required to characterize any system effects (automatic take-off, variable pulsation ratios, time in parlor, incomplete udder evacuations, automatic animal ID detection and validation) and biological effects (stage in lactation, breed, parity, herd effects, cow effects like yields and SCS, etc.). No dataset like this exists, and there is a critical need to describe the trait and any environmental and biological effects that should be included in evaluation models before a detailed recommendation can be made and we can proceed with implementation. These concerns will be addressed by the following specific objectives:

- **Objective 1:** Assemble a high-resolution dataset pertinent to MS representing different dairy breeds, equipment manufacturers, parlor types, and milking management strategies
- **Objective. 2:** Characterize MS for herds grouped by equipment manufacturer and parlor type and assess the impact of additional system effects on the phenotype
- **Objective. 3:** Characterize any biological effects that impact MS, especially concerning udder health
- **Objective. 4:** Standardize MS trait definition and estimate heritability to determine its suitability for selection

Dairy Records Management Systems (DRMS) is a dairy records provider in the USA that supplies herd management software and other services to producers. Every 30 days, DRMS extracts raw milking parlor data from 304 herds. These data comprise milk weights, milking times, breed, parity, meter manufacturer (OEM). This dataset dates to January 2022 and is constantly growing with the addition of new data. As of February 2023, it contained > 40 million observations of individual milkings, representing > 300,000 lactations and > 230,000 cows from 31 different states, 6+ breeds, and 11 OEMs. Data cleaning measures included removing duplicates, restricting raw records to dates from 1 January 2022 to 1 January 2023, requiring a milking duration of greater than zero or less than 15 minutes, a milk weight of greater than 0 and less than 60 lbs, a milking speed of greater than 1 and less than 15 lbs/min, d in milk (DIM) greater than zero, and only including cows with at least 10 observations in each lactation. After data cleaning, the dataset comprised > 22 million records and > 165,000 cows.

## Materials and methods

Data were stratified by breed, milking frequency, and lactation number, and milking speed (lbs/min) calculated for each stratum (shown for conventional non-robot herds in Figure 1). Each data point represents the mean milking speed for that breed-lactation number. Holstein and Jersey milking speed trend similarly, with older animals tending to milk a little slower (it also should be noted that significantly fewer animals are represented in higher lactation numbers). Jerseys milked 3X per day milk significantly faster than 2X. The trends are less clear for the other breeds, but there is far less data available for them (AY = 165 cows, BS = 749, GU = 82, HO = 138,373, JE = 3,873, MS = 51). Primiparous cows do seem to milk slower than 2<sup>nd</sup> and 3<sup>rd</sup> parity cows; this could reflect selection bias with hard milkers being removed from the herd or biological phenomena like the teat sphincter relaxing with age.

## Results

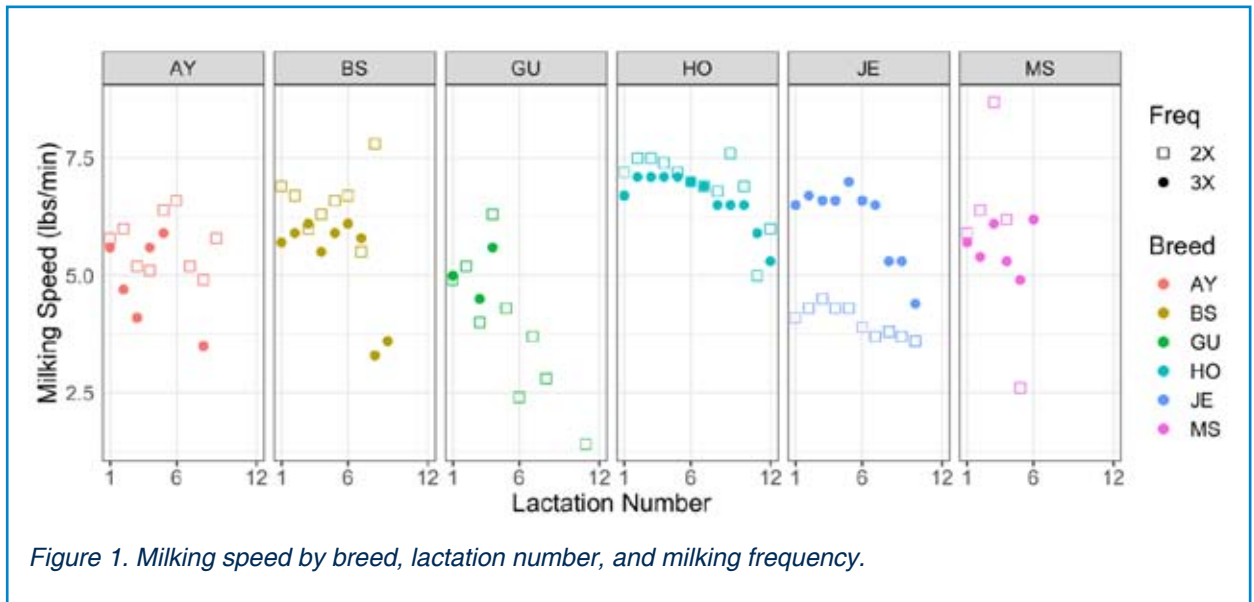


Figure 1. Milking speed by breed, lactation number, and milking frequency.

Milking speed trends were also examined by plotting across days in milk (DIM) to explore any effects of lactation stage (Figure 2). Both Holstein and Jersey milking speed mirror the milk production curve, which is to be expected given the moderately high correlations between MS and milk yield observed both in the literature and in this dataset ( $R^2 = 0.55-0.7$ ). Trends are less clear for other breeds due to data availability.

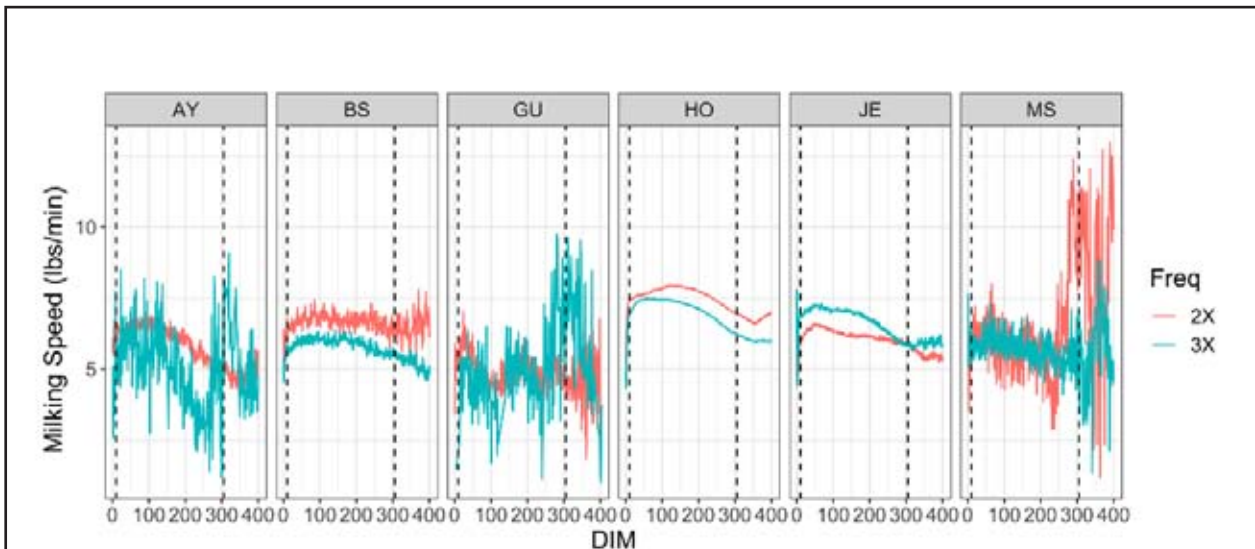


Figure 2. Milking speed by breed, DIM, and milking frequency.

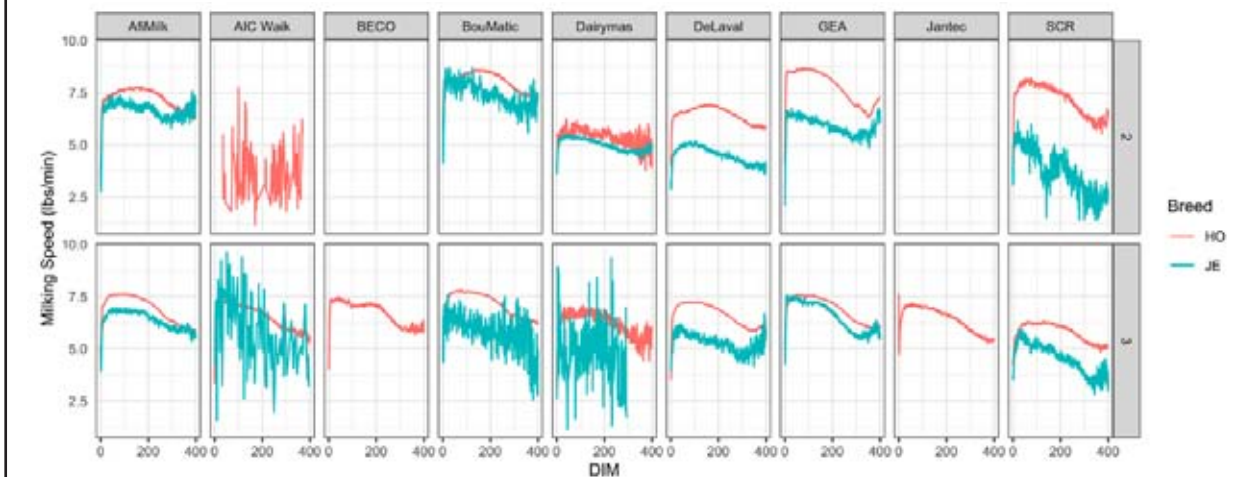


Figure 3. Milking speed, DIM, OEM, and milking frequency (2X, 3X) for Holstein and Jersey. Blank grids indicate that no data was available (e.g., no 2X herds in this dataset used a BECO system).

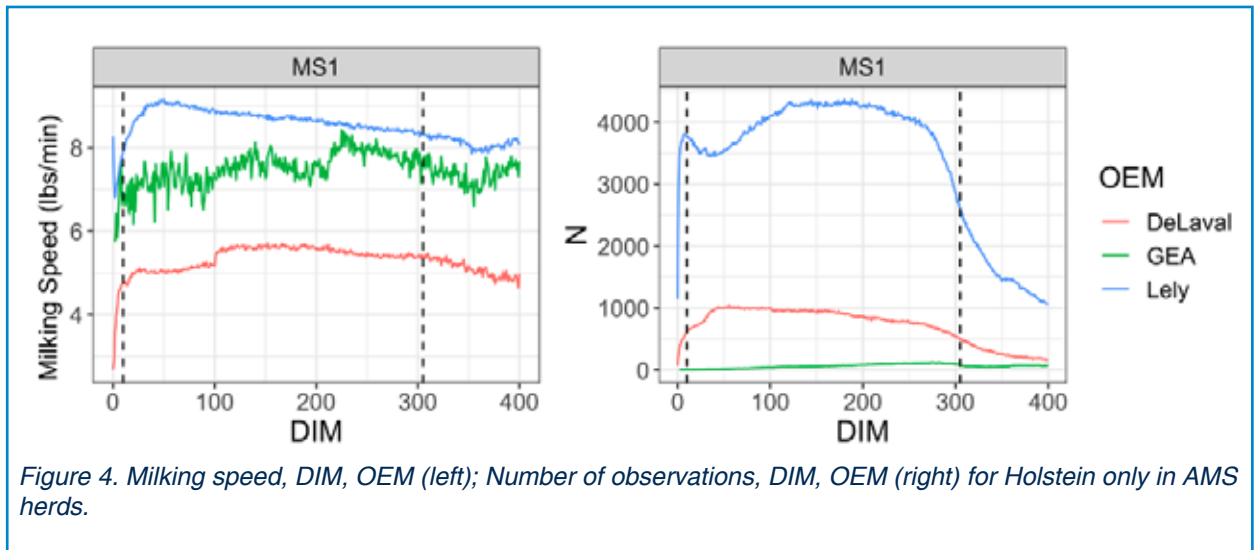


Figure 4. Milking speed, DIM, OEM (left); Number of observations, DIM, OEM (right) for Holstein only in AMS herds.

Trends in milking speed were also examined by OEM for Holstein and Jersey (Figure 3). A clear OEM effect is observed in that speeds vary by meter manufacturer. Smoothness of the curves reflect the amount of data available for each stratum. This pattern can also be observed very clearly by examining the differences in AMS herds (Figure 4). For example, DeLaval and Lely have parallel trends in milking speed, mirroring the milk production curve, but very different speeds overall. This does NOT suggest that cows will milk slower on a DeLaval system! It is an artifact of how the data is collected by each OEM. Every OEM will have their own criteria for when milk flow actually begins and how milking duration is measured (e.g., box time versus milking time). This simply demonstrates that there is a clear OEM effect that will need to be considered in the harmonizing of data collected from different systems.

Milking speed appears to be higher for the first milking of the day across breeds and milking frequencies, as shown in Table 1.

Table 1. Milking speed by milking number, milking frequency, and breed. Milking 1 = first of a 24 hr period, 2 = 2<sup>nd</sup> of a 24 hr period, 3 = 3<sup>rd</sup> of a 24 hr period.

Milking	AY		BS		GU		HO		JE		MS	
	2X	3X	2X	3X	2X	3X	2X	3X	2X	3X	2X	3X
1	5.9	5.2	6.7	5.8	4.9	5.1	7.5	7.0	6.1	6.7	6.2	5.7
2	5.6	5.2	6.4	5.8	4.6	5.1	7.3	7.0	5.9	6.6	6.1	5.6
3	--	5.0	--	5.7	--	4.6	--	6.9	--	6.5	--	5.4

An analysis of milking interval suggests that it has overall little effect on milking speed (Table 2). Milking speed was correlated with the interval prior to that milking event. Because cows in AMS herds have free choice of the robot, they may be milked up to 6X per day. To account for the multiple possible combinations of milking interval, intervals were calculated between the 2nd, 3rd, 4th, 5th, and 6th milkings of the prior day and the 1st milking of the subsequent day.

Table 2. Milking interval and milking speed correlations. MI = Milking Interval, MS = Milking speed.

	HO			JE		
	2X	3X	AMS	2X	3X	AMS
MI21:MS1 <sup>a</sup>	0.01	--	--	0.07	--	--
MI31:MS1	--	-0.03	0.08	--	0.01	0.05
MI41:MS1	--	--	0.06	--	--	0.03
MI51:MS1	--	--	0.05	--	--	0.02
MI61:MS1	--	--	0.01	--	--	0.07
MI12:MS2	0.05	0.04	0.12	0.07	0.17	0.18
MI23:MS3	--	0.04	0.09	--	-0.05	0.10
MI34:MS4	--	--	0.05	--	--	0.05
MI45:MS5	--	--	0.03	--	--	0.00
MI56:MS6	--	--	0.01	--	--	0.03

<sup>a</sup>Notation indicates interval between prior milking and the milking corresponding to MS

## Conclusions

Next steps include higher level modelling to better account for the effects of multiple variables, investigation of the highly variable milking intervals and frequencies for AMS herds, and relationship to udder health parameters. These data will also be used to calculate PTAs for various milking speed phenotypes, including the fixed effects identified as important in this paper.

## Comparing peak of lactation from Automatic Milking Systems and Conventional Milking Systems

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Automatic milking systems (AMS) allows for variable milking frequency for individual cows within a herd, which is not the case for most other milking systems. A more frequent milking is desired at the beginning of the lactation to stimulate the mammary gland to produce more milk during the peak of lactation. The objective of this study was to evaluate the effect of more frequent milkings, as in the AMS, on milk yield at lactation peak. Lactation records were obtained from the Lactanet (Canadian Network for Dairy Excellence) database. A total of 7,706,954 records from herds with AMS and conventional milking systems (i.e., 2 milkings per day) during 4 years (2017-2021) were used for the analysis. Data were grouped by milking system and by parity (primiparous and multiparous). For the analysis the mean of milk production was calculated by 10 days in milk interval (e.g., 10, 20, 30...etc.) and the effect of the milking system was evaluated by a regression analysis. Results showed that at the beginning of the lactation (i.e., 10 days in milk) cows milked in AMS had a significantly lower milk production (2.2 kg/day;  $P < 0.001$ ) than the cows in conventional milking systems. However, when these cows attained the peak of lactation (50 days in milk) they reach the same milk production than those milked in conventional milking systems. During the first 40 days in milk (from 10 to 50 days in milk) cows milked in AMS increased milk production by  $12.06 \pm 0.6$  kg, whereas the increase in the same period was of  $7.7 \pm 0.58$  for cows milked in conventional milking systems. This indicates that for these 40 days period cows in AMS produced an extra of  $4.36 \pm 0.59$  kg of milk ( $P < 0.001$ ) compared with the cows in conventional milking systems. Furthermore, the increase during this period was found for both primiparous and multiparous cows. These findings highlight the importance of monitoring the peaks of lactation in AMS, to adjust the available energy of the diets at the beginning of the lactation to ensure a high milk production by reducing the negative risks in health and reproduction, as it has been reported that the incidence of ketosis is 1.45 times greater in AMS compared with other milking systems.

### Abstract

*Keywords: lactation curves, automatic milking systems, parity*

Automatic milking systems (AMS) allows for variable milking frequency for individual cows and can be adjusted for production level and stage of lactation (Svennersten-Sjaunja and Pettersson, 2008). Research on the effect of milking frequency on milk production is divided. Whereas some studies have reported an increase of milk production with more frequent milkings (Wagner-Storch and Palmer, 2003; Melin *et al.*, 2005) others have reported no effect on milk production (Speroni *et al.*, 2006; Gygas *et al.*, 2007). Furthermore, achieving an optimal milking interval could maximize milk production and minimize any risk of negative effects on udder health (André *et al.*, 2010). With AMS, it is possible to fine-tune milking frequency and number of milkings. It is desirable to have more frequent milkings in the beginning of lactation to stimulate

### Introduction

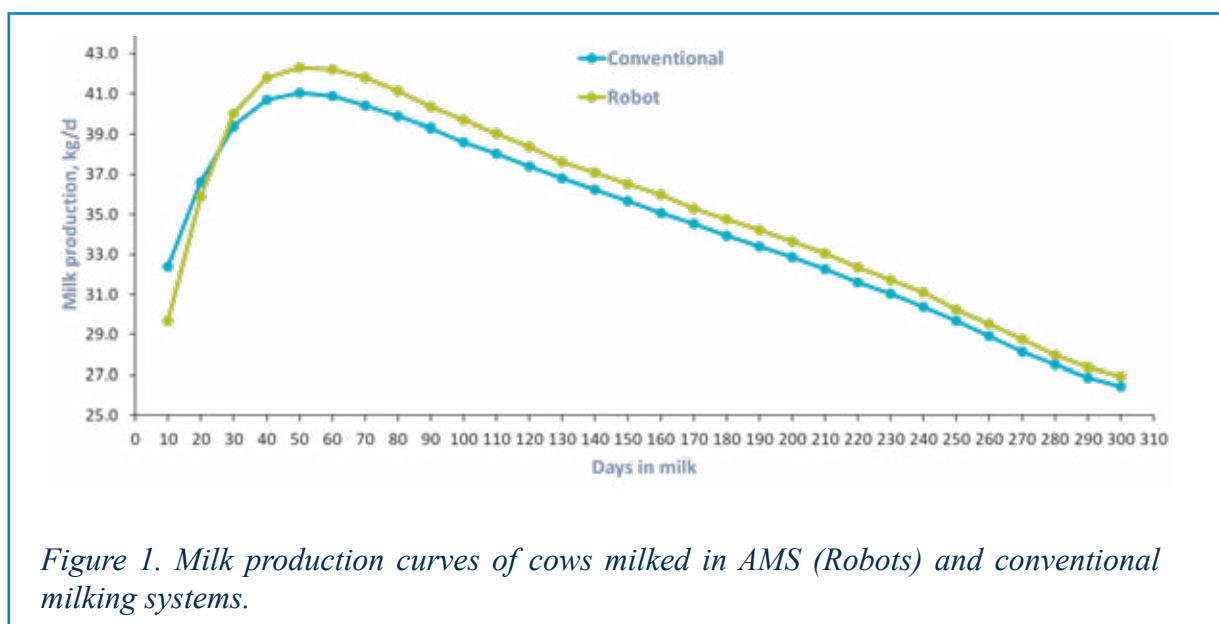
the mammary gland to produce more milk during peak lactation, whereas a decreased number and frequency of milkings is more desired towards late lactation. However, the effect of these variations of the number and frequency of milkings on the peak of lactation is unknown. Therefore, the objective of this study was to evaluate the effect of more frequent milkings, as in the AMS, on milk yield at lactation peak.

## Material and methods

Lactation records were obtained from the Lactanet (Canadian Network for Dairy Excellence) database. A total of 7,706,954 records from herds with AMS and conventional milking systems (i.e., 2 milkings per day) over 4 years (2017-2021) were used for the analysis. There were 516 herds with AMS and 1,766 herds with conventional milking systems. Data was grouped by milking system and by parity (primiparous and multiparous). For the analysis the mean of milk production was calculated by 10 days in milk interval (e.g., 10, 20, 30...etc.) and the effect of the milking system and parity was evaluated by a regression analysis.

## Results and discussion

At the beginning of the lactation (i.e., 10 days in milk) cows milked in AMS had a significant lower milk production than cows milked in conventional milking systems (29.8 vs 32 kg/day;  $P < 0.001$ , respectively). However, when these cows attained the peak of lactation, at around 50 days in milk, they reach the same milk production than those milked in conventional milking systems (Figure 1). During the first 40 days in milk (from 10 to 50 days in milk) cows milked in AMS increased milk production by  $12.06 \pm 0.6$  kg, whereas the increase in the same period was of  $7.7 \pm 0.58$  for cows milked in conventional milking systems. This increase in milk production resulted on an extra  $4.36 \pm 0.59$  kg of milk ( $P < 0.001$ ). Results also showed that the increase in milk production was for both primiparous and multiparous cows, but there were no significant differences among parities ( $P = 0.64$ ). Yet, the difference between the milking systems was significant ( $P < 0.001$ ).



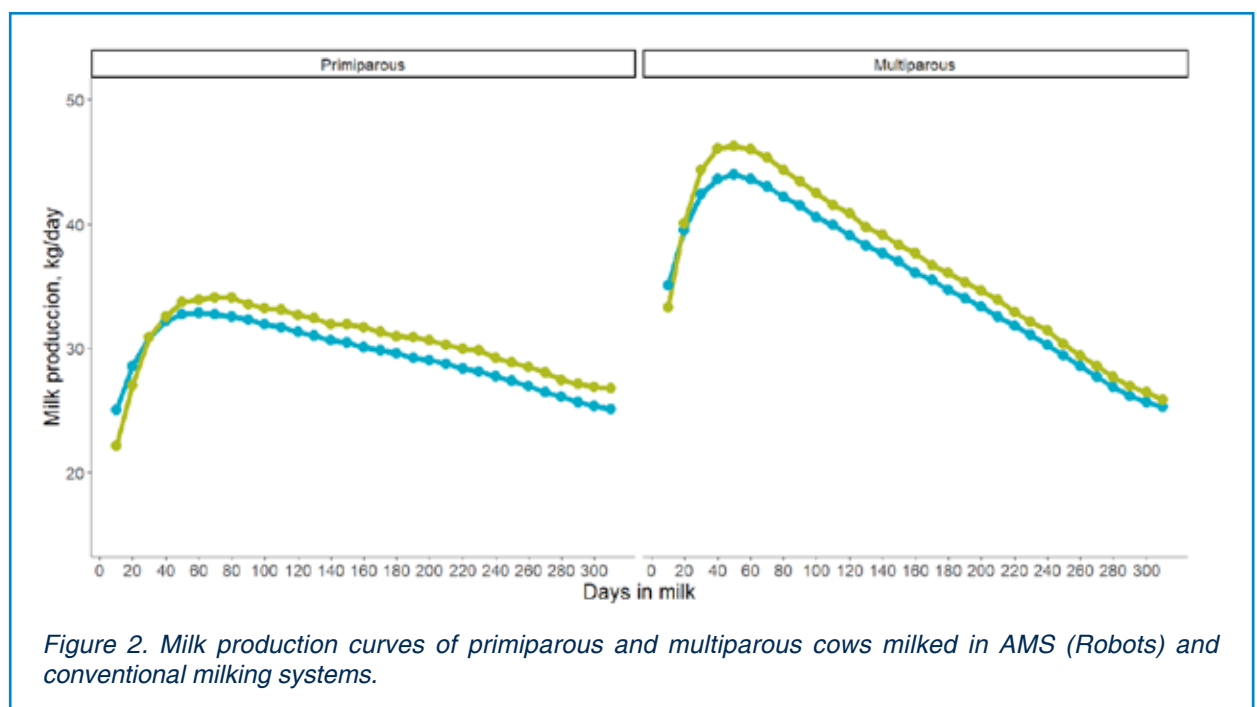


In addition, the daily increase in milk production on the first 60 days in milk for primiparous and multiparous cows milked in AMS was faster than for cows milked in conventional milking systems (55% and 46%, respectively; Figure 2). Primiparous cows milked in AMS had a daily increase in milk production of 0.20 kg milk/day whereas for cows milked in conventional milking systems the daily increase in milk production was 0.13 kg milk/day. For multiparous cows, the daily increase was 0.22 kg milk/day and 0.15 kg milk/day for cows milked in AMS and conventional system, respectively.

These findings highlight the importance of monitoring the peaks of lactation in AMS to provide adequate nutrients to ensure high milk production and reduce negative risks in health and reproduction, as studies have reported that the incidence of ketosis is 1.45 times greater in AMS compared with other milking systems (Tatone *et al.*, 2017). High concentrations of beta-hydroxybutyric acid (BHB) at the beginning of lactation have negative effects on production, reproduction, and health (Ospina *et al.*, 2010; Chapinal *et al.*, 2012; Santschi *et al.*, 2016). These studies reported that cows with elevated BHB (0.20 mmol/l) on the first test date had: an extra 24 days open; a reduction of 2.4 kg of milk on test date; are 27% less likely to be pregnant at 150 days in milk; are 2 times more likely to be culled by 100 days in milk, and are at increased risk of displaced abomasum and clinical ketosis, among others (Ospina *et al.*, 2010; Chapinal *et al.*, 2012; Santschi *et al.*, 2016). Lastly, there are economical losses not only related to the decline in performance but also related to the diagnosis, treatment and in the worst case the dead loss (McArt *et al.*, 2015).

Cows milked in AMS produce less milk at the beginning of the lactation, but then they exceed the production of cows in conventional milking system (2x). This rapid increase in milk production in AMS highlights the importance of monitoring the peaks of lactation in AMS, to adjust the available energy of the diets at the beginning of the lactation, to ensure a high milk production while reducing negative risks in health and reproduction.

## Implications



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## French experience of using ICAR approved method for predicting 24-hour fat percentage and yield from one sampled milking in Automatic Milking Systems

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The use of Automatic Milking Systems is increasing in France, from about 1,550 herds with official records in 2010 to about 3,350 in 2022. The Dairy Cattle Milk Recording Guidelines allows 2 types of robot protocols approved by ICAR, one with at least two milkings per recording test day sampled for components and, to meet the simplification and cost-saving needs of farmers, another with only one milking sampled per recording test day. Regarding the latter, which is used in France since 2017 (Minéry *et al.*, 2018), 24-hour fat percentage and yield are estimated with the ICAR Peeters and Galesloot method (Peeters and Galesloot, 2002).

The aim of this study was to try to improve the prediction of the 24-hour fat percentage and yield by using more complex models described by Peeters and Galesloot. Therefore, we compared the accuracy ( $r^2$ , prediction error and standard deviation of prediction error) of the predictions at a recording test day level for the multiple regression model currently used and for 6 other models also considering the effect of class variables, such as milking interval and fat to protein ratio.

The estimation of regression coefficients and the validation studies were performed on independent updated data sets (with at least two milkings sampled by cow), using a total of 620,272 milkings for 125,905 cows spread over 1,277 French farms from 2017 to 2019.

The results confirmed the relevance of the model currently used but highlighted a possible improvement. Indeed, adding the effect of class variables to the prediction model slightly improved the correlation between the 24-hour reference and the 24-hour prediction for fat percentage and yield, from 0.776 to 0.786 and from 0.910 to 0.913 for fat percentage and fat yield respectively, for the model giving the best results. There was no effect on the prediction error (0.0003% for fat percentage and 0 kg for fat yield) while the standard deviation of the prediction error was slightly reduced, from 0.308 to 0.301% and from 98 to 96g for fat percentage and fat yield respectively.

*Keywords: milk recording, automatic milking systems, 24-hour fat percentage, prediction, accuracy.*

The number of dairy farms in France using Automatic Milking Systems (AMS) in Official Milk Recording increased significantly from 1550 in 2010 to 3550 in 2022 and this number of is relatively stable since 2019. Today the percentage of AMS farms in Official Milk Recording represents 12% of the total of farms in Official Milk Recording

### Abstract

### Introduction

and 14% of cows in Official Milk Recording. The average number of cows by farm is equal to 85 (more than 15 cows compared with traditional farms) and 55% of farms are fitted with one AMS box.

This growth creates difficulties for the Milk Recording Organizations (MRO): cost of milk recording, use of sampling equipment...

On the published literature, several studies have been made to answer some of these issues and challenges for Milk Recording with (Bouloc, 2001; Peeters and Galesloot, 2002; Hand *et al.*, 2006; Leclerc *et al.*, 2012, Bourrigan *et al.*, 2013).

To answer needs and expectations of MRO's and AMS farmers, the French Milk Recording Guidelines proposes 2 types of AMS schemes approved by ICAR:

- at least two sampled milkings per recording test day by MRO's technician=AR scheme or by farmer BR scheme (this is the Gold Standard for genetic evaluation),
- one-sampled milking per recording test day, used since 2017 with a specific identification: AR\* scheme or BR\* scheme by using Peeters and Galesloot's method (defined in Section 2 of current ICAR Guidelines, 2022) for predicting 24-hour fat% and yield. Regarding this AMS milk recording scheme, weighting factors have been defined for genetic evaluation (from the determination coefficient  $r^2$  and the repeatability level of fat% and fat yield traits) and applied since 2020 (Vallee and al, 2021).

For helping technicians, farmers during Milk Recording test day (set up Automatic Milking Samplers, parameters, data transfer,...), today 25 different AMS Standard Operating Procedures (SOP) have been described in the French Milk Recording Guidelines in collaboration with all AMS Manufacturers.

The aims of this study carried out in 2022, consisted to:

- to check and verify the accuracy level of the Peeters and Galesloot's first regression coefficients (defined in 2017) from a new relevant dataset,
- to improve the actual 24-hour performances predicted, by using more complex Peeters and Galesloot's models (7 different models tested) and described in Journal of Dairy Science article (2002),
- to calculate accuracy results on recording test day / 24-hour reference,
  - to evaluate the accuracy of the method on recording test day,
  - to propose potentially changes of the French Milk Recording Guidelines, according to the results achieved.

## Material and methods

### Presentation of the Peeters and Galesloot models tested

The Peeters and Galesloot method is a multiple linear regression declined in different models. The "simple" model allows to estimate 24-hour fat percentage and yield from one-sampled milking by taking into account fat and protein percentage, milk weight and milking interval of the sampled milking and milk weight and milking interval of the previous milking (Table 1). Six more "complex" models (models Ca to Cf), similar to the simple one, also include different classifications of variables such as the time of day of the sampled milking, the parity number, the stage of lactation, the interval preceding the sampled milking and/or the fat% to protein% ratio (Table 2). (Peeters and Galesloot, 2002; ICAR Guidelines., 2022)

Table 1. Peeters and Galesloot's simple model.

$$24\text{-hour Fat\%} = b_0 + b_1 * \text{Fat\%(n)} + b_2 * \text{Prot\%(n)} + b_3 * \text{MI(n)} + b_4 * \text{MI(n-1)} + b_5 * \text{Milk(n)} + b_6 * \text{Milk(n-1)} + e$$

$b_0$  = intercept,  $b_1$  to  $b_6$  = regression coefficients  
 MI = milking interval, Milk = milk weight,  $e$  = residual effect  
 (n) = milking sampled, (n-1) = previous milking

Table 2. Peeters and Galesloot's complex models.

$$24\text{-hour Fat\%}_i = b_{0i} + b_{1i} * \text{Fat\%(n)} + b_{2i} * \text{Prot\%(n)} + b_{3i} * \text{MI(n)} + b_{4i} * \text{MI(n-1)} + b_{5i} * \text{Milk(n)} + b_{6i} * \text{Milk(n-1)} + e_i$$

$b_{0i}$  = intercept,  $b_{1i}$  to  $b_{6i}$  = regression coefficients  
 i = subclass of classification for class variables Cx for x = a, b, c, d, e, f  
 Ca = day time of sampled milking (h) 0-5.59, 6.00-11.59, 12.00-17.59, 18.00-23.59  
 Cb = interval preceding the sampled milking n (min) 0-360, 361-510, 511-700, 701-1440  
 Cc = fat to protein% ratio of the sampled milking 0-1.10, 1.10-1.25, 1.25-1.40, >1.40  
 Cd = parity 1, 2, ≥ 3  
 Ce = lactation stage 1-99, 100-199, ≥200  
 Cf = interval preceding the sampled milking n (min) 0-360, 361-510, 511-700, 701-1440 and fat to protein % ratio of the sampled milking 0-1.10, 1.10-1.25, 1.25-1.40, >1.40

Milkings collected by Milk Recording Organizations over the years 2017 to 2019 in herds where at least two milkings per cow were sampled were used to establish a 24-hour reference population. Data from breeds other than Holstein (71% of the milkings), Montbeliarde (24%), Normande, Simmental and Brown Swiss were deleted due to insufficient numbers, as well as milkings with an milking interval lower than 4 hours, a milk yield lower than 1 kg or higher than 30 kg, a sampling period lower than 12 hours and outlier fat (less than 1.5% and more than 9%) and protein percentage (less than 1% and more than 7%).

Thus a total of 620,272 milkings (described in Table 3) were taken into account for 125,905 cows spread over 1,277 French farms.

The reference dataset was then split into two independent sets, a training data set of 414,394 milkings, used to estimate regression coefficients for the 7 different Peeters and Galesloot models and a validation data set of 205,878 milkings, for which 24-hour fat percentage and yield were predicted. The study consisted in comparing the predicted performances to the 24-hour reference ones by analyzing the accuracy of the predicted values ( $r^2$ , prediction error/bias and standard deviation of prediction error/bias).

Compared to the performances of the simple model, the analysis of the accuracy of the different complex models shows (Table 4):

- a reduction of standard deviation of bias from 0.001% (model Cd) to 0.007% (model Cf) for fat percentage and from 0.42g (model Cd) to 1.83g (model Cf) for fat yield,

### Description of the datasets used in the study

## Results

Table 3. Description of the reference data set.

	Sampled milking				Preceding milking		24-hour reference		
	MY (kg)	Fat% (%)	Prot% (%)	MI (minutes)	MY (kg)	MI (minutes)	MY (kg)	Fat% (%)	Prot% (%)
Mean	11.8	3.99	3.32	587.2	11.4	558.6	30.1	4.02	3.31
Std	3.9	0.78	0.37	169.8	3.7	163.2	8.8	0.65	0.36
Min	1.0	1.50	1.01	60	1.0	60	2.4	1.51	1.64
Max	30.0	8.99	6.83	1440	30.0	1440	73.1	8.85	6.80

Table 4. Correlations ( $r^2$ ), bias and standard deviation of bias between predicted 24-hour fat% and yield and reference 24-hour fat% and yield.

Prediction model	Fat% (%)			Fat yield (g)		
	Bias	Std bias	$r^2$	Bias	Std bias	$r^2$
Uncorrected	-0.034	0.422	0.706	-11	135.96	0.849
Simple model	0.0003	0.308	0.776	-0.11	97.97	0.910
Complex model Ca	0.0002	0.306	0.779	-0.16	97.36	0.911
Complex model Cb	0.0003	0.304	0.781	-0.31	97.03	0.912
Complex model Cc	0.0003	0.305	0.781	-0.07	97.20	0.911
Complex model Cd	0.0003	0.307	0.778	-0.05	97.55	0.911
Complex model Ce	0.0002	0.306	0.778	-0.11	97.48	0.911
Complex model Cf	0.0002	0.301	0.786	-0.30	96.14	0.913

- an improvement of correlations ( $r^2$ ) from +0.2 (models Cd and Ce) to +1.0 (model Cf) point for fat percentage and from +0.01 (models Ca, Cc, Cd and Ce) to +0.3 (model Cf) point for fat yield.

Regardless of the prediction model, a tendency to overestimate fat percentage and underestimate fat yield can be observed, but with a negligible bias overall (less than 0.0003% for fat percentage and less than 0.31g for fat yield).

## Discussion and conclusion

Regarding the accuracy of the Peeters and Galesloot's regression coefficients used in France since 2017 with the simple model, there no difference between the regression coefficients tested during this study in 2022 (overall same level of  $r^2$ , or fat% and fat yield). But it's necessary to check regularly the accuracy level of the regression coefficients (every 4 or 5 years from a new dataset, according to changes of performance, herd management,...).

Regarding the accuracy of 6 Peeters and Galesloot's complex models tested during this study on milk recording test day, the gain of accuracy  $r^2$  (in comparison with the current simple model) is equal to 1.0 point for fat% and 0.3 point for fat yield with a complex model (Cf) which combines milking interval and fat/protein% ratio.

A tendency to overestimate fat% and underestimate fat yield can be observed with Cf complex model but overall with a negligible bias and a reduction of standard deviation of bias to 0.007% for fat% and to 1.83g for fat yield.

After six years of using an ICAR approved method for predicting 24-hour fat% and yield from one-sampled milking in Automatic Milking Systems, the Peeters and Galesloot

method's is widely use today by French Milk Recording Organizations. The goal is to answer the expectations of the farmers to simplify schemes and to reduce the cost of AMS milk recording test day while maintaining a sufficient accuracy for genetic evaluation and cow management purpose.

This new French study about the possibility of improving the accuracy level of the Peeters and Galesloot's method shows that from a new regression formula, adding of milking interval and fat/protein ratio class variable, a gain of accuracy is observed especially for fat%.

Another study (Roelofs *et al.*, 2006) showed that the Peeters and Galesloot's method regression formula was improved to estimate the 24-hour fat% based on one-sampled milking, especially by adding other variable such as month of sampling (pasture effect),..

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## References





## Milk recording in sheep and goats: state of the art and materials used for recording and sampling

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According to the ICAR online survey, the number of dairy sheep and goats in official milk recording reached 890,000 ewes and 410,000 does in 2021. Flocks are large (200-500 females). The milking routine is fast (2-3 minutes / female) and organised by batch of 12 to 24 females milked at the same time. A video showing recording and sampling was presented during the session. Such milking systems often requires 2 or 3 technicians in the milking parlour to achieve recording and sampling activities. Moreover, due to the size of the flocks, sampling is accordingly expensive.

### Abstract

Therefore, to stimulate the development of milk recording and overcome these constraints, the ICAR working group on sheep and goats has constantly promoted simplified designs of milk recording, especially of qualitative milk recording, as one of its major objectives. The recommendations are mainly based on AT or AC methods (i.e. recording of only one milking per day for both milk yield and sampling (this simplified design has consequently strongly increased over the last years to exceed 90%), the sampling of a part of the females (mostly the first parity) and only a part of the test-days (3 samples per female in the middle of the lactation are relevant for genetic purpose). However, the impact of qualitative milk recording remains low, especially in countries with large sheep and goat populations.

Devices used for recording and sampling in sheep and goats are reviewed yearly within the ICAR survey. Most of the devices used are still jars approved by ICAR with the exception status of the guidelines, as they were in use before 1995 and were accepted by the ICAR member organisations at this time. This is the case in most of the countries. Some other countries use devices indifferently in sheep and goats without actual data on precision in either of the species. Until now, there are a limited number of meters that have passed the ICAR test, probably due to difficulties to meet the requirements (low quantity of milk per test-day, high contents, and high viscosity of milk in sheep) regarding the potential market. These agreed milk meters have been moderately used so far in milk recording operations. For some of them, one reason could be the lack of suitability for sampling. Exchanging the experiences of the different stakeholders may help to stimulate the development and adaptation of milk recording and devices in small ruminants.

*Keywords: dairy sheep, dairy goats, milk recording, recording devices, sampling devices, meters, jars.*

## Introduction

The low number of ICAR-certified milk recording devices is a difficulty that ICAR member organisations have to face in sheep and goats. Either the organisations use old-conceived materials agreed by local organisation before 1 January 1995 - mainly jars -, and benefit therefore from the exception status. Or in some cases, they use non-officially agreed materials, neither ICAR-certified, nor benefiting from the “grandfather status”.

This is why a joint session dedicated to small ruminant milk recording devices, co-organised by both the Measuring, Recording and Sampling Devices ICAR sub-committee (MRSD-SC) and the Sheep, Goats and Camelids, ICAR working group (SCG-WG), was planned in ICAR Toledo. This joint session had the ambition to tackle the challenges of milk recording in sheep and goats, (i) through presentations of the different perspectives (point of view of ICAR, the test centres, the manufacturers, and the member organisations), and (ii) through discussion with stakeholders on what we can cooperatively do to stimulate the development and testing of recording and sampling devices for sheep and goats by manufacturers.

This paper presents the point of view of the SCG-WG. We will first present a state of the art of milk recording in sheep and goats, using the yearly ICAR on-line survey (Astruc *et al.*, 2022; ICAR on-line database). We will focus on the impact of milk recording, the spread of the simplified methods of milk recording and the realisation of qualitative recording in sheep and goats. Second, we will describe the issues of recording and sampling in sheep and goats, particularly the issues related to the size of the herds and flocks and to the high speed of the milking routines. Third, we will present the specificities of small ruminant milk, and especially those of sheep milk. We will then come back to the survey to present the devices used in ICAR countries. We will finally draw in conclusion some highlights and perspectives that we consider as key features to develop material dedicated to small ruminants.

## State of the art from yearly survey on sheep and goats milk recording

The terms of reference of the Sheep, Goats and Camelids Working Group (SGC-WG) of ICAR mention that it must conduct and report results of periodic surveys on sheep, goats and camelids performance recording and genetic evaluation, and (ii) maintain relationships with other groups, especially MRSD-SC.

The objective of the survey is to have a state of the art of the situation of milk recording in ICAR countries and to follow how the different recommendations of the working group, as they are suggested in the ICAR guidelines, are considered by member organisations.

Among the topics that are tackled by the ICAR on-line survey, the following are related to this paper: basic information on milk recording, methods of milk recording, optional test for milk composition, milk recording equipment.

## Number of dairy sheep and goats in official milk recording in ICAR countries

When aggregating data from all the countries that have submitted data for the last 10 years, around 900,000 dairy sheep and 460,000 dairy goats are submitted to official milk recording (table 1). The majority, 86% of the dairy sheep and 88% of the dairy goats, is from the three following countries: France, Spain, and Italy. Respectively,

Table 1. Recorded dairy sheep and goats in ICAR member countries (2020-2021).

Countries	Number of recorded ewes (official milk recording)	Number of recorded goats (official milk recording)	Recording methods in dairy sheep	Recording methods in dairy goats
Croatia	7,235	3,622	AT (most) - B4	AT (most) - A4
Czech	1,494	5,152	AT	AC - E
France	334,685 (*)	227,955	AC - D	A4 – AY – AZ - CY - CZ – AT - AC
Italy	161,711	60,326	AT - AC (Sarda)	AT
Latvia		1,296		A4
Portugal	18,052	7,771	A4 (most) – AT	A4 (most) - AT
Serbia		4,846		AT
Slovak	6,643	346	AC	AC
Slovenia	4,624	2,575	AT	AT
Spain	256,480	113,934	AT – AC (Latxa-part)	A4 - AT - AC

(\*) in addition, 553,836 ewes are recorded with D method (non-official milk recording)

9% and 10% of the dairy sheep and dairy goat populations are recorded (official milk recording). In addition to official milk recording, France has 550,000 dairy sheep in D method. There is a relative stability of recorded animals over time.

The table 1 shows the different methods used. The simplified methods (those with one recorded milking per day) have been highly recommended by the SGC-WG for many years. The simplified methods used in sheep and goats are AT, AC, AY, AZ, CY, CZ, while the non-simplified (two recorded milking per day) methods are A4, B4, E (ICAR guidelines – section 16). We estimate that simplified designs concern 98% of the recorded dairy sheep and 58% of the recorded dairy goats. The D method (non-official design) is used in France in commercial flocks (sheep). Simplified milk recording methods are more used in sheep than in goats.

**Importance of simplified recording methods**

Qualitative recording is globally generalised in goats. In dairy sheep, in countries with large population size (Spain, Italy, France), qualitative recording is partial, while in countries with smaller population size, qualitative recording is generalised.

**Use of qualitative recording (optional recording)**

The spread of simplified designs of milk recording (quite generalised in sheep and majority in goats) and the limitation of qualitative recording to a part of the animals (especially in large populations of dairy sheep) may be explained by two main reasons. First, the large flocks/herds size (300-500 animals) makes milk recording more expensive because of the cost of many milk analyses. Second, the high speed of the

**The issues of recording and sampling in sheep and goats**

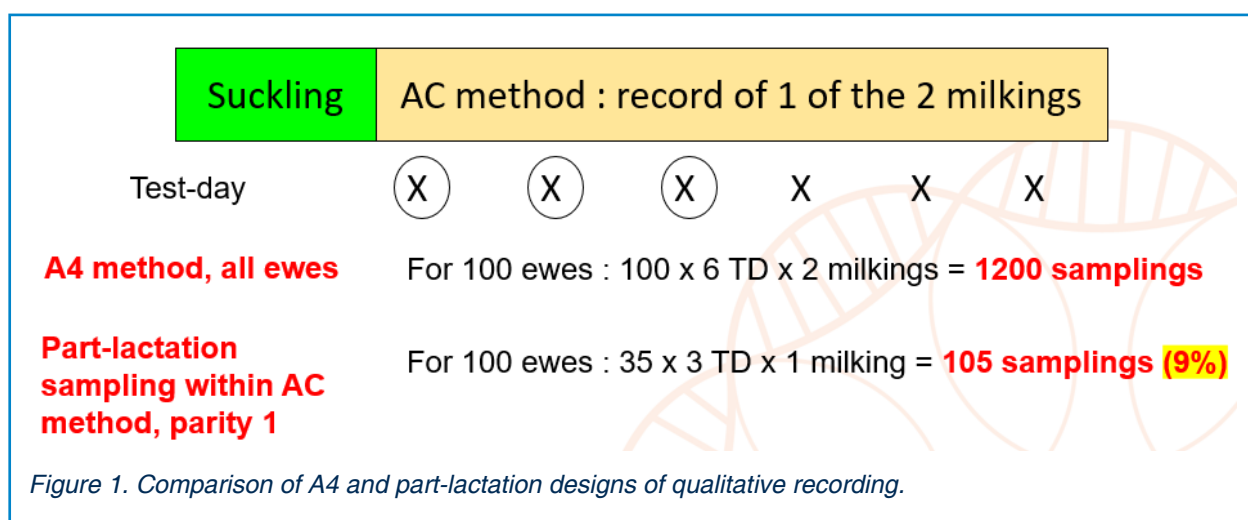
milking routine (1 sampling every 15-20 seconds) requires more often one or more additional technician(s) to realise the sampling.

It is therefore necessary to rationalise and to think the milk recording designs in terms of cost-benefit for the breeding program efficiency.

There are three ways to simplify the qualitative milk recording. First, qualitative recording is an optional disposition in the guidelines. That is why some breeds do not realise sampling. However, as soon as a breeding program is efficient on milk yield, it becomes necessary to include fat and protein contents information in the selection criteria. When qualitative recording is necessary, it is recommended to reduce the part of the animals sampled within the farm. For example, only the ewes/does in parity 1 (or parities 1 and 2) may be sampled. As a third strategy to rationalise qualitative recording, it is possible to use the part-lactation sampling method. This design consists in sampling only the middle of the lactation, which is the part of the lactation with the highest genetic relevance (the most heritable and the most highly correlated with the whole lactation). This method allows to reduce by half the number of samples. It is implemented in dairy sheep, in France, Italy and Spain. Such simplifications imply an acceptable decrease in the accuracy of the EBVs, compared to the gain in cost.

The figure 1 gives an illustration of the interest of the part-lactation sampling applied to the AC method in terms of number of analyses. Milk recording is realised on one milking only, 3 test-days (TD) out of 6 are sampled, and the sampling is applied to the first parity only. In the A4 methods (considered as the reference), 1,200 samplings are done for 100 females (100 females x 6 TD x 2 milkings). In comparison, in the described simplified design, 105 samplings are done for the same 100 females (100 females x 3 TD x 1 milking x 35% in parity 1). This means a decrease in 91% of the number of samplings.

We must have in mind the consequences of such simplified designs. As few samples are realised, there are few measures of content in an animal lifetime (between 2 and 6 measures). Such a decrease in number of measures leads to a decrease in the heritability, especially the heritability of fat content (from 0.50 to 0.35 in the case of France). Genetic progress depends on the accuracy of the EBVs. Yet it is economically unthinkable (on large populations) to increase the number of measures to compensate this lower heritability. Therefore, each measure must be precise enough. Relaxing the precision of an individual measure would lead to a lower efficiency of the selection, which is obviously not expected from the geneticist point of view. This highlights the



fact that the devices must be accurate, and sampling must be accurately representative of the milking.

However, small ruminant milk (and especially sheep milk) has “unfavourable” specificities regarding the recording and sampling devices.

If milk contents in goats are quite similar to those in dairy cattle, sheep milk components are high. Fat content is on average 70-75 g/l (up to 130-140 g/l in late lactation stages). Protein content is on average 50-55 g/l (up to 100-110 g/l in late lactation stages). Sheep milk has a high viscosity and there is a lot of foam (Figure 2). In addition, in sheep and goats, the quantity of milk per milking is small (500 ml – 4000 ml) rendering the sampling is more difficult.

All these characteristics make the recording of yield, and above all the sampling, more difficult to realise with accuracy. To try to overcome these difficulties, requirements in the ICAR guidelines (ICAR guidelines – section 11) have been relaxed for sheep and goats over the last 20 years, by adapting the limits of error. The last changes were accepted in 2023. For milk yield, the limit of error for bias is 1.5 time higher in sheep and goats compared to cattle. For fat percentage, the limit of error for bias is twice higher in sheep and goats than in cattle, and the limit of error for standard deviation is 3 times and twice higher, respectively in sheep and goats than in cattle.

Despite these relaxed requirements, few materials have been tested by ICAR. To date, the materials certified by ICAR are the MM25SG from DeLaval (low line) in sheep and goats, the Lactocorder from WMB (high line) in sheep and goats, the ARG1 jars from Sarl ELCABE (high line) in sheep and goats. The Afifree from Afikim is no longer sold.

## Specificity of small ruminant milk



*Figure 2. Foam above the milk in a jar during a milk recording in a Lacaune flock in southern France.*

Table 2. Recording and sampling devices used in dairy sheep and goats in ICAR member countries (2020-2021).

Countries	Goats		Sheep	
	(Portable) Jars	Meters	(Portable) Jars	Meters
Croatia		Waikato MK4		Waikato MK4
Czech		Tru-Test (Mini)		Tru-Test (Mini)
France		Tru-Test (50%) Lactocorder WMB (50%) Few DeLaval MM25SG(<5)	Gély (~3,000)	Lactocorder WMB (<5), MM25SG(<5)
Italy	MIBO, Miele, Italiana, ROYAL (1/3)	Waikato MKV (1/3), Tru-Test HI, EMM (5%) [Lactocorder WMB, DeLaval MM25SG]	MIBO, ROYAL (90%)	Waikato MKV, EMM (5%) [Lactocorder WMB, Afifree, DeLaval MM25SG]
Portugal	Westfalia, Vitlab	Sneder Mayfra, Tru-Test		Tru-Test, Flaco
Slovak	Fisher Slovakia,	Tru-Test	Fisher Slovakia, Berango / Milkovis	
Slovenia		Waikato MK4, Tru-Test		Waikato MK4, Tru-Test
Spain	Esneider	Tru-Test, DeLaval	Berango (model Esneider) MIBO (model Lattometri) Gruponor	DeLaval MM25SG, Westfalia, Afikim, Flaco, GEA

EMM = (on-farm) Electronic Milk Meter.

### The devices used for milk recording and sampling in sheep and goats

According to the yearly on-line survey, the devices used for the milk recording operations in dairy sheep and goats are displayed in the table 2.

A major part of the devices used are portable jars, particularly in sheep, approved through the exception status (as they were approved by the ICAR member organisations before 1 January 1995). There are still few on-farm electronic milk meters used for milk recording operations, except in some breeds in Spain. The ICAR certified devices are not widespread, except for the Lactocorder in goats in France (around 50% of the recording operations) and the MM25SG in Spain.

### Conclusion and perspectives

As the sizes of the herds and flocks are large and as the speed of the milking routine is high, the development of milk recording in such large populations of sheep and goats is possible with simplified recording designs. Sampling operation is a key concern for expanding milk recording in sheep and goats. Sampling devices must be friendly and allow a fast pace that does not exceed 20 seconds. Getting good accuracy in recording yield and sampling milk is a challenge for the devices, especially in sheep for which milk quantity is low and fat content is twice as high as in cattle. ICAR has adapted its guidelines to sheep and goats, by relaxing the limits of accuracy, and by separating sheep and goats. Despite that, most of the devices used are jars “agreed” through

the exception status. New requirements, voted by the ICAR General Assembly in Toledo in 2023 (ICAR guidelines – section 11) might help manufacturers to produce new materials for sheep and goats. The key factor to stimulate the market is to have friendly sampling devices.

**Astruc J.M., Carta A., Negrini R., Simčič, M., Špehar M., Ugarte E., Mosconi C., 2022.** Milk recording in sheep and goat: state of the art using the data from the ICAR on-line yearly survey. Proceedings of the 45th ICAR Annual Conference held in Montréal, Canada, 30 May – 3 June 2022. ICAR Technical Series no. 26.

**ICAR Guidelines – Section 11 Milk Recording Devices.**  
<https://www.icar.org/Guidelines/11-Milk-Recording-Devices.pdf>

**ICAR guidelines – Section 16 Dairy Sheep and Goats.**  
<https://www.icar.org/Guidelines/16-Dairy-Sheep-and-Goats.pdf>

**ICAR on-line database for cow, sheep and goat milk recording.**  
<https://my.icar.org/>

## References

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## Overviews of milk recording and recording devices in sheep and goats in Italy

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Sheep and goat farming has a long history in Italy, thanks to the unique environment with a large part of hills and mountains and a dry climate where these two species can thrive better than cattle. The most common rearing system is grazing on pasture with some periods in stable. Many dairy products are linked to Italian breeds and environment, such as Pecorino cheese. The latest demographic census, done by the Italian Ministry of Health Veterinary Services, reports about 6.1 million sheep and 0.9 million goats belonging to more than 100 breeds distributed in 81,262 and 51,056 flocks, respectively; the main breeds are Sarda, Massese, Valle del Belice, Langhe, and Comisana for sheep and Saanen, Camosciata delle Alpi, Sarda, Aspromontana for goats.

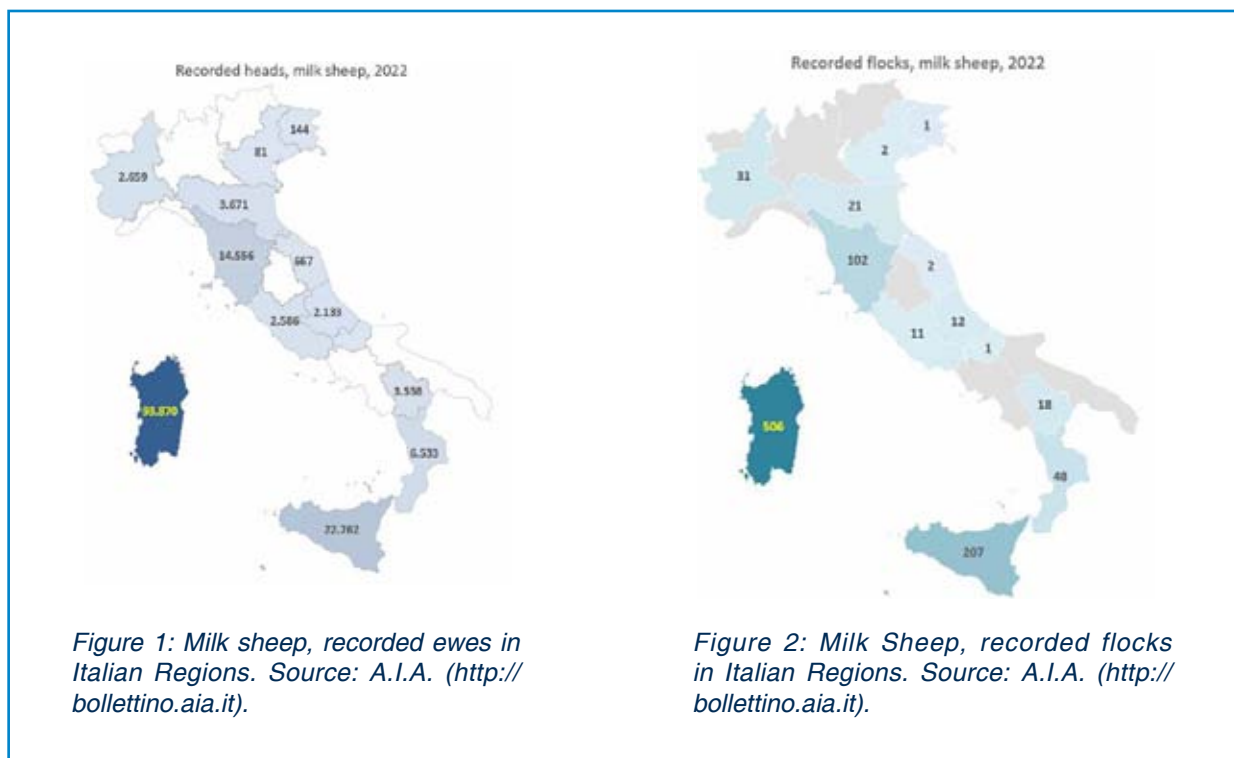
### Introduction

Official milk performance recording for sheep and goats has been active in Italy since the second half of the last century and is run by the Italian DHI organization (A.I.A., Associazione Italiana Allevatori) using ICAR approved recording methods. Official technicians perform milk recording methods monthly ("A" method, recording every 4 weeks). The methodologies used are AC4 and AT4 for sheep and AT4 for goats. Milk performance recording is done using ICAR-approved devices. In 2022, (A.I.A., <http://bollettino.aia.it>) 153,367 ewes and 55,671 goats were recorded for milk production in 962 and 642 flocks, respectively, spending about 10,000 working days. Milk analyses were more than 255,000 (69,000 in primiparous Sarda sheep only and 186,000 for goats).

### Milk performance recording

In 2022, 153,367 ewes were officially recorded for milk production in 962 flocks. Figures 1 and 2 show the distribution of recorded ewes and flocks in the period.

### Milk sheep



Central and Southern Italy, along with the islands (Sicily and Sardinia) have the highest concentration of heads compared to northern Italy. Sardinia is the region with the highest number of heads (93,870).

The distribution of recorded flocks follows the proportions of recorded ewes; Sardinia has the highest number of recorded flocks (more than 50%), followed by Sicily. The distribution of ewes and flocks by breed is shown in Table 1, in which only breeds with more than 100 heads are included.

The most important breed is Sarda (72% of total recorded ewes and 61.2% of total flocks), followed by Valle del Belice and Lacaune. The average number of heads per flock in Sarda is 182.8. Recorded milk productions by breed are shown in Table 2.

Table 1. Distribution of ewes and flocks by breed.

Breed	Recorded ewes	% on total	Recorded flocks	% on total	Average ewes per flock
Sarda	107.694	72,0	589	61,2	182,8
Valle del Belice	11.649	7,8	184	19,1	63,3
Lacaune	7.487	5,0	55	5,7	136,1
Massese	6.700	4,5	83	8,6	80,7
Comisana	3.846	2,6	41	4,3	93,8
Delle Langhe	2.369	1,6	29	3,0	81,6
Assaf	1.325	0,9	12	1,2	110,4
Nera di Arbus	1.226	0,8	36	3,7	34,0
Carsolina	200	0,1	1	0,1	200,0
Barbaresca	101	0,1	3	0,3	33,6

Source: A.I.A. (<http://bollettino.aia.it>)

Table 2. Recorded milk productions by breed.

Breed	Primiparous			Secondiparous			Third Parity And Over			All Ewes		
	Milk Lt.	Fat %	Protein %	Milk Lt.	Fat %	Protein %	Milk Lt.	Fat %	Protein %	Milk Lt.	Fat %	Protein %
Lacaune	215			321			330			304		
Assaf	187			276			302			271		
Sarda	153	5.08	5.01	231			237			225		
Valle Del Belice	152			234			227			225		
Massese	117			125			132			129		
Nera Di Arbus	101			181			189			177		
Delle Langhe	87			138			154			142		
Comisana	76	6.51	5.06	173			151			156		
Pinzirita	75	6.07	5.31	120	5.86	4.80	129	5.96	4.78	117	5.93	4.85
Noticiana	53	6.47	5.28	105	5.70	5.23	101	5.93	5.10	97	5.82	5.20
Carolina				119			106			114		

Source: A.I.A. (<http://bollettino.aia.it>)

The most productive breed is Lacaune, followed by Sarda, Valle del Belice and Massese. Milk analyses are not done in all breeds: the main activity is on Sarda primiparous ewes, and on other breeds like Comisana, Pinzirita, and Noticiana. Among native breeds, Sarda and Valle del Belice are the best milk producers.

In 2022, 55,671 goats were performance recorded in 642 flocks. Figures 3 and 4 show the distribution of recorded goats and flocks in the period.

Compared to sheep, goats are more evenly distributed in the country. Northern Italy has a good number of goats, even if southern and insular regions have more than 50% of recorded heads. (Figure 4)

As before, the number of recorded flocks is proportional to recorded heads. The distribution of goats and flocks by breed is shown in Table 3, in which only breeds with more than 30 heads are included.

The number of recorded goat breeds is higher than sheep. In this situation, five breeds share about 80% of total heads (Camosciata delle Alpi, Sarda, Saanen and Aspromontana). Recorded milk productions by breed are shown in Table 4. The most productive breed is Saanen, followed by Camosciata delle Alpi, Bionda dell'Adamello and Roccaverano. Milk analyses are done on almost all the recorded breeds.

## Dairy goats

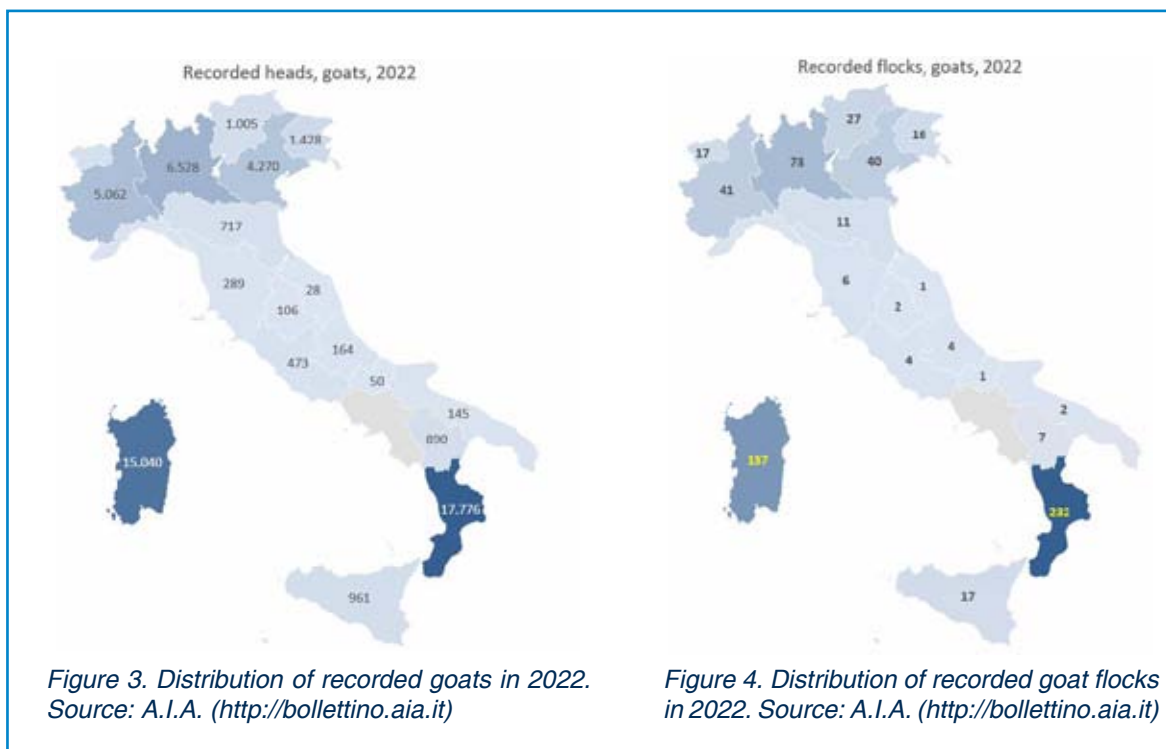


Table 3. Distribution of goats and flocks by breed.

Breed	Recorded heads	% on total	Recorded flocks	% on total	Average heads per flock
Camosciata Delle Alpi	12.775	22,9	222	34,6	57,5
Sarda	12.551	22,5	118	18,4	106,4
Saanen	9.180	16,5	127	19,8	72,3
Aspromontana	8.005	14,4	93	14,5	86,1
Rustica di Calabria	4.697	8,4	103	16,0	45,6
Nicastrese	3.618	6,5	65	10,1	55,7
Murciana	1.122	2,0	17	2,6	66,0
Sarda Primitiva	505	0,9	15	2,3	33,7
Messinese	401	0,7	10	1,6	40,1
Maltese	291	0,5	26	4,0	11,2
Argentata Dell'etna	267	0,5	11	1,7	24,3
Roccoverano	212	0,4	6	0,9	35,3
Verzaschese	109	0,2	6	0,9	18,2
Bionda Adamello	54	0,1	4	0,6	13,5
Girgentana	49	0,1	3	0,5	16,3
Garganica	46	0,1	2	0,3	23,0
Rossa Mediterranea (Derivata Di Siria)	46	0,1	3	0,5	15,3
Jonica	37	0,1	2	0,3	18,5

Source: A.I.A. (<http://bollettino.aia.it>)

Table 4. Recorded milk productions by breed.

Breed	Primiparous			Secondiparous			Third parity and over			All goats		
	Milk Kg.	Fat %	Protein %	Milk Kg.	Fat %	Protein %	Milk Kg.	Fat %	Protein %	Milk Kg.	Fat %	Protein %
Saanen	420	3,52	3,30	621	3,55	3,34	639	3,40	3,30	584	3,48	3,32
Camosciata Delle Alpi	405	3,72	3,37	588	3,60	3,41	618	3,55	3,39	554	3,60	3,39
Bionda Adamello	386	3,45	3,02	402	3,11	2,96	550	2,83	2,83	479	2,98	2,89
Roccoverano	351	3,87	3,17	460	3,48	3,14	459	3,46	3,30	449	3,50	3,24
Murciana	237	4,75	3,53	430	4,60	3,53	327	4,36	3,40	368	4,52	3,48
Maltese	208	4,49	3,45	324	3,89	3,42	338	4,17	3,48	314	4,08	3,45
Verzaschese	196	3,83	3,23	345	3,87	3,39	345	3,51	3,19	303	3,75	3,30
Nicastrese	191	4,66	3,77	191	5,17	3,87	197	5,14	3,84	195	5,11	3,85
Jonica	157	4,46	3,82	263	5,25	3,87	292	5,08	3,73	283	5,10	3,76
Rossa												
Mediterranea (Derivata Di Siria)	146	4,67	3,48	189	4,40	3,80	182	4,02	3,50	169	4,37	3,59
Garganica	144	4,92	3,47	180	4,22	3,39	189	4,34	3,46	177	4,41	3,44
Aspromontana	128	4,33	3,15	173	4,45	3,71	185	4,54	3,71	180	4,51	3,71
Sarda	115	4,62	3,74	156	5,67	4,31	157	5,57	4,36	153	5,02	3,98
Rustica di Calabria	90	5,30	3,94	128	5,30	3,94	140	4,99	3,80	133	5,14	3,87
Sarda Primitiva	79	4,90	3,90	125			140			127	4,90	3,90

### The importance of correctly working milk recording devices and milking plants: AIA's Milking Control Service

The Italian DHI organization (AIA) established and developed a national service called SCM (Milking Control Service) since 1970. SCM personnel comprises more than 100 highly qualified technicians providing different services in the field of milking and milk recording activity. According to ISO regulation, SCM checks the efficiency of milking systems both in the absence (dry test) or presence (wet test) of milked animals. SCM's technicians inform owners on incorrect settings or calibration, on every working issue and, where possible, assist the farmer to fix them or to contact manufacturers for technical corrections.

These activities are crucial for milk production and animal welfare: for example, correcting vacuum levels above certain limits avoids animal stress and mammary gland' susceptibility to mastitis and increases milk yields. On the side of milk recording, SCM performs calibration checks for both milk meters installed in farms or used by technicians. With these tests is possible to detect, if any should occur, milk registration errors and whenever possible it is performed a correction of working biases or an adjustment of milk meters components.

In addition, all the calibration check' equipment in allocation to SCM's technicians, are calibrated annually in a centralized SCM laboratory in Maccarese, near Rome. Furthermore, SCM service identifies through numbered sticky labels all the milk meters used in farms for performance recording. Within the EU-funded LEO (Livestock Environment Opendata) project, SCM acquired new-generation VaDia kit, a vacuum multisensor for evaluation of milking routine and machine efficiency and Lactocorder TT devices to analyse milking curves, animal milk ejection efficiency, and check milking routines.

The goal is to use these advanced tools to enhance service testing (e.g., assessing pulsator settings and faults) and to examine the milking systems and management efficiency thoroughly, giving farmers early alerts, advice, and extension services to improve animal welfare and milk quality and yield. The SCM will then gather innovative information and data with state-of-the-art instruments, offering the farmer integrated, practical, broad-spectrum support.

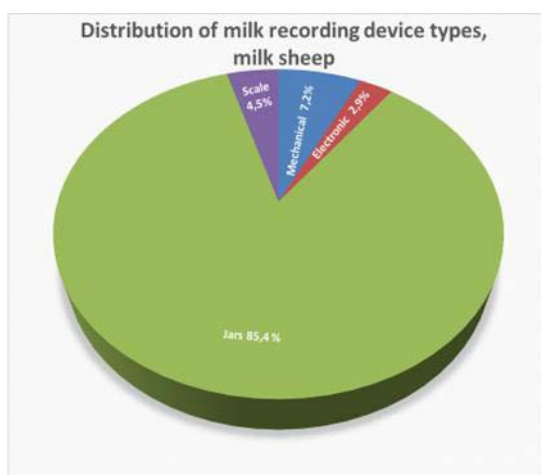


Figure 5. Percentage of milk recording devices categories used in milk performance recording for sheep in 2022

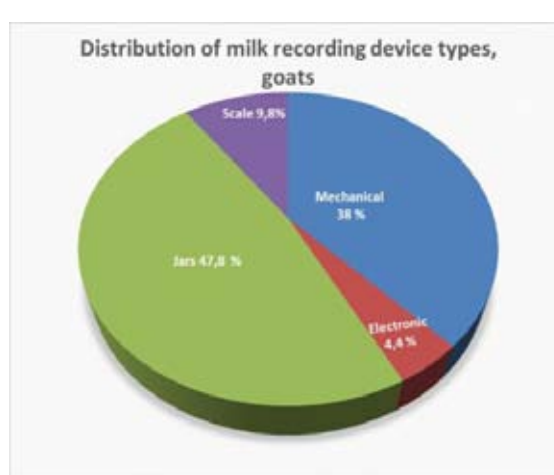


Figure 6. Percentage of milk recording devices categories used in milk performance recording for goats in 2022

As mentioned before, SCM service identifies all milk recording devices used in Italy, both fixed in-farm meters or portable devices to be installed on the day of the performance recording. It is therefore possible to make some statistics on the working devices situation. The following figures show the percentage of each device type category used in sheep and goat performance recording.

Jars are the most common type of milk recording in both species but are more used in sheep (85.4% of all milk recording device types) while in goats they represent slightly less than 50%; moreover, mechanical meters are proportionally more used in goats (38%) than in sheep (7.2%, one fifth of those in goats). Scales are proportionally more widespread in goats (9.8%), while in sheep they account for half this proportion. Lastly, electronic meters are less than 5% of all device types, with a lower value in dairy sheep.

A pilot study was conducted to check if any problem of electronic milk meters could occur in calibration and subsequent milk measuring biases. For this purpose, the pilot study was set up by AIA SCM service in a Sarda flock in Tuscany. The milking plant was a milk parlour line 12 + 12, low milk line equipped with MM25 De Laval milk meters (Azienda Mesina, Pergine Valdarno, Arezzo (Tuscany)). To get rid of any effect of line length and better manage the installed devices, six MM25, three for each line (placed in start, centre and end of the line) were chosen. These devices were restored to factory default and in addition, an acid washing of the plant was done to remove any dirt in pipelines. 10 animals per meter were milked, with a total of 60 milkings. A procedure to check the milk meters calibration with real milk yields was performed. The procedure, for each milked animal, consisted in:

- Connecting 6 portable jars (MIBO) with the outlet nipple of the MM25 to correctly collect the milk, taking care that connection followed the connecting pipe inclination as reported on the manufacturer's instruction.
- Reading milk yield on the MM25.
- Collect the related milk from MIBO jar to a bucket and record the milk weights, a digital calibrated scale was used to weigh the milk collected by the jar;
- Make a comparison between recorded production and meter measurement and calculate, for each meter, the average difference between weighed and MM25 milk. Usually, this average is used to check the calibration.

For each meter and each milked animal, a difference in weight between weighted (scale) and measured milk was calculated and expressed as % deviation on scale weight (taken as reference measure). Overall results are presented in table 5.

Table 5. Overall results of the pilot study.

Meter #	Milked ewes	Recorded milk (g), average		Difference Scale - MM25, average	
		MM25	Scale (Reference)	g	% on Scale
1	10	582	543,1	-38,9	-7,61
2	10	567	548,1	-18,9	-9,39
3	10	551	530,5	-20,5	-13,12
4	10	564	564,1	0,1	-0,10
5	10	644	641,9	-2,1	-0,11
6	10	498	494,8	-3,2	-0,99

## Milk recording devices: types and distribution

## Pilot study: Influence of calibration on performance recording results

As a result, over the 6 meters used, 3 had an average % deviation within 1%, while the other 3 had very large average % deviations (Meters 1,2,3, values in bold). Following such results, a single-animal analysis was performed for the biased meters.

The evaluation of the measures of single animals in the biased meters was performed. For meters 1 and 3 it was found that primiparous ewes were milked in the trial. These primiparous, according to the farmer and the SCM technician, caused some problems in milking routine because they were not yet used to mechanical milking, and they could kick and make body movements that could cause vacuum problems and abnormal milking: in table 6 the situation of meter number 1 is reported.

Table 6. Situation of a meter is reported.

Animal #	MM25, g	Scale, g	Diff MM25 - Scale, g	Diff MM25 - Scale, % on Scale
1	700	668	-32	-4,79
2	620	614	-6	-0,98
3	230	236	6	2,54
4	690	674	-16	-2,37
5	700	685	-15	-2,19
6	580	575	-5	-0,87
7	530	446	-84	-18,83
8	660	593	-67	-11,30
9	520	420	-100	-23,81
10	590	520	-70	-13,46
<b>Average</b>	<b>582</b>	<b>543,1</b>	<b>-38,9</b>	<b>-7,61</b>

It was found that animal 1 to 6 were pluriparous ewes, while 7 to 10 were primiparous ewes. Primiparous animals, even having regular yields, showed a significant overestimation of measured milk, that increased the overall bias (-7.61%) of the meter. When animals 7 to 10 were removed from the sample, the overall bias just went down to allowable values (-1.44%), as reported in table 7.

This means that if primiparous are included in the sample for calibration, there could be a risk of not correctly calibrate the meter.

Table 7. Overall bias variation to allowable values.

Animal #	MM25, g	Scale, g	Diff MM25 - Scale, g	Diff MM25 - Scale, % on Scale
1	700	668	-32	-4,79
2	620	614	-6	-0,98
3	230	236	6	2,54
4	690	674	-16	-2,37
5	700	685	-15	-2,19
6	580	575	-5	-0,87
<b>Average</b>	<b>586,7</b>	<b>575,3</b>	<b>-11,3</b>	<b>-1,44</b>



Table 8. Variation of one value out of ten that was heavily overestimated.

Animal #	MM25, g	Scale, g	Diff MM25 - Scale, g	Diff MM25 - Scale, % on Scale
1	380	366	-14	-3,83
2	660	657	-3	-0,46
3	230	224	-6	-2,68
4	230	228	-2	-0,88
5	1050	1093	43	3,93
6	220	126	-94	-74,60
7	630	642	12	1,87
8	740	657	-83	-12,63
9	480	476	-4	-0,84
10	1050	1012	-38	-3,75
Average	567	548,1	-18,9	-9,39

Another issue was found studying the single readings in meter number two. In this case, as reported in table 8, there was just one value out of ten that was heavily overestimated.

Animal # 6 was studied and identified as a low-producing animal due to trauma consequences; it was inspected and very low milk flow from udder was verified. In this case, it seemed like the meter was influenced by low milk flow and/or low milk yield, that resulted in a big individual and group bias. Should animal # 6 not be considered, meter bias would be reduced from -9.39 to -2.14%.

Results seem to indicate that if low producing ewes are included in the sample for calibration, there is a risk to calibrate with a new bias not eliminating the error. Results from this study showed that new devices (in this case, restored to factory default), if correctly installed, are working well; however, the choice of animals to be included in data for calibration is essential because if primiparous ewes or low yield ewes are included in the set of milked animals used for calibration, they can increase the error.

Sheep and goat milk performance recording in Italy, carried on by A.I.A. (Italian Breeders Association, Italian national DHI) have a consolidated importance due to the broad diffusion of the two species and their capacity to thrive in hilly and dry environments, particularly in the southern and insular part of the country. Performance recording is carried on using jars, scales and mechanical milk meters, with a minor use of electronic meters. All meters and milking plants used for performance recording are routinely checked and verified for calibration by A.I.A.'s Milking Control Service (SCM) that, using brand new advanced instruments like VaDia and Lactocorder, can add innovative services for the farmers. Regarding electronic milk meters, their calibration could not be effective if some categories of animals (primiparous, less used to mechanical milking) or low-producing heads are included in the sample used for calibration.

## Conclusions

**A.I.A. Bollettino OnLine Controlli sulla Produttività del Latte - 2021/2022** (official milk performance recording results). <http://bollettino.aia.it>

## References



## Dtreo, a flexible cloud-based data-recording platform for data-driven decisions in sheep and goat industries

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Sheep and goat production systems are extremely valuable sources of income, food and by-products, especially when considering their ability to use land with few alternative agricultural applications. These livestock systems positively impact local socioeconomic activities, both in developed and developing countries.

Regardless of the geographical context, on-farm data recording is one of the main challenges sheep and goat farming faces in the context of sustainable and profitable livestock production systems. The availability of good quality data, collected by standardised systems, is crucial for the development and implementation of technical interventions and genetic services. Data flow through the supply chain can support market structure and has an impact on farmers, traders, processors, businesses, and revenues.

A good record-keeping system should be suited to the conditions in which it is used and to the expected use of the collected data. If properly defined, a good system can enable farmers to make informed data-driven business and management decisions.

The cloud-based platform, Dtreo, has been developed for data capture, storage, and reporting in many different farming scenarios. The platform transforms livestock performance data collected at the farm level into actionable information, supporting farmers in making data-driven decisions and connecting producers to markets. Thanks to its flexible structure, Dtreo is customizable in terms of data collected, validation criteria, and user interface language. This applies not only to small ruminants, but also to pigs, cattle, and aquaculture recording requirements. The platform has also been considered for supply chains either in vertical enterprises or horizontally across an industry.

To date, the Dtreo platform has proven to be a valuable support tool in sheep and goat community-based breeding programs in Ethiopia and India, as well as in advanced small ruminant operations in developed countries.

Our paper highlights how the Dtreo platform has supported sheep and goat farmers with data and information to drive decision making.

### Abstract

*Keywords: sheep, goat, performance recording, database.*

## Introduction

Globally, about 20.8% of dairy products come from sheep and goats and they make up 1.3% and 1.9%, respectively, of the total milk produced (Mazinani & Rude, 2020). Sheep and goat meat consumption are fourth and fifth after pork, poultry, and beef meat. Wool, being four to seven times more expensive to produce than manmade fibres, is now often marketed as a luxury product (Doyle et al., 2021).

Sheep and goat production systems are therefore extremely valuable sources of income, food and by-products, especially when considering their ability to use land with few alternative agricultural applications. They play a remarkable role in the agro economy of countries in the Mediterranean area, and developing countries in Africa, Asia, and Latin America.

Regardless of the geographical context, on-farm data recording, processing, and storing, is one of the main challenges that sheep and goat farming faces in the context of a sustainable and profitable agribusiness.

In developing countries, the lack of infrastructure, the absence of basic tools to capture data, and the highly fragmented production system limit the opportunity to provide support to farmers, develop advisory services and establish genetic improvement programs (Santos et al., 2021). In developed countries, the absence of standardized record-keeping systems and low accuracy of data recording have been recognised as factors limiting profitable sheep and goat production and the efficiency of genetic selection programs (Aldridge et al., 2018; Salaris et al., 2018). Due to the generally small scale of the breeding initiatives for small ruminants, it is hard to distribute the development and operating costs of a bespoke data platform over modest numbers of animals with low individual value. For small ruminants, automatic weighing and milk recording systems may capture live weight and daily milk records quite efficiently but are not easily customisable to also capture other functional aspects of the animals of interest when making individual animal management, culling and genetic selection decisions.

A good record-keeping system should be suited to the conditions in which it is used and to the expected use of the collected data. If properly defined, a good system can enable farmers and the wider supply chain to make informed data-driven decisions and generate value. Flexible solutions are therefore needed to fulfil the needs of the farmers in different production systems.

The cloud-based data and information platform Dtreo ([www.dtreo.io](http://www.dtreo.io)) has been developed to overcome the aforementioned issues, supporting data collection, storage, analyses, and reporting in different situations. In this manuscript we describe the main features of the platform, providing case studies and examples in which Dtreo has been shown to be a valuable support tool to farmers and communities in both developed and developing countries.

Dtreo captures, stores, and reports individual animal level data. It has been developed in the Microsoft stack of technologies and delivered in the cloud, affording a high level of data storage security. Dtreo is accessed via an internet browser and mobile application, making it highly accessible and flexible, so changes can be implemented almost immediately.

The platform allows pedigree, performance, health, and environment data recording through forms, direct entry, or files. Data entry is tailored to the user's data collection requirements. Dtreo is not hard-coded, and it can be configured for many different data capture situations. The data, recorded either online or offline (where connectivity is limited, with support of mobile app smart device software), is transferred into a designed Microsoft Azure table storage and Cosmos DB SQL API which uses entities (e.g., location, flock/herd, animal, etc.) and events (e.g., birth, weaning, sales, etc) which are customizable by the user.

A hierarchical order of (multiple) entities and sub-entities can be defined by the user based on the specifics of the operation, then events are associated with the lower level of sub-entity.

Before being saved, the data is validated by applying user-defined filters for the event, quarantined if necessary, and ultimately stored.

Quantitative geneticists and livestock breeders rely on accurately recorded phenotypes to drive genetic progress. However, phenotypic data must often be collected by commercial suppliers who may have little interest in genetic improvement. One of Dtreo's strengths lies in its capacity to balance the needs of both 1) commercial suppliers, who value the ability to make management decisions based on analytics produced from phenotypic data, as well as 2) stud breeders, who value the genetic evaluations generated by accurately recorded data.

In Dtreo, clean and normalised data are therefore available for internal analysis within the platform or externally (e.g., in a genetic evaluation or delivered to a third party).

Dtreo reporting exploits the visualisation capability of Microsoft's PowerBI. PowerBI aggregates and transforms a user's data into pictures as graphs, cards, decision-trees, maps, tables, and other visuals. Moreover, bespoke analytics aimed at supporting data-driven decisions are implemented on the platform, depending upon users' requirements.

Dtreo can easily be contextualized to create any type of input data for other software, if needed, or to integrate third party data. The platform's user interface has been translated into various languages (English, French, Portuguese, Hindi, Amharic, Arabic to date) to make it easier to use.

Thanks to bespoke permission settings, Dtreo is suitable for both individuals and collectives of farmers.

The Ethiopian government, in collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA), the International Livestock Research Institute (ILRI) and the Brazilian Agricultural Research Corporation (EMBRAPA), has initiated sheep and goat community-based breeding programs (CBBP) in different locations of the country to support communities of smallholder farmers in improving flock performance and addressing market demand (Haile *et al.*, 2020).

Despite the different production and ecological systems in which the communities operate, the use of Dtreo, supported by the New Zealand Ministry of Foreign Affairs

## Dtreo Platform overview

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## Case studies

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### Community-based breeding program (Ethiopia)

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and Trade (MFAT), has allowed the collection, storage, and reporting of uniform data to be used in selection decisions (Haile et al., 2019). Initially, 19 local enumerators/technicians were trained and equipped with tablets, with data capture starting from 2019.

Birth, 6-month, and yearling weights, as well as functional conformation, body scores and litter size data were collected by these technicians, who also managed animal identification and breeding program activities.

In each community, the households' flocks were pooled and treated as one entity. The outcome from data analyses in Dtreo was used in a two-stage ram selection process: between 4 and 6 months of age, based on the adjusted weight of lambs and twinning rate of ewes; and before the reproductive age based on a set of agreed selection traits. To improve acceptance of the breeding program by the community and to build commitment, a selection committee (whose members were appointed by the community) oversaw the final selection of the young sire candidates.

At early 2023, a total of approximately 100,000 animals with over 67,000 lambing/kidding records, 125,500 live weight records at different ages, and 23,000 milk records were available on Dtreo. As described in detail by Haile et al. (2020), the CBBP has positively impacted animal performance as well as market participation by the communities involved, improving their income. Thanks to its success in Ethiopia, the possibility of implementing CBBPs in other pastoral communities is well and truly underway (Getachew et al., 2018). However, investments from both the public and the private sector in strategic areas around CBBPs (e.g., technology, infrastructure, organisation) are of crucial importance for the long-term sustainability of the programme (Haile et al., 2019).

### **Project Mesha (India)**

Project Mesha, a programme of the Aga Khan RSP Foundation with further funding from the Bill and Melinda Gates Foundation, commenced in 2016. It aims to improve the quality of life of marginalized communities by improving the productivity of their livestock (goats), empowering women goat keepers in the Muzaffarpur district of Bihar state in India, and improving incomes (Schurink et al., 2022). As a part of the project, in 2018 a community-based goat breeding program was implemented focusing on goat identification, performance recording, and the selection of superior male kids for breeding (Nimbkar et al., 2021). The program initially involved 4 recording villages (with at least 200 breeding does each) and expanded to 8 villages in 2020 and 16 in 2021. The 16 recording villages are expected to generate selected breeding bucks for recording as well as non-recording villages in the vicinity.

Dtreo has been used as both a recording and evaluation system. The user interface has been customised to allow the collection of the following data: animal identification, location, ownership, kid weight at birth, 3 months, 6 months, adult (doe) weight, dam's chest girth, dam's condition at the time of assessment, dam's litter size history, and dam's kid-survival history.

Hindi (and English) was implemented as the user interface language to facilitate data recording performed by the veterinarians and para-workers of the Project Mesha field team. The mobile Dtreo application platform has enabled the field data collectors to collect data offline with ease, on their tablets or mobile phones while in the villages. The data, after being validated and stored, is used by Dtreo in the calculation of an overall index score for buck kids. The criteria used in the scoring system include the 100 days weight of each buck kid plus four traits of the kid's dam: chest girth, condition at the time of assessment, litter size, and kid survival. The outcomes are made available through a Dtreo report to the field team for primary selection.

At early 2023, 91 bucks have been selected based on the index scores and placed for mating. The average daily gain up to 100 days of age of the progeny of selected bucks was found to be 26.8% higher than the progeny of free-roaming bucks (weighted average based on number of progenies per buck). Improved traits of the progeny sired by the breeding bucks as opposed to the progeny born from mating does to the roaming bucks in the villages has motivated several villages to join the program. There is also an increased demand for selected breeding bucks within the villages for mating.

Dtreo has proven useful for private operations in the New Zealand dairy sheep industry. Dairy sheep is a rapidly growing industry in New Zealand; however, compared to other livestock industries, the field is still relatively niche, with few established options for flock recording. Since 2021, one of the largest sheep milking groups in the southern hemisphere has been using Dtreo as their breeding platform to integrate data collection on-farm, genetic evaluations, and selection indexes. By leveraging Dtreo's strengths, accurate genetic evaluations have been provided, with high level of engagement from commercial suppliers involved with the dairy sheep operation. Consequently, Dtreo is now set with the goal of assisting commercial suppliers to improve phenotypic culling decisions using analytic tools, and novel traits data recording.

### *Dairy Sheep operations (New Zealand)*

Thanks to its flexibility, the Dtreo platform has been shown to be a valuable support tool for other livestock systems e.g., cattle, pigs, and aquaculture also. Brief examples of how Dtreo supports farmers in cattle and pig operations are provided below.

### *Other species*

Several private-owned beef cattle operations in developed countries have invested in Dtreo as a decision support tool for the management of their business.

### *Cattle*

Data collected at farm level includes pedigree, weights (at birth, weaning, yearling age, and at slaughter), and disease and treatment events, as well as mating, sale, and slaughter dates. These are validated, stored, and summarised in real-time reports for supporting the decision-making process. The reports are location, management group, breed (in case of multi breed or crossbred), and animal specific, according to each farmer's need.

The possibility of handling groups of sires simplifies the mating strategies and pedigree recording for farmers using stock bulls in their herds. The integration of third party data allows the upload of breeding values (including GEBVs) computed by external genetic evaluation centres or breed societies. This information is processed by Dtreo providing reports tailored to the user requirements.

Dtreo has allowed these beef operators to discover insights about their data that were not possible previously. They specified relevant analytics to be promptly developed through the flexible structure of the platform, uncovering new information and supporting more informed decisions.

## Pigs

Dtreo has been adopted as the data collection and information platform by PigBoost, a collaboration for boosting development of the pig industry in Uganda. The project involves Vetline Services, with support from the Pig Improvement Company (PIC), the Roslin Institute (UK), Makerere University (Uganda), and AbacusBio. The initiative is aimed at empowering pig farmers in Uganda by improving animal production, health, and welfare through a digital transformation of the pig production supply chain.

Dtreo has been customized to store animal identification, weights, artificial insemination data, farrowing, and health data which are directly uploaded by farmers and Vetline Services technicians. With real-time monitoring of animal performances at the farm level, Dtreo enables pig farmers and Vetline to make data-driven decisions about animal management and breeding.

By connecting different actors of the supply chain, Dtreo has increased transparency and ensured the return of value to farmers.

## Conclusions

The amount and the quality of available data affects all the steps of any livestock supply chain: from management decisions at farm level, implementation of genetic improvement strategies, to market access.

A flexible digital database system combining performance data recording, analysis, and reporting has been shown to increase livestock productivity and enhance the competitiveness of farmers for different species and situations.

Farmers in both developing and developed countries are supported in the decision-making process by the availability of informative and straightforward reports, obtained by analysing validated data.

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## Challenges on milk recording and sampling in the Latxa dairy sheep breeding program

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### Abstract

Latxa is a local dairy sheep breed located in the south of the Pyrenean Mountains, mainly in the Basque Autonomous Community and the Foral Community of Navarra and it has a strong genetic link with the sheep population called Manech on the French side of the Pyrenees.

The improvement program of the breed began in 1981 and in these 40 years it has been adapted to the needs of the moment, with the general objective of improving its productivity to increase the economic profitability of the breeders, maintaining the traditional production system, rusticity and adaptation to the environment that characterizes this breed.

Nowadays, the breeding goal of the Breeding Program is more balanced and focuses on the improvement of the milk yield, fat and protein percentage and udder morphology. At the research level, functional longevity and adaptation to climate change are being studied.

This paper is a review the methods and equipment used for milk recording operations in the Latxa breed, with an emphasis on the main challenges face regarding the specificity of the dairy sheep milking routine and on the main prospects for the future. It will as well give the general lessons and experiences drawn from the other Spanish dairy sheep breeds involved in a breeding program with milk recording.

*Keywords: Latxa, dairy sheep, milk recording, breed association, Spain.*

Latxa is a local dairy sheep breed of the Basque Autonomous Community and the Foral Community of Navarra, in the north of Spain and on the border with France. The census in 2022 was of 135.143 sheep in 5.530 flocks in the Basque Community and 125.970 sheep in 1.444 flocks in Navarra.

In the last 15 years there has been a big decrease in the number of sheep but not in number of flocks and nowadays 400 flocks are milking flocks and the rest have Latxa sheep as a complement to another activity, mainly the industry.

### Introduction

Table 1. Evolution of number of Latxa sheep and flocks.

Year	2005	2010	2015	2021
Ewes	409.730	364.169	301.038	261.113
Flocks	6.934	7.747	7.149	6.974

## Breeding program

The breeding program is managed by CONFELAC, a Confederation of the four member breeders' associations: ASLANA in Navarra, AGORALA in Araba, ELE in Gipuzkoa and ACOL in Bizkaia.

The selection program is aimed at milking flocks and although it is considered a single breed, there are three recognized varieties, being the distribution of number of animals and breeders in the Herdbook in 2022 as follow:

- Latxa Blond Face: 41.181 sheep and 95 breeders in 2022.
- Euskadi Latxa Back Face: 22.580 sheep and 70 breeders.
- Navarra Latxa Black Face: 16.584 sheep and 42 breeders.

The milk recording started in 1981 and in 1999 the sampling was introduced to improve the milk composition (fat and protein percentage) and since 2001 the udder morphology is the third improvement character.

Regarding to the genetic evaluation, in 2022 the genomic evaluation was introduced to evaluate all the characters.

## Milk recording and sampling

In 2022 the milk recording was made in 169 flocks that had 63.288 sheep and 39.692 lactations were calculated, but the evolution in the number of animals has been different for each variety:

- There is a stability in Blond Face variety.
- Stable but small population in Navarra Black Face.
- Decrease in Euskadi Black Face population.

The milk recording methodology used is AC4 in 60% of the flocks and AT4 in the 40% and the meters are volumetric in all of them except in 5 flocks where farmer's individual electronic meters are used.

All the sheep are identified with ear tag and electronically by ruminal bolus and the microchip reader device used in the milk recording is the "Gesreader" of Datamars.

Regarding to the sampling for the milk composition analysis, in 2022 58.134 samples were taken in 111 flocks, being the 65% of them. Due to the high cost of the sample collection and analytics, it cannot be done in all animals and in 2022:

- In 47 flocks all the milking sheep were sampled up to the day 130 of lactation.
- In 32 flocks the sheep on first and second lactation were sampled up to the day 130 of lactation
- In 32 flocks sheep in first lactation were sampled up to the day 130 of lactation.

The samples are identified at the time of collection with a sequential number and they are analysed in ALVO laboratory, in Navarra.

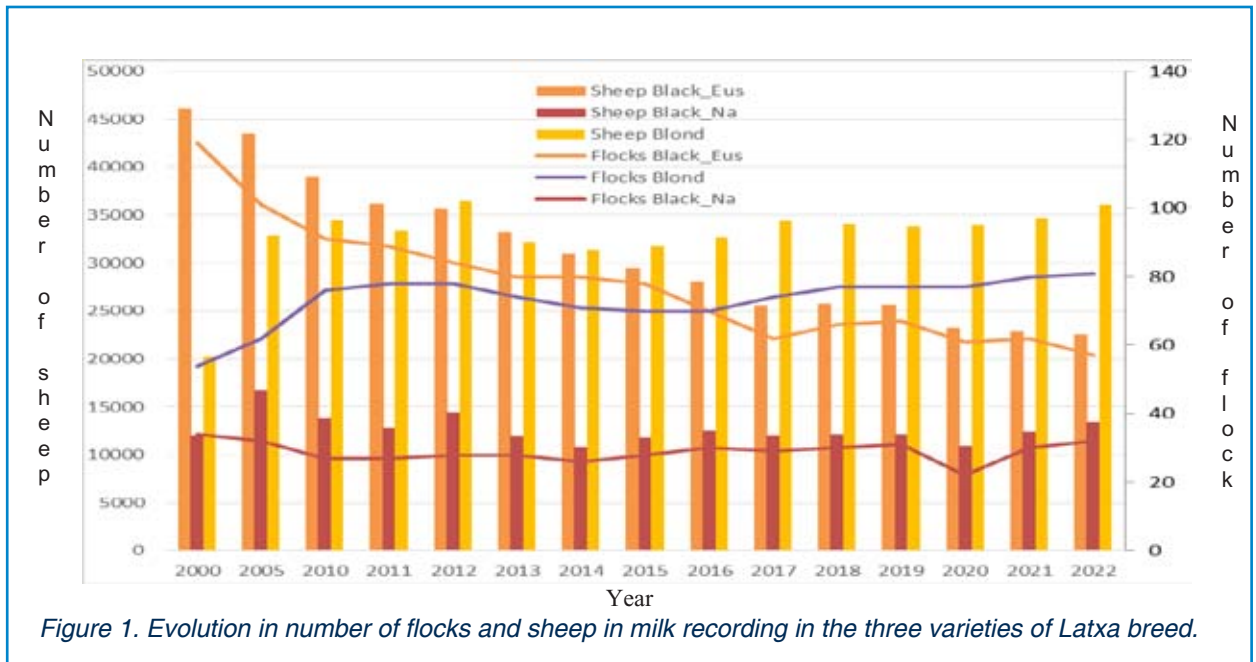


Figure 1. Evolution in number of flocks and sheep in milk recording in the three varieties of Latxa breed.



Figure 2. Pictures of the milk recording devices. Top left: Volumetric milk meter; In the middle: Microchip reader; Top right: Ear tag and ruminal bolus.

Breeders collect birth data in the birth book or through the SIRA electronic device. 25% do so using the SIRA reader, which generates a file that is automatically loaded into the central database of the breeders association.

**Data flow**

The technicians make the milk recording using the Gesreader and then all the information is unloaded in a specific program in a laptop and then dumped into the central database of the breeders association.



Figure 3. Sampling in electronic meter milking machine.



Figure 4. SIRA microchip reader used by breeders.

### Difficulties in milk recording and sampling

The most significant difficulties identified are due to the great variability of milking machines:

- 30% of the milking machines are high-line (most of them new machines) but the meters for milk recording in use today are designed for low-line milking machines and due to the high cost of each meter it is not possible to have both types for each technician.
- Some flocks have individual identification of animals in the milking machine and sheep must be identified with electronic ear tag. But the microchip reader used for milk recording can't read several animals first and then enter the milk yield and it is difficult to read the ear tag from the milking pit.
- Several problems with electronic meters: most of them are not approved by the ICAR, some of them don't measure with good accuracy and the sampling is very difficult and not representative.

Table 2. Other Spanish breeds significant data.

	Manchega	Churra	Assaf
Ewes	900.000 (600.000 milking)	300.000 (100.000 milking)	800.000
Flocks	1.000	400	1.100
Ewes in HerdBook	171.028	93.000 21.980 milk recording	140.000
Breeders in HerdBook	130	131 38 milk recording	125
Calculated lactations	119.546		74.101
Samples	51.311 in 18 breeders	51.064 all breeders	53.000 all breeders first lact.
Breeders Electronic meters	51	2 but not using	36
Udder morphology	58.046 in 123 breeders	5.202 in 15 breeders	

In addition to the Latxa, there are other dairy sheep breeds in Spain and the most important are the local breed Manchega in Castilla-La Mancha and Churra in Castilla y León. The non-autochthonous breed with the largest census is the Assaf.

## Other Spanish breeds

As a summary, the main challenges on milk recording and sampling in the Latxa breed are the following:

- Specific meters for milk recording and sampling in high-line milking machines.
- New readers for milk recording with electronic Ear Tags or bolus indifferently.
- Milking machines and electronic meters adapted and approved for milk recording and sampling.
- Individual sample identification with animal number.
- Improve information flow having more electronic readers for breeders and a web database accessible for breeders.

## Conclusions





## Infrared models for the prediction of cow colostrum immunoglobulins G concentration: phenotypic variability and relationship with colostrum yield

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The concentration of immunoglobulins G (IgG) defines the narrow-sense quality of cows' colostrum. Administering colostrum with IgG < 50 g/L is not recommended for newborns due to the insufficient amount of antibodies and concrete risk of failure of passive transfer of immunity. In a former project, we investigated the predictive ability of FTIR for the prediction of IgG using 530 colostrum samples harvested within 6 h from parturition in 9 Holstein farms. To develop infrared prediction models radial immunodiffusion kits were used for IgG determination (gold method) and spectra were collected through FOSS machineries used for official milk analyses (FOSS A/S, Denmark). Regression-based approaches, from PLS up to machine learning algorithms, were used to train and test the models and prediction accuracies showing  $R^2$  in external validation ( $R^2_v$ ) range from 0.70 to 0.85 and a root mean square error (RMSE<sub>v</sub>) around 13 g/L. With this background, the COLOXINF project was born in 2022 in collaboration with the Breeders Association of the Veneto Region (Italy). A total number of more than 4,000 samples were harvested in 95 farms following the previous protocol and the colostrum yield (CY, L) at first milking was recorded. IgG was predicted using the best performing model ( $R^2_v = 0.84$ ; RMSE<sub>v</sub> = 13.4 g/L) and, using exclusively purebred Holstein cows, we estimated IgG for each CY level (I: ≤3 L, II: 3-4 L, III: 4-6 L, and IV: ≥6.1 L). Prior to the statistical analyses, lactations were grouped (parity 1, 2, 3, 4, and ≥5), and samples with IgG deviating more than 3 SD from the mean and with CY outside the range 0.1-15.00 L were eliminated. The mixed model accounted for the random effect of the herd and fixed effect of parity, calving season (SEAS), CY level, and interactions of parity with both CY and SEAS. In a second analysis, we studied the variability of CY adjusting for parity, SEAS, and parity x SEAS interaction, with the herd as random. IgG and CY were normally distributed, averaged 102.16 g/L and 4.63 L, and were negatively correlated (-0.18). IgG was the lowest in primiparous (83.71±2.40 g/L) and the highest in parity 5 (117.15±2.75 g/L) and varied according to CY: 110.44±2.30, 104.95±2.48, 98.50±2.47, and 93.23±2.53 g/L for class I, II, III, and IV. Estimates suggested that there is dilution and that, regardless of the parity, low-producing cows (in class I) deliver colostrum with greater IgG, e.g., 17.21 g/L more compared to IV. The significant interaction between parity and CY demonstrated that the greatest IgG was provided by the less-yielding cows in parity 4 (123.30±3.32 g/L) and 5 (124.06±3.58 g/L): considering that at least 200 g of IgG must be provided to calves at the first meal, it derives that just 1.6 L of colostrum collected from these cows is sufficient to cover the requirements. As regards CY, the lowest and greatest yield were found in parity 1 (4.19±0.24 L) and 2 (5.42±0.26 L), with the latter being not significantly different from parity 3, 4, and 5.

### Abstract

*Keywords: passive transfer of immunity, animal health, young stock survival, infrared spectroscopy, novel phenotype, immunoglobulin G.*

## Introduction

The quality of bovine colostrum relies on concentration of immunoglobulins G (IgG, g/L) which can be assessed through various lab methods that differ in terms of accuracy, costs, and facility of use and implementation. Colostrum administration procedures, the quantity delivered, and its IgG level determine the risk of passive transfer of immunity in calves. Colostrum with concentration of IgG below 50 g/L is of insufficient quality for neonates, particularly if the intake is less than 4 L. Moreover, ideally the first meal should occur as soon as possible after birth and no later than 6 h of life. Colostrum IgG has been studied widely in recent years, particularly in cattle. This trait is usually determined in the first colostrum (i.e. collected between 0 and 6 h after calving) using the gold standard radial immunodiffusion assay (RID). In the field, indirect measure of colostrum quality is provided by instruments like colostrometers and refractometers (Costa *et al.*, 2023). Other indirect methods may exploit the Fourier transform infrared spectroscopy (FTIR) and could be promising and accurate for IgG determination in colostrum (Franzoi *et al.*, 2022). In fact, FTIR represents the most popular machinery of milk official analyses and is successfully used worldwide. The present study aimed to evaluate if the narrow-sense colostrum quality – IgG concentration – can be predicted from the colostrum spectra collected by the Milkoscan FT7 (FOSS A/S, Denmark). For this purpose, various regression-based approaches, from partial least square regressions up to machine learning algorithms, were used to train and test the models. Subsequently, the best model was selected and used in a prediction set that consisted of 3,379 individual colostrum spectra. Samples were collected from 95 farms that joined the COLOXINF project funded by the Breeders Association of the Veneto Region (Italy) in January 2022. For each sample in the prediction set, the quantity of colostrum yielded at first milking (CY, L) was recorded.

## Material and methods

### FTIR performance

#### Data

The experimental design and sampling protocol have been widely described in Costa *et al.* (2021). The farmers involved in the first trial were 9 and provided the initial samples used for developing the prediction model. Using bovine-specific kits, colostrum IgG was determined as described by Costa *et al.* (2021) and the spectra were collected at the laboratory of ARAV (Vicenza, Italy) equipped with the Milkoscan FT7 (FOSS A/S, Denmark) on the same samples. The spectra acquisition took place on diluted aliquots (1 colostrum:1 pure deionized water).

### Calibration and validation

Both the PLS and the Random Forest Ensemble method were used in regression for evaluating colostrum IgG predictability using the FTIR spectrum, net of water absorption regions, as predictor. The dataset was randomly split into a testing set (70%) and a validation set (30%) and the model performance in external validation included the coefficient of determination ( $R^2_v$ ) and the root mean square error (RMSE<sub>v</sub>). Preliminarily, spectral wavelengths in transmittance were converted to absorbance by taking the log<sub>10</sub> of the reciprocal. A self-built script was created using the packages *tidyverse*, *caret* and *pls* available in the R software to carry out calibrations and model validations (Liaw and Wiener, 2002; R Core Team, 2022).

In January 2022 the COLOXINF project of the Breeders Association of the Veneto Region (ARAV, Vicenza, Italy) started in collaboration with the University of Padova. The aim of the project was to collect more than 4,000 colostrum samples to retrieve spectra and predict IgG using the most performing model developed earlier (i.e., with the best performance in external validation). Using the protocol adopted by farmers in the previous experiment, individual colostrum samples were collected in 95 farms for approximately 12 months. Only samples obtained within 6 h from calving were considered and, when possible, the CY of the donor cow at first milking was recorded.

Only purebred Holstein cows were retained ( $n = 2,728$ ) and samples whose infrared-predicted IgG deviated more than 3 SD from the mean and with CY outside the range 0.1-15.00 L were removed. Prior to the statistical analyses, records were grouped for both parity (1, 2, 3, 4, and  $\geq 5$ ) and CY, with 4 levels identified:  $\leq 3$  L (I), 3-4 L (II), 4-6 L (III),  $\geq 6.1$  L (IV). An analysis of variance was used to evaluate the dilution effect of IgG by adjusting for the CY level. The mixed model accounted for the random effect of the herd and fixed effect of parity, calving season, CY level, and first-order interactions of parity with both CY and season. In a second analysis, the variability of CY was studied to estimate least squares means (LSM) for the fixed effects, namely parity, calving season, and parity x season interaction. Herd was included in the model as random.

Both the PLS and the Random Forest showed FTIR spectrum to be promising when attempting to predict the punctual concentration of IgG in cow's colostrum. While the first performed very good ( $R^2v = 0.85$ ,  $RMSEv = 13$  g/L), Random Forest, instead, resulted in a lower  $R^2v$  (0.74) although the same coefficient in training was high (0.97). When using Random Forest, predicted IgG values were more dispersed in testing than training (Figure 1) and the out-of-bag error, indicator of prediction error under bootstrapping, was equal to 0.08.

Given the apparent risk of overfitting with Random Forest, PLS was chosen for the IgG determination in the prediction set. According to the weight assigned to the wavelengths, the most important regions of the spectrum have been identified as suggested by Caponigro *et al.* (2023) using the variable importance in projection (VIP), where VIP scores  $> 1$  belong to the most important predictors (Figure 2).

Both IgG and CY were normally distributed, averaged 102.16 g/L and 4.63 L, and were negatively correlated (-0.18). IgG was significantly affected by all the fixed effects, except for the interaction between parity and CY level ( $P=0.061$ ; Figure 3). IgG was the lowest in primiparous and the highest in oldest cows (Table 1). In the case of CY level, LSM of IgG were all significantly different, being equal to  $110.44 \pm 2.30$ ,  $104.95 \pm 2.48$ ,  $98.50 \pm 2.47$ , and  $93.23 \pm 2.53$  g/L for class I, II, III, and IV. The analysis of variance revealed that autumn calving cows are those yielding the top-quality colostrum (Table 1).

Estimates given in Figure 3 suggest that there is a dilution effect. In particular, regardless of the parity, low-producing cows (CY class I) seem to deliver colostrum with greater IgG, i.e., with 17.21 g/L more compared to those in class IV. The LSM of the interaction between parity and CY (Figure 3) highlighted that the greatest amount of IgG was provided by the less-yielding cows in parity 4 ( $123.30 \pm 3.32$  g/L) and 5 ( $124.06 \pm 3.58$

## Prediction set

### Phenotypes

### Statistical analysis

## Results

### FT-MIR performance

### Variability of colostrum traits

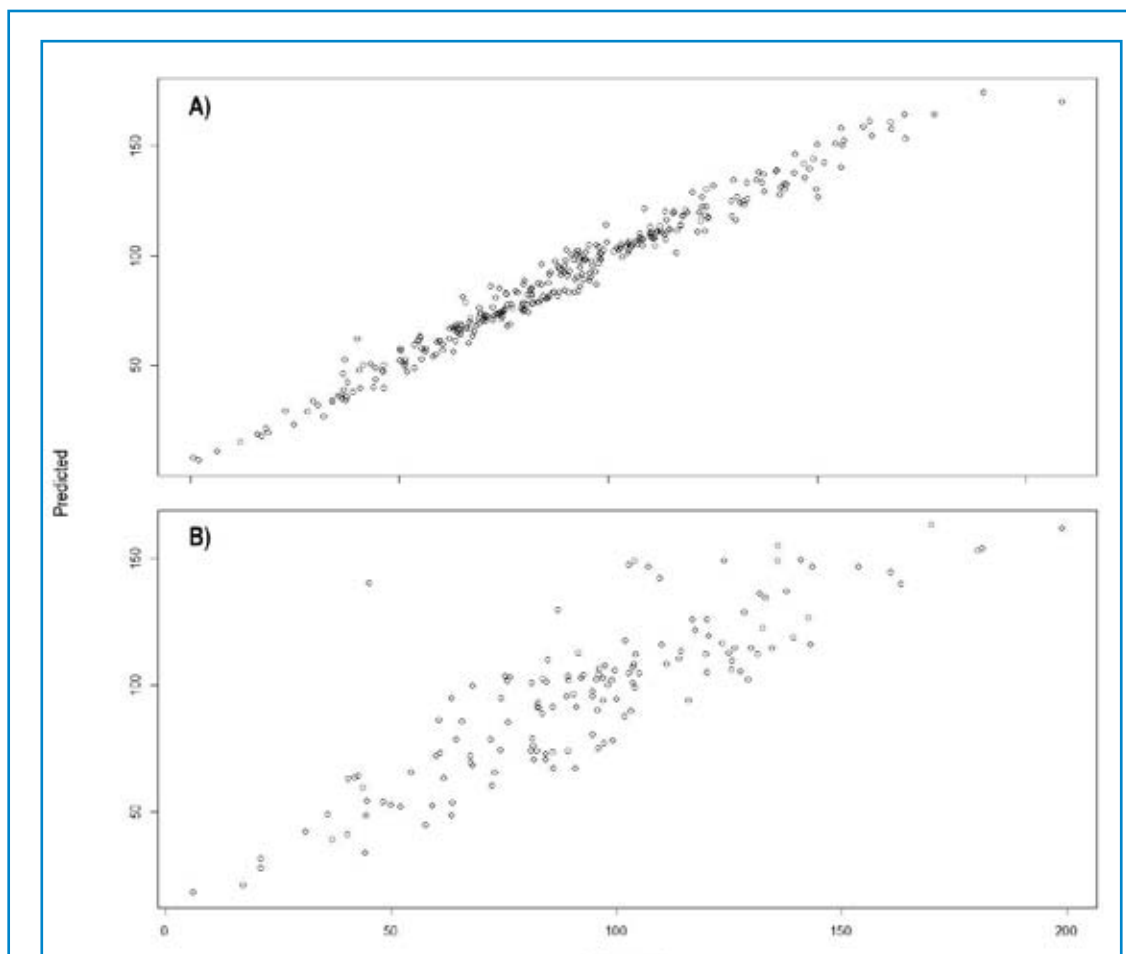


Figure 1. Predicted vs measured IgG concentration in A) training and B) testing set.

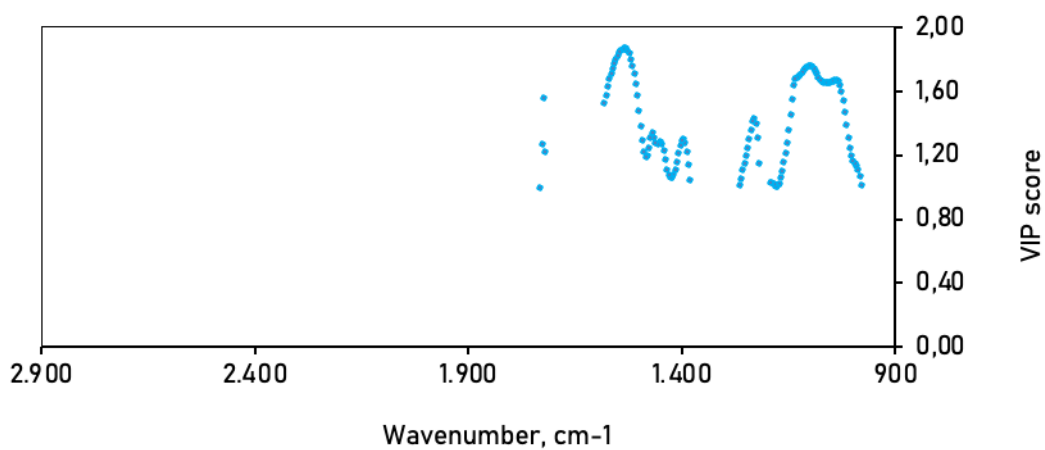


Figure 2. The most important (VIP score > 1) wavenumbers used for prediction of IgG via PLS.

Table 1. LSM<sup>1</sup> of parity and calving season estimated for IgG concentration (g/L) and CY (L).

Trait	Parity					Calving season			
	1	2	3	4	≥ 5	Winter	Spring	Summer	Autumn
IgG	83.71 <sup>d</sup>	93.14 <sup>c</sup>	105.06 <sup>b</sup>	112.74 <sup>a</sup>	117.15 <sup>a</sup>	100.86 <sup>b</sup>	98.45 <sup>ab</sup>	102.84 <sup>b</sup>	109.00 <sup>a</sup>
CY	4.19 <sup>b</sup>	5.42 <sup>a</sup>	4.96 <sup>a</sup>	4.93 <sup>ab</sup>	4.89 <sup>ab</sup>	4.48 <sup>b</sup>	5.34 <sup>ab</sup>	5.12 <sup>a</sup>	4.50 <sup>b</sup>

<sup>1</sup> Different letters within trait and within effect indicate significantly different estimates (P<0.05).

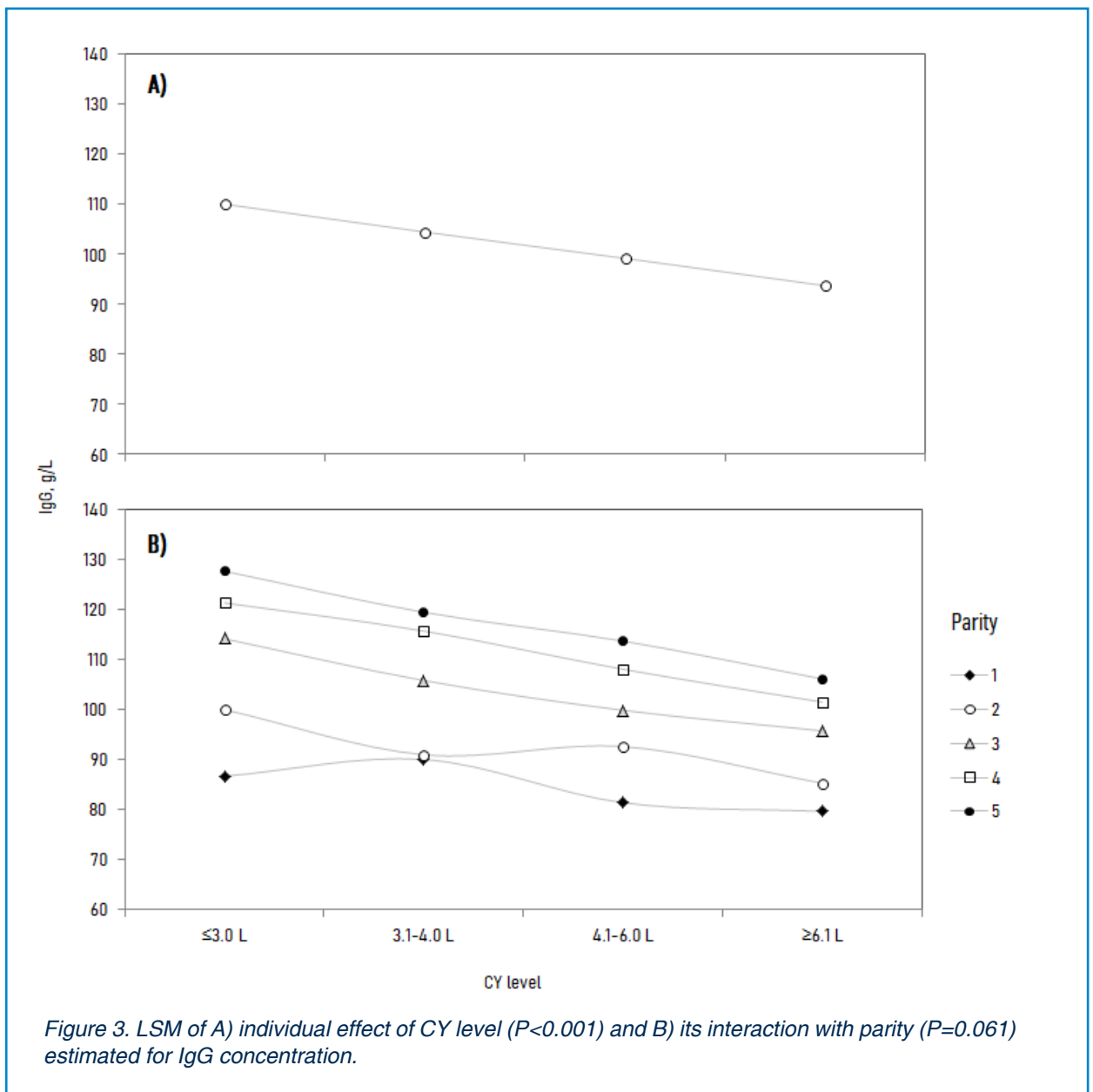


Figure 3. LSM of A) individual effect of CY level (P<0.001) and B) its interaction with parity (P=0.061) estimated for IgG concentration.

g/L). Considering that at least 200 g of IgG must be provided to calves at the first meal, it derives that just 1.6 L of colostrum collected from these cows can be considered as sufficient to cover the requirements. This volume is quite lower compared to the conventional amounts recommended (Godden *et al.*, 2019). In fact, considering that the minimum acceptable IgG concentration corresponds to 50 g/L, in general guidelines talk about at least 4 L of colostrum administered at first meal within 6 h from birth to satisfy the calf's IgG requirement (Godden *et al.*, 2019; Costa *et al.*, 2021).

Parity and calving season but not their interaction significantly affected CY ( $P=0.468$ ). Estimated LSM are presented in Table 1 and indicate that the lowest and greatest amount of colostrum is found in first- ( $4.19\pm 0.24$  L) and second-lactation cows ( $5.42\pm 0.26$  L) and LSM of parity 3, 4, and 5 fall between them (Table 1). Cows calving in summer are those delivering more colostrum compared to other seasons, with on average 5.12 L delivered at first milking. These findings are in line with Gavin *et al.* (2018) who observed that cases where the parturient cow produces low CY are more frequent in winter. As reviewed by Costa *et al.* (2023), the photoperiod plays an important role in dairy cattle metabolism, regulations, and physiology, including lactogenesis. In fact, CY is reported to progressively increase with the number of light hours, i.e., in the period from spring to summer in the northern hemisphere (Zarei *et al.*, 2017; Gavin *et al.*, 2018; Costa *et al.*, 2023).

The present study opens the room for the use of FTIR for colostrum IgG prediction and paves the way for more studies willing to explore the relationship between IgG concentration and CY in dairy cows. In fact, this can be considered as the first study attempting to investigate the variability of CY at first milking (<6 h from calving) on a large number of animals and herds adopting a standard protocol.

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## In-line real-time milk composition analysis by tunable laser spectroscopy for dairy farms

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In this work we discuss latest advancements of spectroscopic sensing based on tunable laser spectroscopy in the SWIR/MIR spectral region for individual animal-level milk composition monitoring in dairy farms. In-line milk composition monitoring in dairy farms is challenging for a number of reasons - aggressive environment, rapid milk composition dynamics during a single milking cycle, turbulent flow and challenges, associated with the fundamentals of liquid spectroscopy such as large spectral bandwidth requirements, etc. To address these challenges in our work, spectroscopic sensing was performed using BROLIS HerdLine tunable laser-based sensor, operating in the 1.9-2.5 micron wavelength range, with a very fast spectral acquisition rate of ~1000 spectra/s and a capability of sensing milk fat, milk protein and lactose in-flow within the milking line. This spectral region is known for high specificity and sensitivity vs shorter wavelengths, and the choice of the laser allows access to maximum spectral power density possible. Rapid spectral acquisition allows tracking dynamic composition change throughout the individual milking cycle. We deployed 136 sensors across 4 different commercial dairy farms with a total cow number of over 2800 cows. Depending, on the milking infrastructure used in the farm, a single sensor serves from 12 to 60 cows. Cows under investigation were being milked 2-3 times per day. Data was continuously collected for a period of several months and yielded impressive results with regard to Root- Mean-Square-Error-of-Prediction (RMSEP) of 0.2% for milk fat, 0.15% for milk protein and 0.25% for lactose. Such high accuracy provides high confidence for the discussed technology to seek ICAR certification in the very near future. In addition, collected data consisted of all individual animal milkings throughout the monitoring period and was used to build a data model for individual animal and herd-level management not only from the point-of-view of farm economics but also in terms of animal health monitoring. Early insights into the HerdLine health model demonstrate the potential ability to detect health deterioration earlier compared to the standard operating procedures currently in use. We believe, our work demonstrates first larger scale deployment of laser-based MIR spectroscopic sensor technology for dairy farms, with a real ability of real-time high-accuracy individual animal level milk composition analysis

### Abstract

*Keywords: spectroscopy, milk composition analysis, in-line sensing, mid-infrared, herd monitoring, laser spectroscopy.*

## Introduction

Infrared spectroscopy is a well-established and known laboratory technique for analysing composition of different materials and is routinely used as a “gold standard” in a variety of industries - food, petrochemicals, pharma, etc. Typical spectroscopic techniques involve direct absorption spectroscopy, which is most represented by expensive table-top Fourier- transform infrared spectrometers (FTIR), or instruments, based on Raman spectroscopy. Dairy industry is among the industries where FTIR is a standard laboratory tool for milk composition analysis and is routinely used throughout the world, when it comes to the necessity of high accuracy that is necessary for proper herd management and milk quality assessment. Downside of the technique is that it is restricted to the lab use and requires milk sampling – i.e., it is an off-line technique, and most often also off-site. Having a technology that could bring the laboratory accuracy to the farm, would not require sampling, and could analyse milk composition real-time and in-line would be hugely beneficial. In this work, we present a widely wavelength tunable laser based spectroscopic sensor for real-time in-line milk analysis. The laser is designed to work in the 1.9-2.5 micron spectral band, allowing access to 1st overtone and combination ro-vibrational absorption bands of different molecules [1]. In this work we focus on milk fat, milk protein and lactose monitoring in dairy farms.

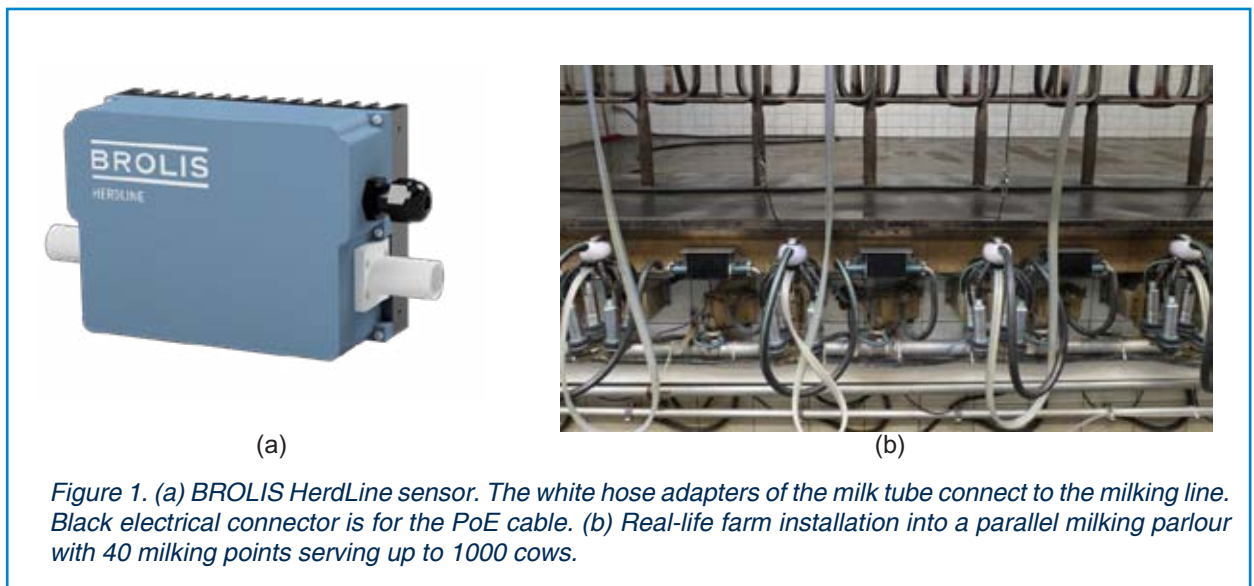
## HerdLine dairy farm study

### *Spectroscopic sensing hardware and farm installation*

This work utilizes BROLIS HerdLine sensor (Figure 1a), which is based on a patented swept- wavelength laser technology, with internal wavelength and output power locking within every spectral sweep [2, 3]. The laser houses BROLIS in-house GaSb laser chip and GaSb photodetector chip for signal collection. GaSb-based gain-chip is designed to have gain- bandwidth of in excess of 300 nm and continuous wave output power of multiple mW. The laser is strictly single mode, with a linewidth of sub MHz range. The laser line is then rapidly swept across the gain-bandwidth at a speed of 1000 spectra per second, with a resolution of 1 nm. This enables the possibility of tracking the composition dynamics within every individual milking. The sensor has a flow through tube, which connects directly to the milking line, and laser is scanning the milk flow through the tube in transmission mode. As the light passes the milk flow, it is collected by a GaSb photodetector with a cut-off wavelength of around 2.7 microns. Collected signals are converted into absorbance, and further processed to deconvolute individual concentrations of target analytes. Depending on the milking parlour, a single sensor can serve from 12 to 60 cows. The cleaning of the milk tube is performed at the same time as the milking line is washed and is designed to withstand both high temperature and high/low pH agents. Figure 1b illustrates a real-life dairy farm installation of HerdLine sensors. Powering, reading and controlling the sensors is performed via a single power-over- ethernet (PoE) cable, which connects to the local farm server. The data from the local farm server is then further streamed to the cloud infrastructure, where it is aggregated and converted into data that is streamed back to the farmer via the HerdLine software. Complete hardware set that was deployed at different farms consisted of HerdLine sensors, mounting racks, cabling and a local server.

### *Sensor performance in the dairy farms*

This study is based on 136 HerdLine sensors, deployed in 4 dairy farms in Lithuania and another European location with around 2800 dairy cows in total. Cows under investigation were milked 2-3 times a day. Reference sampling was performed in two different ways. In 3 farms, reference sampling was performed manually by collecting small volumes of milk in short time intervals during a milking cycling, to average for the intrinsic composition dynamics of the milking cycle. Collected samples were then sent to a governmental laboratory UAB “Pieno tyrimai” for certified testing. As it will be evident from the results- manual sampling for control measurements yield excess



error in averaging composition levels, particularly for milk fat concentration. In another location, the milk of the entire milking cycle was collected in a collection tank and then the sample was taken from that tank as a representative average sample and analyzed in the EUROFINS laboratory. In this location the sensors collected data for milk fat and protein only and not for lactose. Sensors collected data in the farms from over 6 months to 3 months of each dairy cow. Figure 2 shows sensor performance in a farm, where control samples were collected manually. Data represents 8 days of operation. RMSEP for fats is 0.37%, proteins 0.18% and lactose 0.28%. Note that all this data corresponds to an in-line, in-flow measurement. Figure 3 shows data for fats and proteins in site 2, where control samples were taken from the collection tank. The data also corresponds to 8 days of operation for proper comparison. RMSEP obtained for fats and proteins is notably better – 0.2% and 0.1 % respectively. We attribute this to the fact that using manual control sampling technique is prone error as it has direct influence on the averaging of the concentrations of the constituents of the entire milking cycle. Fat concentration changes dynamically during the milking cycle as shown in Figure 4, as measured by the HerdLine sensor. HerdLine’s rapid spectral capture ability allows to see the intrinsic dynamics and perform proper averaging, yielding a high-accuracy in-line in-flow measurement.

Possibility of monitoring individual cow’s milk composition allows aggregating data and constructing data trends over extended periods of time. Such trends are valuable for both individual animal and herd status monitoring, when it comes to health, nutrition, or precision selection. Figure 5 shows a 6-month data trend of an individual animal. Data demonstrates that accurate concentration measurements of milk composition allow detecting onset of acidosis/ketosis much earlier compared to activity tracker technologies. For example, the sensor depicted in Figure 5 indicated acidosis risk immediately after installation for animal No. 60257 as seen by the fat-to-protein ratio, which was not indicated by any other alternative technology. Furthermore, after

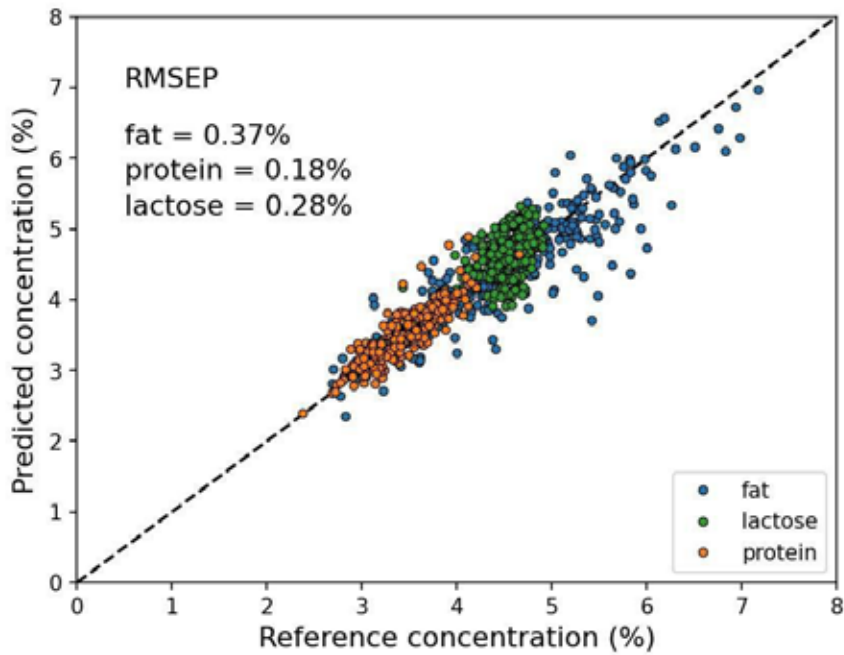


Figure 2. HerdLine sensor performance in site 1 with manual control sampling technique used. Data is plotted for all 24 sensors.

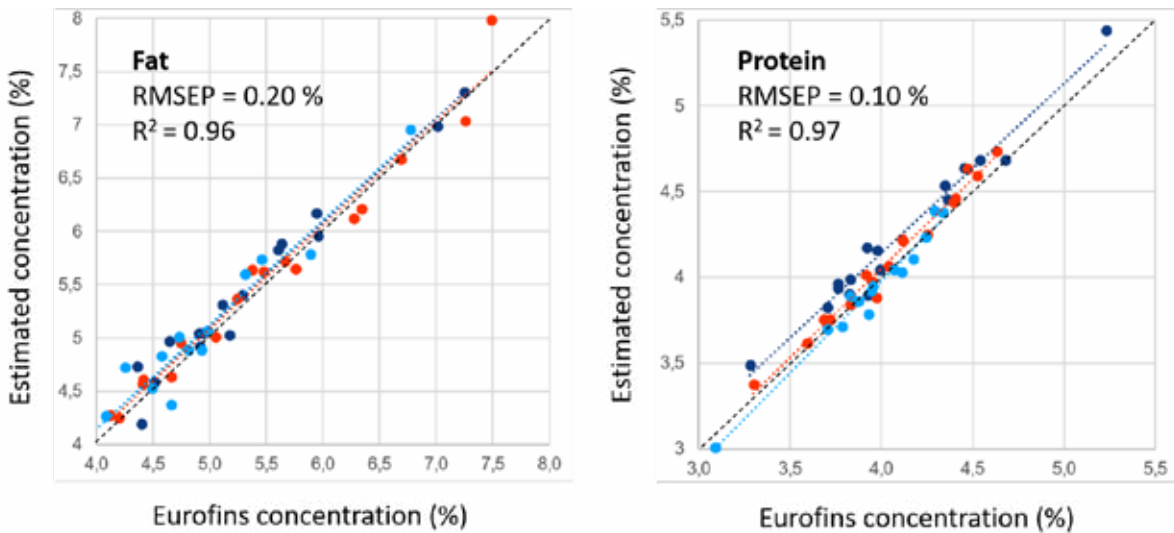


Figure 3. HerdLine sensor performance in site 4, where control samples were taken from the collection tank and analyzed in Eurofins laboratory.

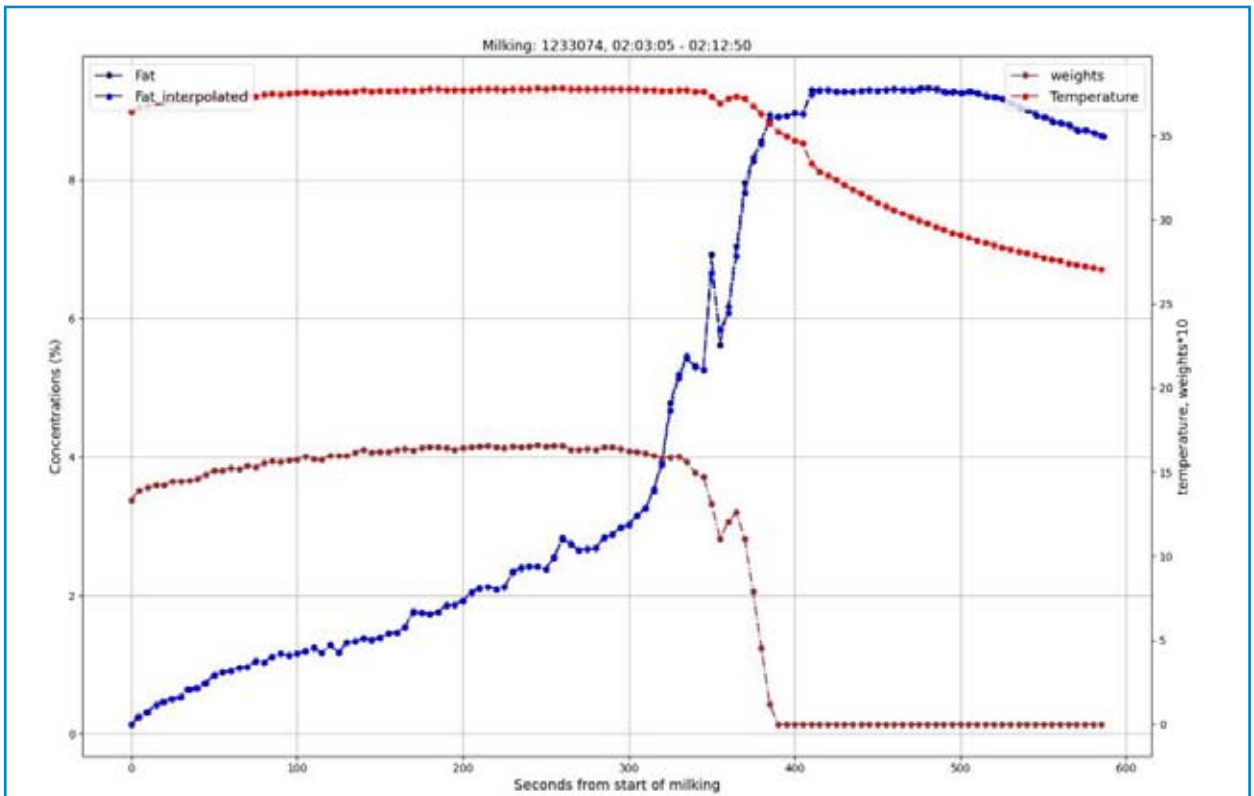


Figure 4. Fat concentration dynamics within single milking cycle as measured with a HerdLine sensor.

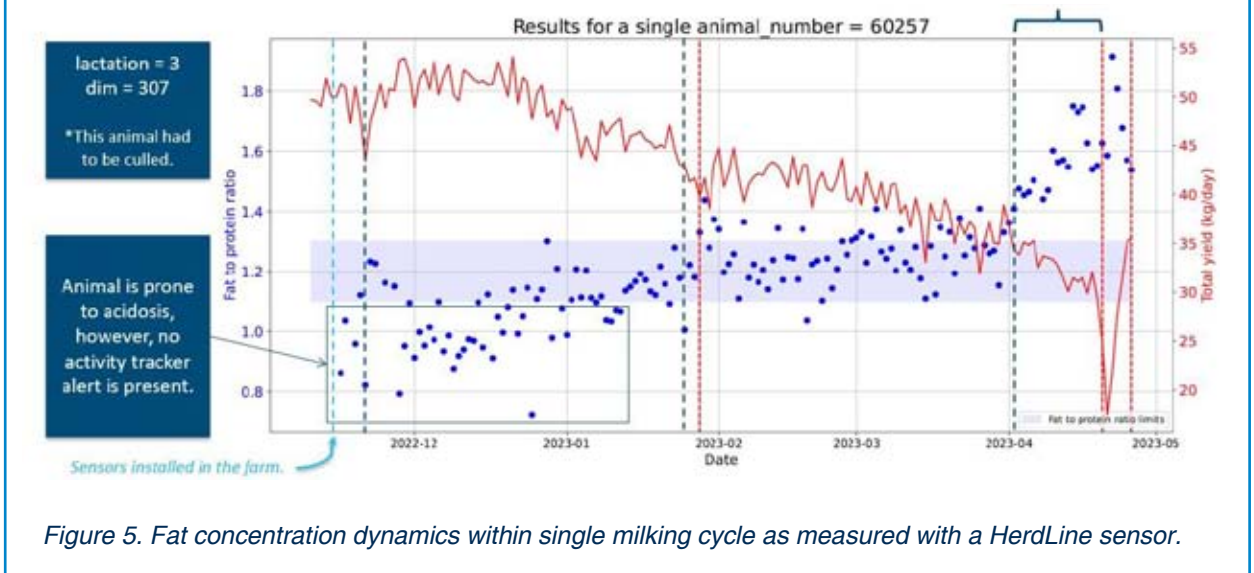


Figure 5. Fat concentration dynamics within single milking cycle as measured with a HerdLine sensor.

5 months, the cow went to ketosis state, as indicated by an increased fat-to-protein ratio. Activity tracker sent a warning only 17 days later, when the milk yield was already down by 50%. This is a powerful illustration of how precision in-line sensing technology can help dairy farmers manage the herd health in-time. In a similar manner, data trends from individual animals within the same farm can be aggregated to provide a trend for the entire herd.

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[2.] **US 11,201,453 B2.**

[3.] **US 11,177, 630 B2.**

## A time-series analysis of Alpine and Saanen goat milk productivity trends in Taiwan

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In order to project amounts and fluctuations in goat milk and milk components in Taiwan, it is necessary to analyze long-term animal data with multiple lactations. This study analyses the trend and seasonality of goat milk production and its components between 2018 and 2022. A total of 20,738 lactation records were collected from 2,376 Alpine goats and 522 Saanen goats from January 2018 to December 2022. In each record, the goat's lactation total milk, fat, and protein yields were calculated. Time series decomposition was used to determine milk productivity's trend and seasonal pattern. The results showed distinct trends and seasonality between goat breeds and lactation numbers. We observed similar seasonal and amplitude patterns across all lactations for fat, protein, and lactose yield, respectively. Higher lactation numbers also showed a larger seasonality amplitude for all yields (milk, fat, protein, and lactose). Additionally, different patterns were observed for all yields between Alpine and Saanen goats regardless of lactation. The results could be used for advising management decisions according to farm and breed productivity goals. In addition, trend and seasonal patterns can be utilized in Taiwan goat milk industry to forecast milk, milk component, and component production by specific breeds of goats.

### Abstract

*Keywords: dairy goat, milk yield, time series analysis.*

Milk yield has a substantial impact on the economic profitability of dairy goat farms. Therefore a great deal of attention is paid to the analysis of this indicator as well as the factors which influence goat milk production. At farm level the milk production and milk components are influenced by factors which impact the whole goat herd but at the same time there are factors which affect individual animals. In order to make managerial decisions, it can be desirable to assess the yield of individual goats in the context of the herd at a concrete farm. The basis for yield evaluation is modelling of a 200-day lactation yield. Using a mathematical model for the description of the lactation curve leads to the need for finding a suitable regression function for fitting of measurements of daily yield, which are performed mostly only once a month. The Wood function is the most preferred method for solution of this nonlinear regression problem (Wood, 1967).

### Introduction

To evaluate phenomena observed in the long term, which have an individual impact and occur less frequently, it is desirable to standardize the estimated 200-day lactation yield. A timeline may be convenient to capture correctly trend, seasonal, and cyclic components. The existence of these components needs to be tested at a chosen

level of significance with suitable statistical tests. Subsequently, the productivity can be stripped of substantial factors. The main goal of this study is to propose a suitable solution to the issue of yield correction in selected animals, which will make comparisons possible in the long term.

## Material and methods

### Animals and dataset

We used a dataset provided to us by the Taiwan Dairy Herd Improvement of Goat. The dataset included Holstein lactation records with following variables: milk yield (kg/goat for a whole lactation), fat percentage (%) and yield (kg/goat for a whole lactation), protein percentage and yield (kg/goat per lactation), lactose percentage and yield (kg/goat per lactation), lactation starting date, parity, location, and lactation length (d) across an 5-year period. A total of 20,738 lactation records were collected from 2,376 Alpine goats and 522 Saanen goats from January 2018 to December 2022. In each record, the goat's lactation total milk, fat, and protein yields were calculated.

### Time-series data decomposition

The time-series milk production data were decomposed into trend, season, and error to facilitate separate examination for each of them. Additive time-series data consisted of trend, season, and irregular (error) components, and the model is given as follows:

$$y_t = T_t + S_t + I_t$$

where  $y_t$  is the milk production value at time  $t$ ,  $T_t$  is the trend cycle component at time  $t$ ,  $S_t$  is the seasonal component at time  $t$ , and  $I_t$  is the irregular (remainder) component at time  $t$ .

## Results and discussion

Each decomposed time series contained monthly trend and seasonality components.

### Milk yield per lactation

Upon decomposition of the actual data into trends, it was revealed that a consistently increasing trend in Alpine goat milk production could be observed over the period 2018–2019, and the milk production reached its plateau in 2020 and gradually decreased since 2021 (Figure 1). The Saanen goat milk also had similar increase over the period 2018–2019, but were slightly decrease to 700 kg per lactation and remained relatively steady since then (Figure 2). When the data was decomposed into a seasonal component, a seasonal pattern was clearly shown, with the predominant peak in milk production per lactation occurring in Alpine goat kidding in December and Saanen goat kidding between March and September.

The mean Alpine milk production of the present study 700 kg per lactation was inferior to the national average of French Alpine goat herds (949 kg/goat; IDELE, 2017),



showing considerable potential for improvement of Taiwan dairy goat productivity. Multiple studies have reported the influence of month and season of kidding on peak milk yield, lactation persistence, and lactation milk yield of dairy goats (Montaldo *et al.*, 1997; León *et al.*, 2012). The higher milk yield in spring kidders was likely driven by the longer photoperiod during mid lactation, as December kidding would result in peak production in March when the photoperiod gradually increased, whereas November kidding would result in peak production in February when the photoperiod is reduced to nearly minimum. Goats that kidded in winter reached peak lactation earlier and slowly reduced daily yield in the subsequent months. Conversely, goats that kidded in early spring reached peak lactation much later.

For lactation milk yield, long term trends suggested a similar pattern between primiparous and multiparous Alpine goat. A plateau of 700 kg milk yield per lactation for multiparous Alpine goat was reached since mid 2019 to 2020, while the primiparous Alpine goat can only reached a trend of 650 kg per lactation (Figure 1B). The milk yield per lactation difference between primiparous and multiparous Saanen goat (650 kg vs 800 kg) was even larger than those in Alpine goat (Figure 2B). The seasonal components revealed that multiparous goat has different pattern and larger amplitude than those in primiparous goat.

Slightly distinct groups with closer long-term trend and seasonality patterns among lactations and locations. The improved classification of the original and the decomposition parameters can assist with herd and cow management decisions demonstrating the importance of seasonal patterns in production variables according to geographical location and parity.

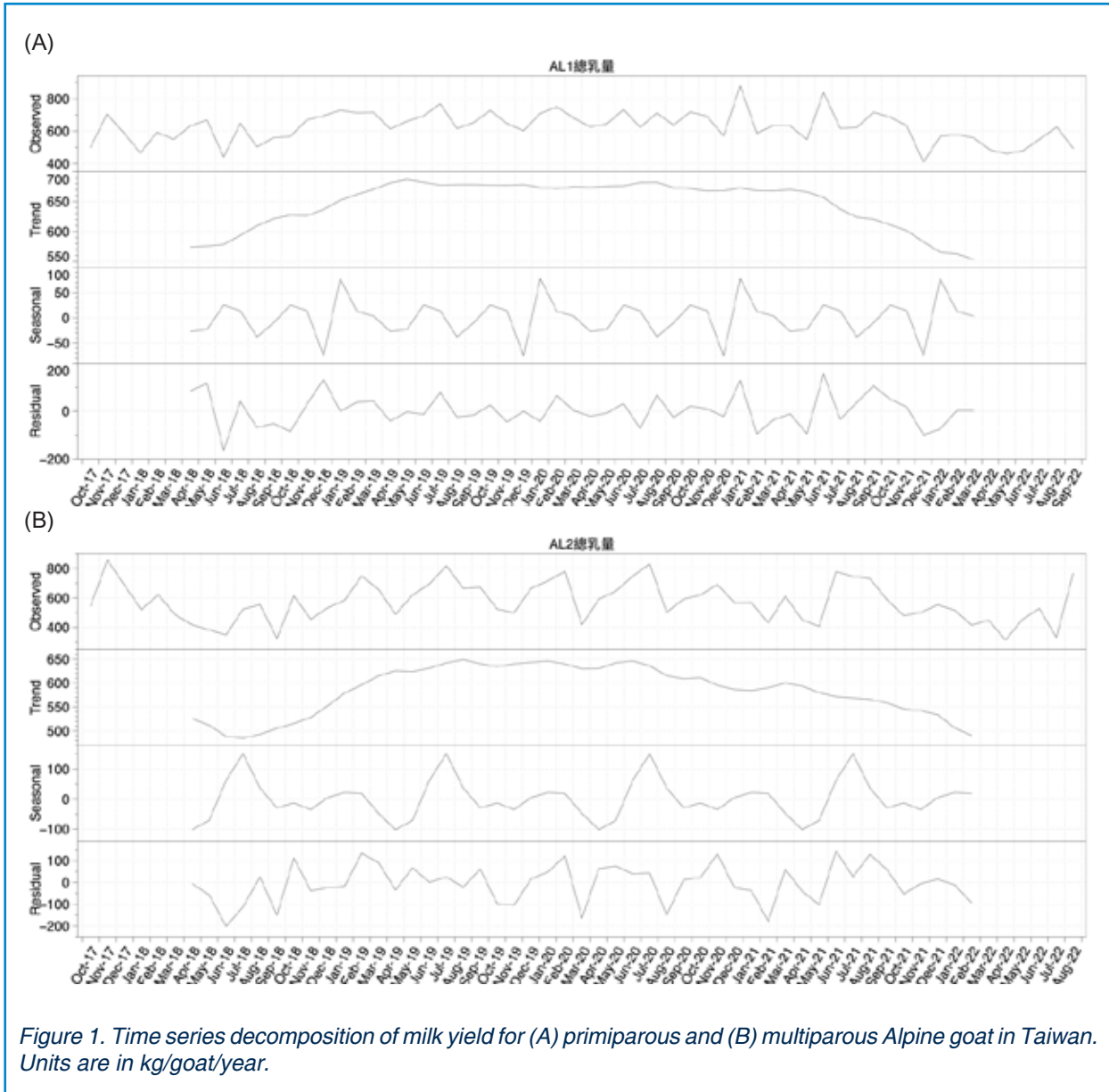
For fat, protein, and lactose yield, a consistently increasing trend in Alpine and Saanen goat milk could be observed over the period 2018–2020, when it reached its plateau in 2020 and gradually decreased since late 2021 (Figure 3-6). We observed similar seasonal and amplitude patterns across all lactations for fat, protein, and lactose yield, respectively. The overall trend for fat content is increasing since 2018, but protein, and lactose content were also increased since 2018 to 2019 and then and stabilized since then. Milk from spring kidders had a higher fat, protein, and lactose lactation yield than autumn kidders. Thus, this suggested that the decline in total milk solids yield during winter lactation was, to some extent, a synchronization effect of the decreased milk yield with decreasing photoperiod. There might be other environmental factors causing the seasonal variations such as feed and forage quality, air temperature, relative humidity, rainfall, and solar radiation, they have been suggested as significant effects on milk yield and milk physicochemical composition (Nardone *et al.*, 2010; Salari *et al.*, 2016; Clark and García, 2017).

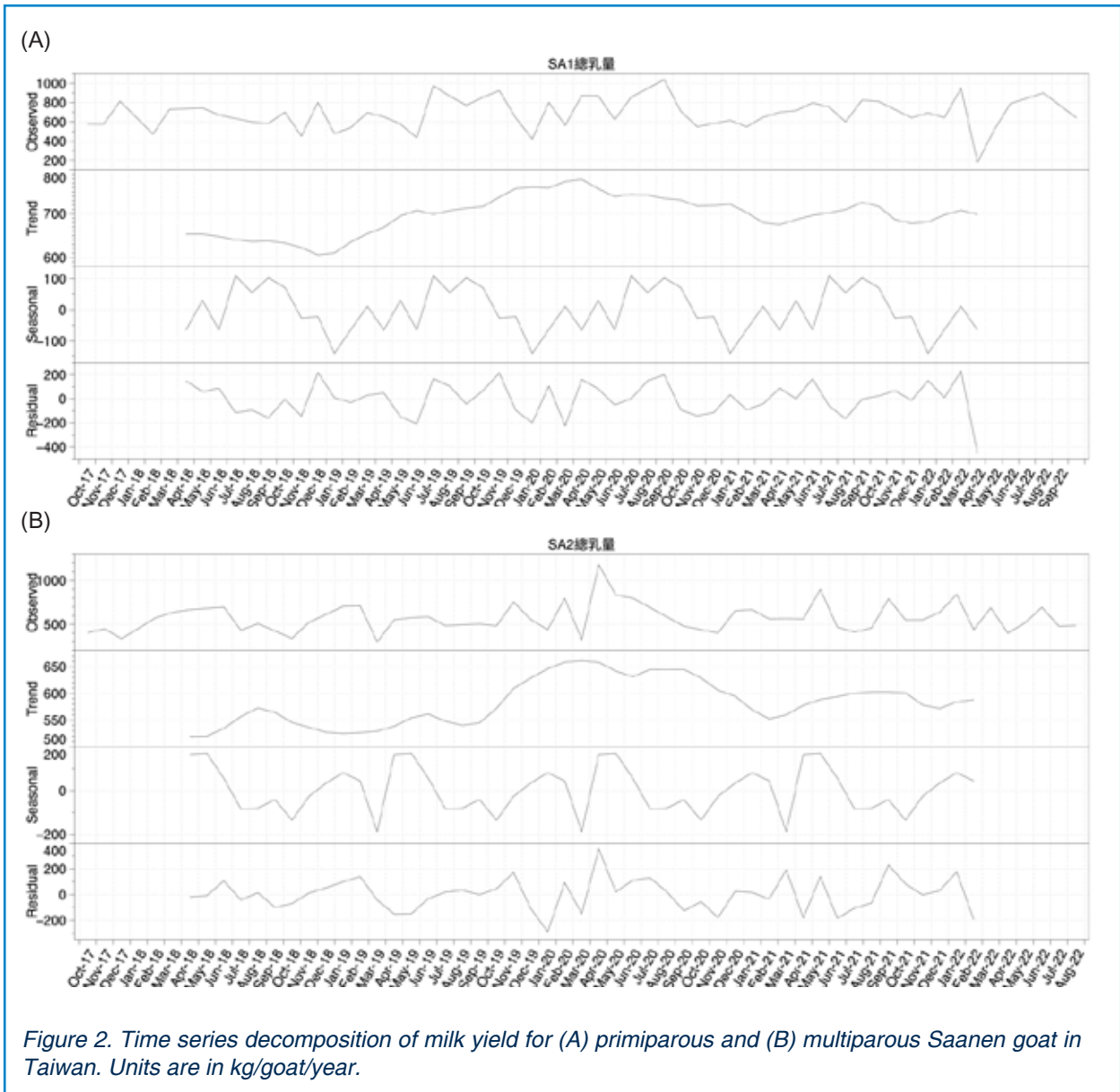
#### ***Yield and content of fat, protein, and lactose per lactation***

Dairy goat farmers in Taiwan are aiming to continue increasing milk production over the next 5 year. Our results showed that month of kidding had a considerable effect on lactation curves of dairy goats in Taiwan, indicating that light manipulation, a cost-effective and straightforward method (García- Hernandez *et al.*, 2007; Russo *et al.*, 2013), could accelerate increments in the national herd productivity. Moreover, this study has identified significant interactions between month of kidding and parity number, suggesting that the effects of such factors on milk traits are not independent of each other and that interactive effects should be considered when analyzing individual performance. From a higher-level perspective, most of these results are consistent

#### ***Industry implications***

with previous findings. However, this study not only gives additional information on how nongenetic and environmental factors can affect milk production of commercial dairy goats but, most importantly, also has produced new knowledge regarding productive traits of dairy goats raised in intensive feeding systems and managed in multiple kidding seasons per year.





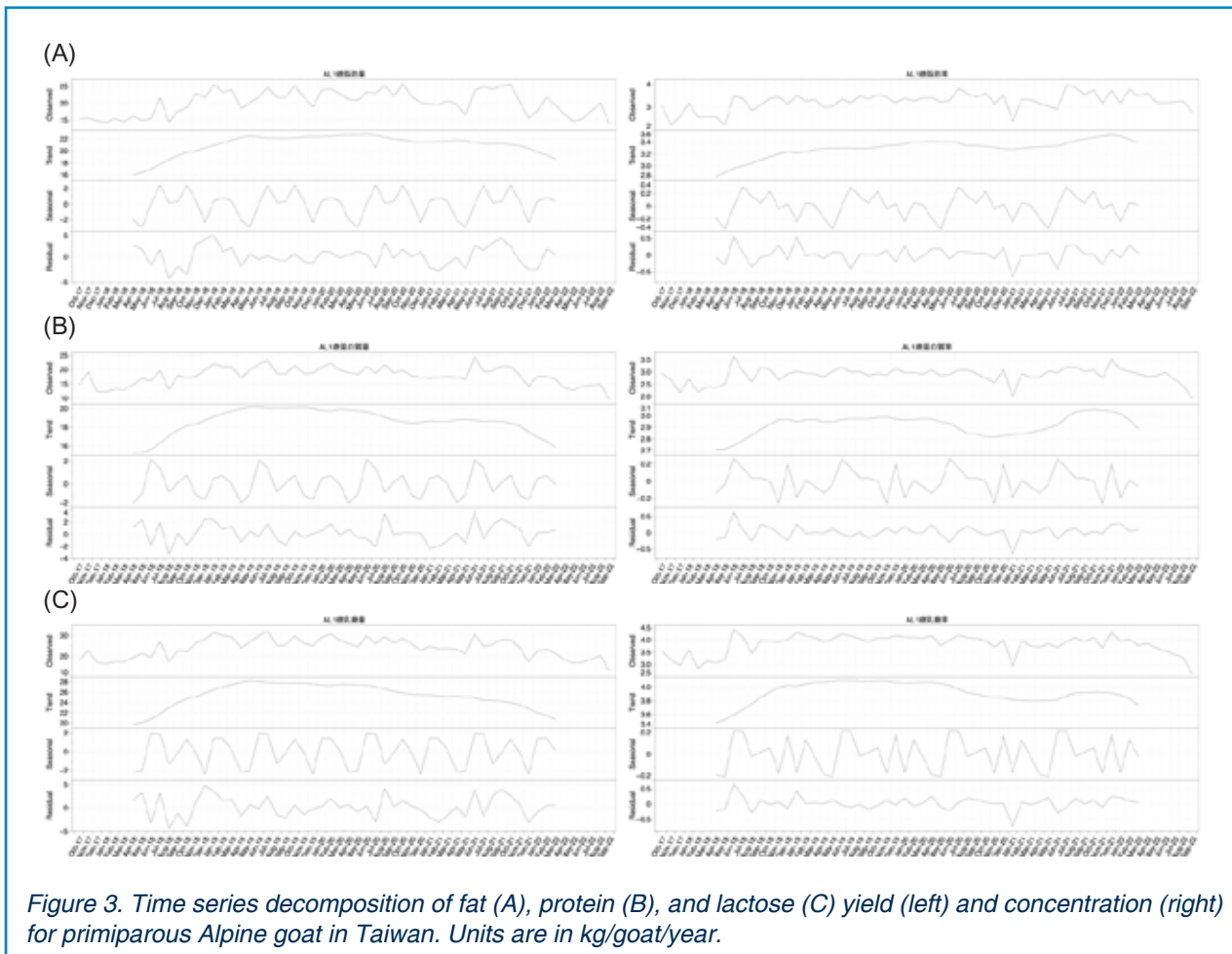
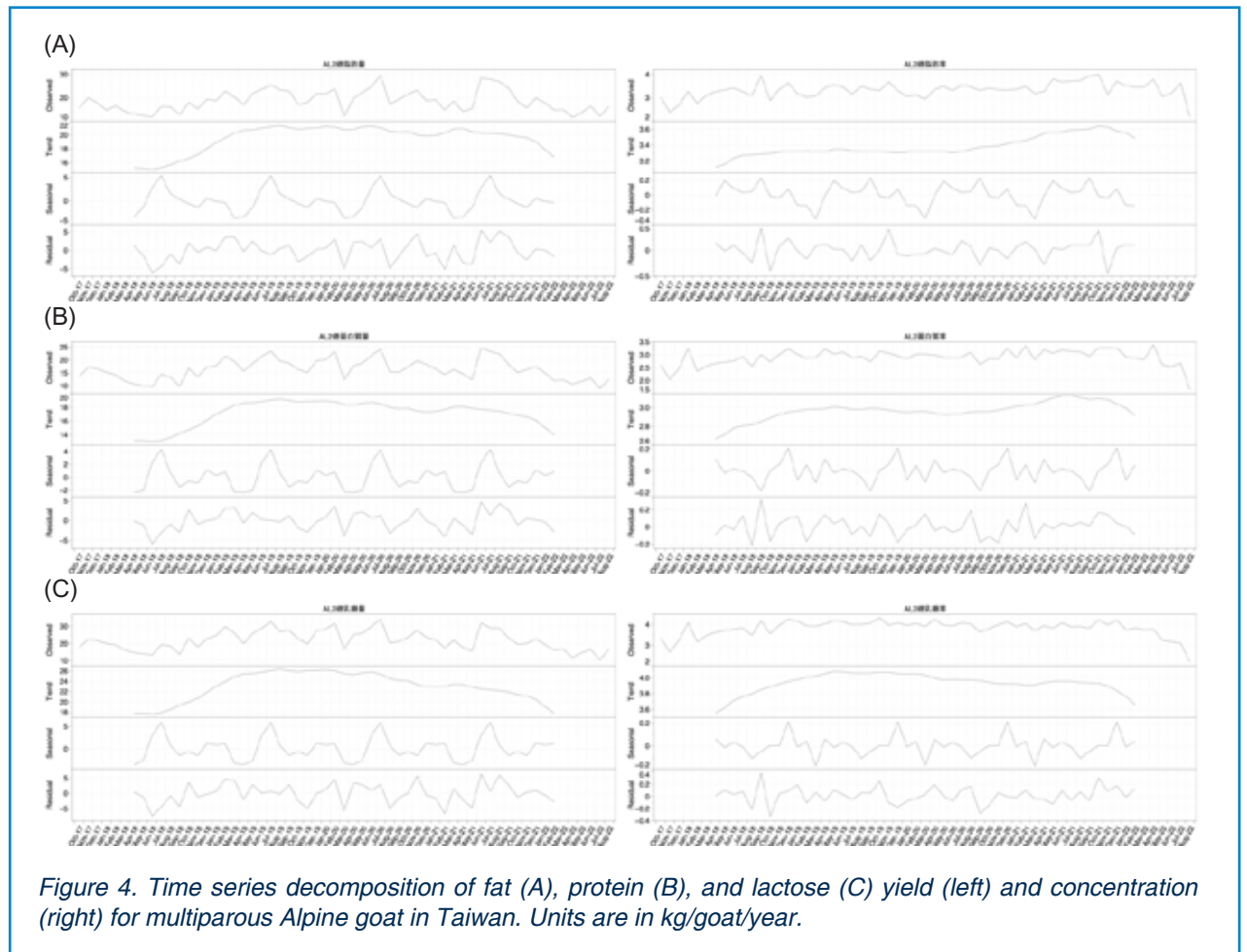


Figure 3. Time series decomposition of fat (A), protein (B), and lactose (C) yield (left) and concentration (right) for primiparous Alpine goat in Taiwan. Units are in kg/goat/year.



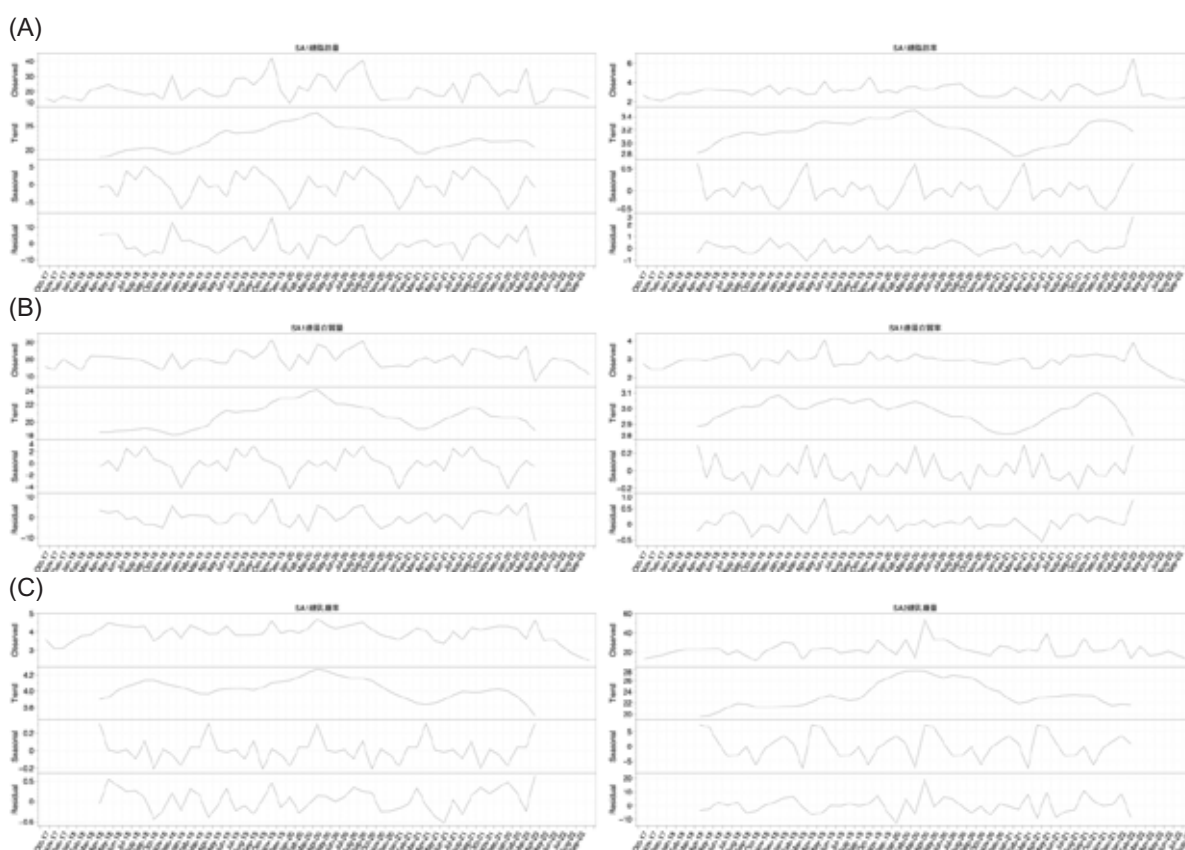


Figure 5. Time series decomposition of fat (A), protein (B), and lactose (C) yield (left) and concentration (right) for primiparous Saanen goat in Taiwan. Units are in kg/goat/year.

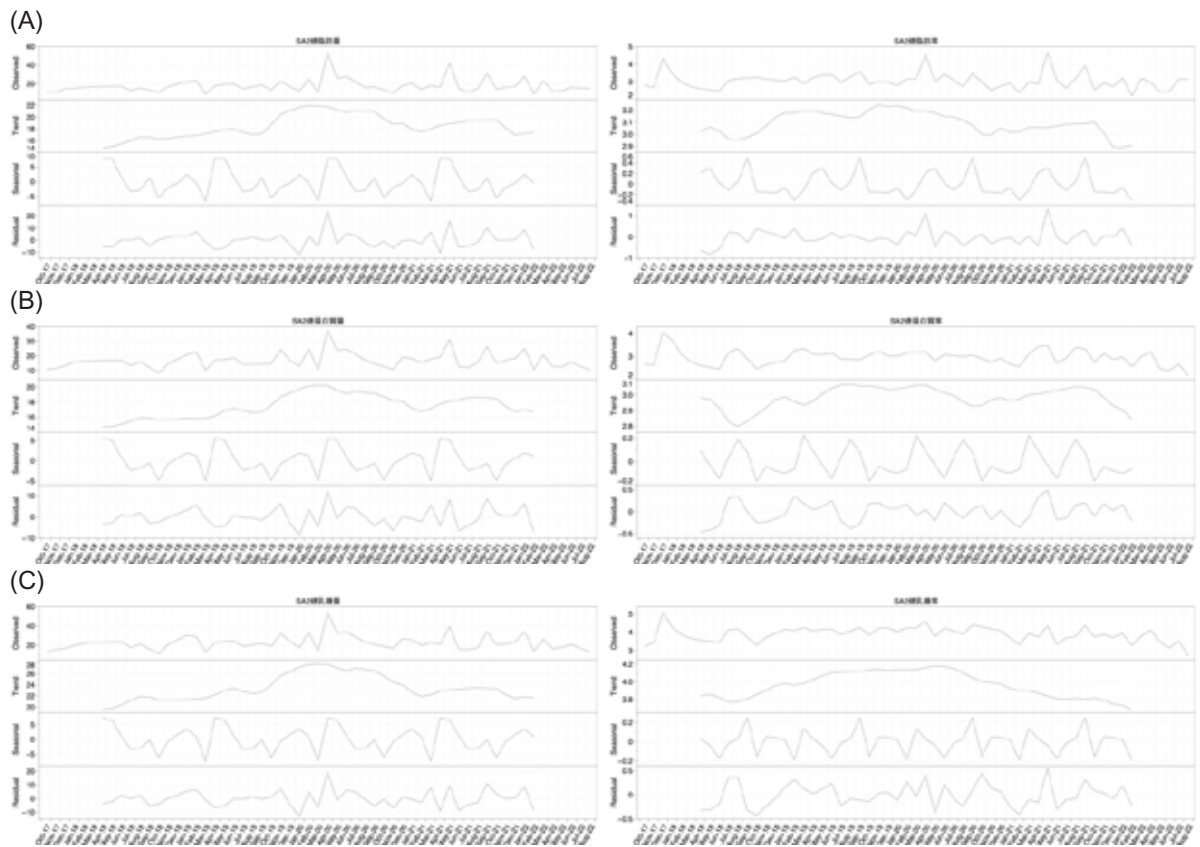


Figure 6. Time series decomposition of fat (A), protein (B), and lactose (C) yield (left) and concentration (right) for multiparous Saanen goat in Taiwan. Units are in kg/goat/year.

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## GenoCells for dairy goats: identification of the most contributing goats to the bulk tank somatic cell count by genotyping tank milk

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Efficient monitoring of bulk tank somatic cell count (SCC) is essential for dairy goat farmers to optimize milk price and herd's health. GenoCells® is a revolutionary technology that uses DNA analysis to determine with high accuracy the animals contributing to the bulk tank SCC. This method has already been tested with cows and found to be as accurate as traditional flux cytometry method. This new technology allows more flexibility and frequency than the traditional method of SSC on individual sample, creating great interest among dairy goat farmers. To validate the reliability of the GenoCells® method for goats, and assess its potential added value for farmers, a trial is currently led in eight dairy goat farms of the Pays-de-la-Loire French geographic zone.

### Abstract

*Keywords: dairy goats, cells, DNA, milk tank, individual cells responsibility.*

In recent year, there has been a growing interest among dairy goat farmers in Somatic Cell Count (SCC) monitoring due to the worsening sanitary conditions on farms. It is opposite to the technical optimization that is wanted by the new farmers and necessary to respond to the contemporary's society requirement: animal welfare and sanitary security (Koop *et al.*, 2010). The milk recording operations, that allow to get the SCC of the milk, are being as binding as the herds grow. Moreover, they don't allow the frequency and the flexibility that would be useful to apply an efficient monitoring of the somatic cells. A new method was created to identify animals that contribute the most to the bulk tank SCC by the analysis of tank milk (Blard *et al.*, 2012; Georges, Blard and Coppieters, 2014). This method which uses a linear model to determine the contribution of each animal to the DNA found in tank milk, only requires that all the animals are genotyped. The GenoCells® service has been launch in France for the dairy cows by Seenovia on the 1st of January, 2018, and has since been adopted by users in the United Kingdom, Germany, the USA and Ireland (Lenormand *et al.*, 2019; Perrin and Marg-Haufe, 2019). However, it remains unclear whether it's reliable for goats and it provides any technical added value for farmers. To address these questions, a trial was conducted in eight commercial dairy goat farms.

### Introduction

## Material and methods

Eight farms of varying sizes (50 to 700 goats) and varying levels of bulk tank SCC were selected to represent a diverse range of herd situations. Thus, the trial would be able to experience GenoCells® in numerous types of herds. The genotyping of milk (bulk tank) and cartilage (goats) samples were performed using the Goat\_IGGC\_65K\_v2 chip of Illumina. Statistical analysis applied on genotypes was performed to determine the responsibility of each goat for the cells found in the bulk tank sample. The GenoCells® cells responsibilities are compared to the reference method: the milk recording operation using Tru-test or Lactocorder to collect and measure milk and The Fossomatic FC machine to measure somatic cell count by flow cytometry technology. The cells responsibility for each goat was calculated using the somatic cell count and milk production of the entire herd. 13 comparison were made in 2022.

## Results

The linear model between the cells responsibilities determined by GenoCells® and by the milk recording operation showed a strong correlation (adjusted  $R^2 > 0.9$ ), as shown in Figure 1 and Figure 2. Although, some differences appear between the results provided by the milk recording operation and those coming from Genocells®. This observation is more marked in the most and least contributing individuals. However, the aim of this method is to provide a powerful and flexible tool for the management of the herd. Thus, the main target of the method is not the low contributors, but rather the high contributors to the tank cell. Furthermore, this picture should be nuanced as these

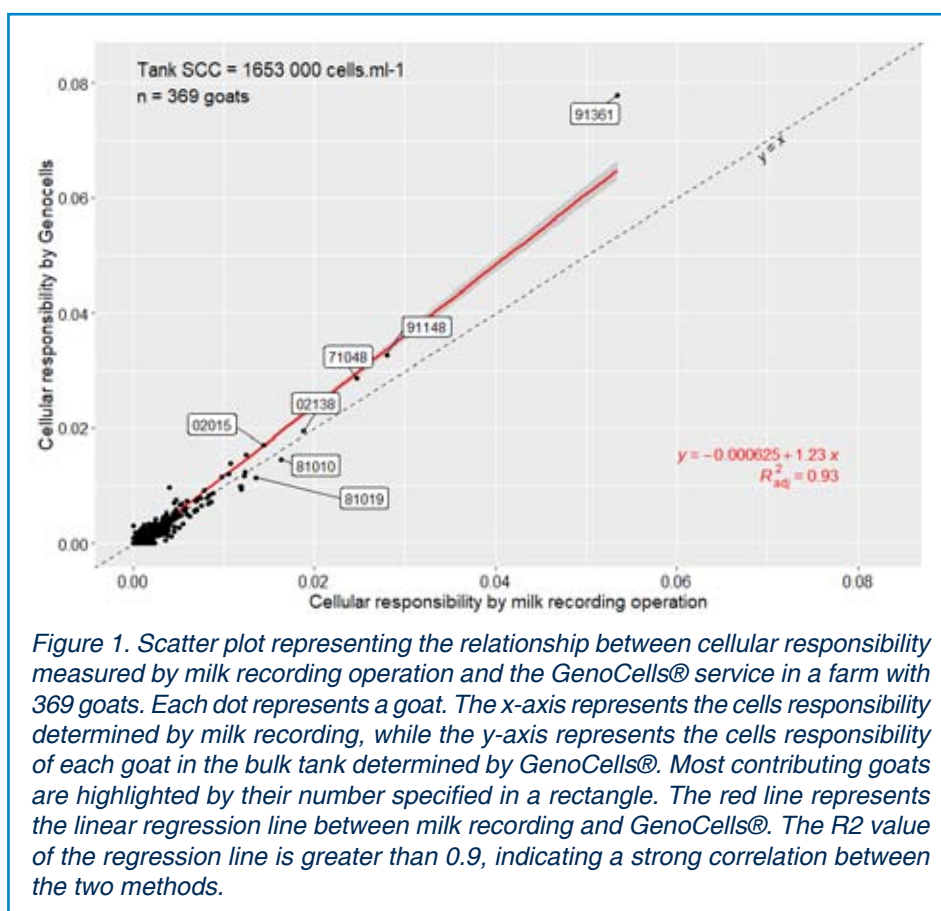
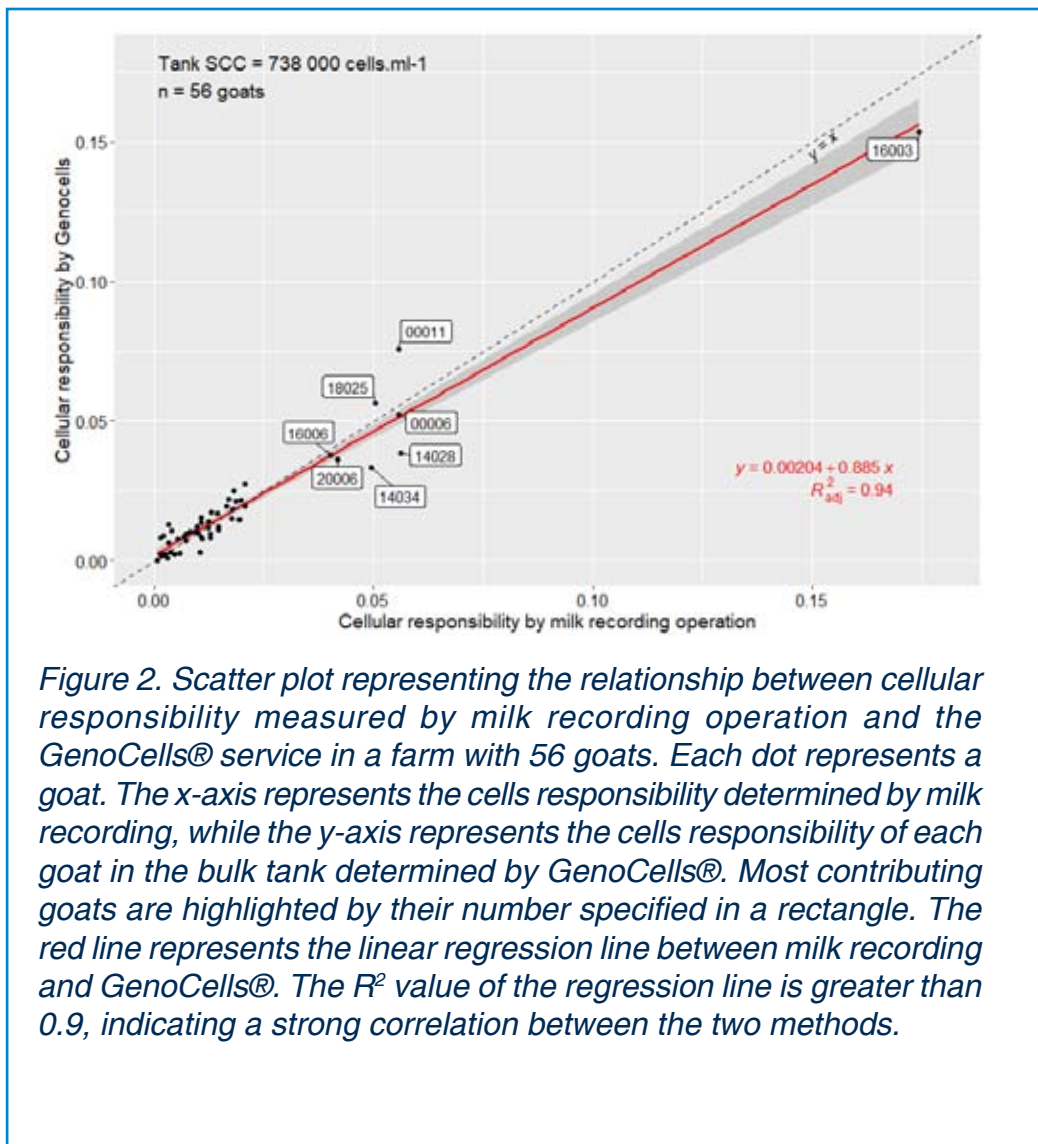


Figure 1. Scatter plot representing the relationship between cellular responsibility measured by milk recording operation and the GenoCells® service in a farm with 369 goats. Each dot represents a goat. The x-axis represents the cells responsibility determined by milk recording, while the y-axis represents the cells responsibility of each goat in the bulk tank determined by GenoCells®. Most contributing goats are highlighted by their number specified in a rectangle. The red line represents the linear regression line between milk recording and GenoCells®. The R2 value of the regression line is greater than 0.9, indicating a strong correlation between the two methods.



*Figure 2. Scatter plot representing the relationship between cellular responsibility measured by milk recording operation and the GenoCells® service in a farm with 56 goats. Each dot represents a goat. The x-axis represents the cells responsibility determined by milk recording, while the y-axis represents the cells responsibility of each goat in the bulk tank determined by GenoCells®. Most contributing goats are highlighted by their number specified in a rectangle. The red line represents the linear regression line between milk recording and GenoCells®. The  $R^2$  value of the regression line is greater than 0.9, indicating a strong correlation between the two methods.*

differences are small and do not represent real clinical differences. Furthermore, the results were consistent across herds of varying sizes and bulk tank somatic cell count levels ranging from  $738 \times 10^3$  CE.ml<sup>-1</sup> and 56 goats to  $1653 \times 10^3$  CE.ml<sup>-1</sup> and 369 goats.

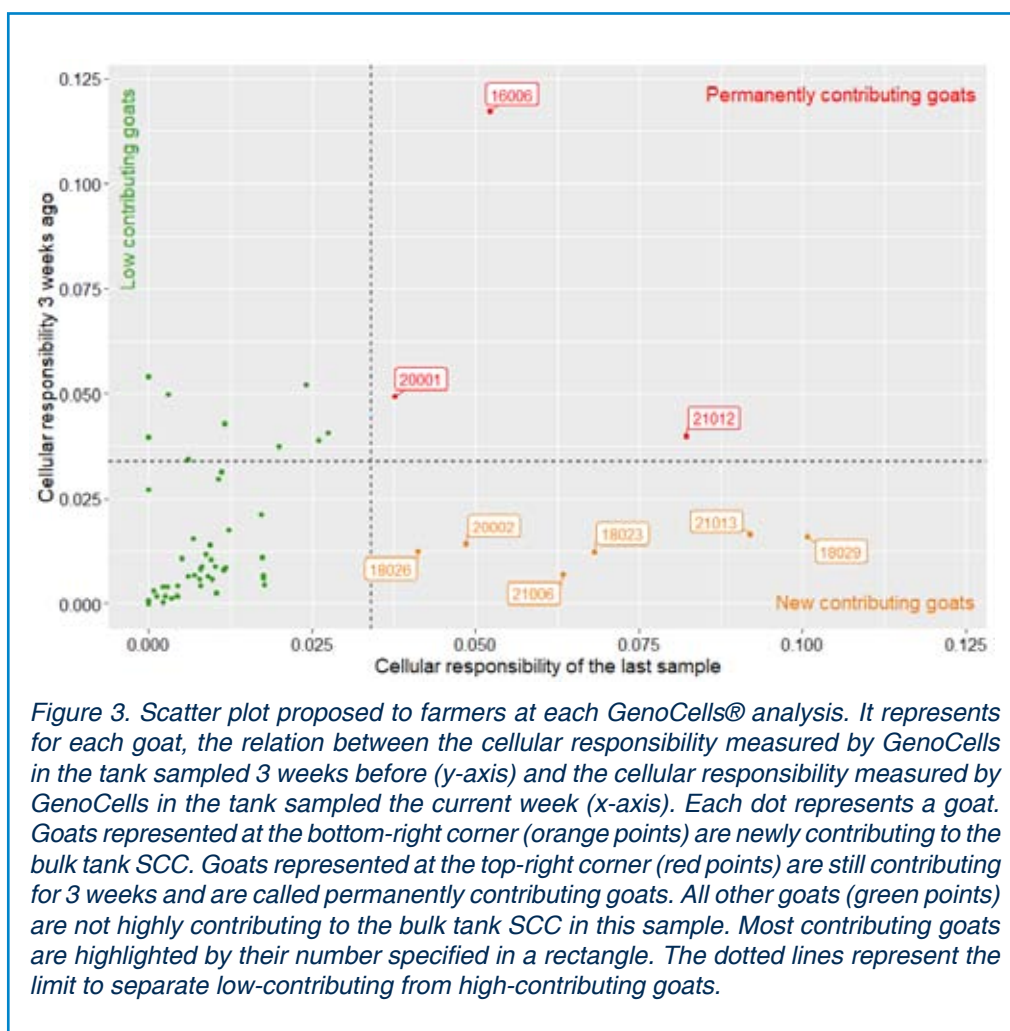
The eight farms of the trial represent a diversity of practices that impact directly their relation to the somatic cells: from 50 to 700 goats (from low to high sanitary pressure), from delivery farms to transformers (with or without prices penalty on somatic cell criteria) and from good sanitary level to bad one. Because the method needs all the goats to be genotyped, the genomic selection is an essential added value to appreciate

### Evaluation of the technical added value for farmers

with GenoCells®. Therefore, the farmer selected are also more or less interested in genetic and genomic tools.

To allow the farmers to feel the value of GenoCells®, recurrent bulk tank samples were analyzed for GenoCells® (every 3 weeks) from June 2022 and it will last until the end of 2023 (following the processes described higher). Farmers receive a result containing the evolution of the cells responsibility of the goats who contributed the most the last two samples, as presented in the Figure 3.

Moreover, genomic information about goats (indexes) are a real value of GenoCells® for farmers. Therefore, Capgenes, the French genetic selection organism, is included in the project. To evaluate the value of genomic information, Capgenes send the genomic indexes to the farmers, including for young goats.



The results of two years of GenoCells® testing will be analyzed to determine the real added value of GenoCells® for dairy goat farmers and thereafter build a profitable offer for them.

## Results

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In conclusion the GenoCells® technology allows for efficient monitoring of SCC in dairy goat herds and the trial results suggest its reliability for dairy goats. The assessment of the technical and economic added value for farmers is ongoing and could lead to a valuable tool for herd management and optimization of milk production.

## Conclusion and perspectives

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## Valorization of extensive breeding of the Spanish Merino Sheep through the quality wool improvement: Preliminary results of GWAS of fiber diameter from Whole Genome Sequences

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The autochthonous Spanish Merino is one of the most emblematic breeds in Spain for its productive importance and history. This breed was created and improved to become the sheep with the highest wool quality in the world. During the last forty years, due to the wool market crisis, the breeders crossed the Spanish Natives Merino with other merino breeds focused in meat production, which caused a decrease in the wool quality. Not often, there are around 130,000 Spanish pure merino that still have the high-quality wool inherited from the historical lines. Currently its production system continues to have a high level of extensification, so the reevaluation of its breeding has a high value in the maintenance of the pasture and/or mountain ecosystems where it is managed. For the present study, wool quality from a population of 6,200 purebred Spanish Merino (registered in the flock-book) were analysed using the Australian OFDA 2000 to determine classical wool quality criteria such as average fiber diameter and its coefficient of variation, in addition to other very important criteria for the textile industry such the comfort factor, staple length, fibre curvature or spin Fineness. The evaluation of these animals determined that the Merino breed still has a high potential to produce fine quality wool but is currently highly heterogeneous. For this reason, in addition to the classical genetic evaluations for the selection of breeding animals, the aim is to search for molecular markers that improve the reliability of these evaluations and make possible an early selection of these breeding animals. In this first approach, the most extreme animals in terms of fiber fineness were selected (44 animals with the highest fineness and 31 animals with the highest diameter). The Whole Genome Sequences of animals were obtained at an average coverage of 4.5x (Neogen, Ayr, Scotland), mapped against the Rambouillet 2.0 reference genome. Subsequently, sequences with high quality levels were used for variant calling. The GWAS performed determined the presence of 17 regions associated with fiber diameter distributed in 8 chromosomes. After the ontological assay, 39 genes were detected. Of them, the 92 % were related with protein coding and the rest with the synthesis of different types of RNA. These preliminary results require further studies using a larger population to validate them.

### Abstract

*Keywords: Spanish Merino, GWAS, NGS, fiber diameter, wool.*

## Introduction

The native Spanish Merino is one of the most emblematic breeds in Spain, not only for its economic importance but also for its impact on our history since its origin. It is believed that Romans started to improve this breed in the Iberian Peninsula looking for white fleece and fine wool 2,000 years ago. Thus, we could say that the origin of the Merino was focused on the wool quality of the animals (Sánchez 1970). Exportation of animals out of the Peninsula were forbidden until the XVII-XVIII centuries when animals were exported to other countries where the animals were used to improve local breeds (Laguna 1986). This is the reason because the Spanish Merino is considering the origin of the worldwide Merino and Merino-derived breeds (Ciani *et al.*, 2015; Granero *et al.*, 2022).

During the 1960s decade, due to the international wool market crisis, the Spanish merino census decrease drastically, and the breeders looked to change the selection objective to animals with mixed meat-wool aptitude. To achieve this objective, many breeders were crossed its sheep with foreign breeds with greater meat aptitude, such as the Merino Precoz, Fleischschaf, Landschaf, Île de France, among others. Those crossings, carried out on the Spanish Merino Type, caused the improvement of its meat production but also caused a decrease in its wool quality. Even most pure breeders stopped including wool quality as a selection objective and only several flock, characterized as historical lines, keep the morphotype, genetic and phenotype of the original animals with the exceptional wool quality (Granero *et al.*, 2022). Due to this, currently, although there are more than 3,2 million of sheep in Spain with a racial pattern closely to the Spanish Merino (Spanish Merino Type), only approximately 130.000 are actually pure Spanish Merino and are registered in the flock-book as pure by the National Association of Merino Sheep Breeders (ACME).

In Spain, it is common practice that high quality wool from purebred Merino sheep is mixed with normal quality wool from crossbred sheep, penalizing these elite fleeces, and causing a depreciation in the price of the wool. Besides, the Spanish Merino production system continues to have a high level of extensification. The animals are bred in mountain pastures or in the especial Iberian habitat called “Dehesa” in semi-liberty conditions. So, the revaluation of the wool quality as an elite product are closely linked to the maintenance of the pasture and/or mountain ecosystems where it is managed.

Currently, ACME together with SAT Merino Breeders and the textile company group Holistex are working under the “Merino Genuino®” project to revalorize the Spanish Merino wool. In that sense, ACME recently implement a breeding program aimed at improving wool characteristics typical of the original Merino.

In this sense, a wool quality control nucleus has been organized, in which around 7000 fleeces a year are analysed using the ODA-2000 machine, having already carried out three genetic evaluations for the characteristics of the fleece. A further step is the search for genetic markers associated to these demanded phenotypes to improve the reliability of these valuations. New molecular tools such as Next Generation Sequency have allowed to deep in the knowledge of whole sheep Genome. Although genomic tools have been previously used to looking for genes related to wool quality, this is to our knowledge the first work carried out on the genuine Spanish Merino breed.



In the present study, 6,200 purebred Spanish Merino (all registered in the flock-book) belonging to 24 herds were used. Wool of all the sheep were collected from the same costal area. The samples were sent to the Centre of Selection and Reproduction Animals (CENSYRA) in Badajoz where were analysed with Australian OFDA 2000. Fiber Diameter (FD), Standard Deviation (SD), Coefficient of Variation (CV%), Comfort Factor (CF%) and Staple Length (SL) were measured.

In this first approach, the most extreme animals according to the Breeding FD values were selected (44 animals with the highest fineness and 31 animals with the highest diameter). DNA were purified from blood samples. After the quality control made with the Thermo Scientific™ NanoDrop™ One (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), the DNA were sequenced using the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, California, USA) in NEOGEN Genomics (Lincoln, NE, USA).

The quality control of the raw data was conducted with fastqc V0.11.9 software. The remaining high-quality sequences were aligned with the GCA\_016772045.1\_ARS-UI\_Ramb\_v2.0 sheep genome using the Burrows-Wheeler Aligner (BWA) software (Li and Durbin 2009). Duplicates were removed with Picard software (<http://broadinstitute.github.io/picard/>). Finally, only the quality sequences were retained with Samtools v1.12 software. Finally, the genotypes were called with bcf tools to generate the vcf file (Li 2011).

To the GWAS, the Multivariate Linear Mixed Model of Gemma software were used correcting by the genomic matrix (Zhou and Stephens 2012, 2014).

Statistical data of the wool parameters studied in the present work are shown in Table 1. The FD ranged between 15.2  $\mu$  to 28.5  $\mu$  with an average of 22.2  $\mu$  which means that the breed has animals with extra fine wool highly appreciated in the market, but also animals have a fine wool and even of low quality. Not often, the CV% of 12% indicate the relatively high variability within the breed. A previous characterization made by Arrebola (2002) in this same population showed a FD value of 21.31  $\mu$  (max 25  $\mu$  and min 18  $\mu$ ) and a CV% of 7%. The evolution in this two decades showed a little increase of the FD and its CV%.

The animals showed elevated CF% values with an average of 95.7 which implies that approximately the 85% of the Spanish Merino Sheep have CF% values over 90. The SL showed very differences within the population ranging from 15 to 85 mm (average of 42 mm). These results are significantly different to those obtained at the beginning of the century, when the SL averaged in 69.2 mm (Arrebola 2002). So, SL is one of the main traits that should be solved to the wool revalorization. The evaluation of these animals determined that the Merino breed still has a high potential to produce fine quality wool but is currently highly heterogeneous.

## Material and methods

### Animal Selection and Phenotype characterization

### Sequenciation

### Sequences quality control, alignment, and variant calling

### Genome wide association study

## Results and discussion

Table 1. Descriptive statistical of the wool parameters related to Fiber Diameter (FD) and Staple Length analysed (SD), obtained from the Spanish Merino population analysed.

Sample	Min	Max	Mean
Fiber Diameter ( $\mu$ )	15.2	28.5	22.2
Standard Deviation FD ( $\mu$ )	2.3	6.6	3.8
Coefficient of Variation FD (%)	12	29	17.48
Comfort Factor (%)	70.8	100	95.7
Staple Length (mm)	15	85	42

In this preliminary GWAS analysis, focused on the fiber diameter, our results show the presence of 17 regions associated with FD, distributed in eight chromosomes (1, 2, 3, 4, 10, 11, 19 and 22; figure 1). The chromosome 1 accumulated 9 of these 17 regions. After the ontological assay, 39 genes were detected. Of them, the 92 % were related with protein coding and the rest with the synthesis of different types of RNA. These preliminary results require using a larger population to validate them and further studies going deeper into the implicated genes.

## Conclusions

The revaluation of this natural fiber will allow, on the one hand, the protection of the natural areas where the animals are raised and, on the other hand, the creation of a quality product that competes with the best wools in the world. The Spanish Merino Type has lost much of its wool quality due to crossbreeding for meat production purposes. However, the pure Spanish Merino was left out of these practices and has proven to have a high potential to produce quality wool, although it has also lost some wool quality and, above all, its variability has increased in recent decades. The search for markers related to wool parameters will help in the selection of animals with this elite wool.

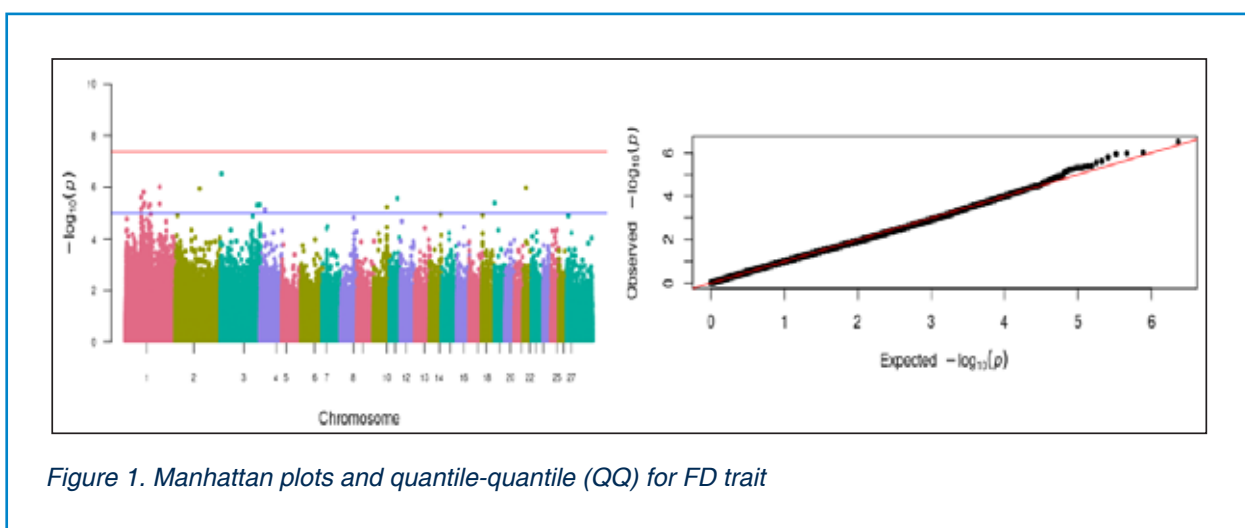


Figure 1. Manhattan plots and quantile-quantile (QQ) for FD trait

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## The state of the art about the development of mid-infrared based fatty acids predictions and their applications along the dairy food chain

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Milk mid-Infrared (MIR) spectrometry has been used since the 1980's to analyse milk components. Today advanced analytical techniques and powerful data processing tools allow additional value to be derived from this spectral information. For instance, milk MIR spectra can be used to quantify the fatty acid (FA) composition in dairy milk. Research into milk FA prediction has the potential to significantly impact the dairy chain, from analysis of the nutritional value of milk and also in management benefits to the farmer. However, the application of this technology at the farm level is currently limited. The ExtraMIR project aims to reduce that gap and support the dairy chain in future market needs. This research investigates the variability and reliability of reference data sets for statistical FA modelling to predict the milk FA composition using MIR spectra. Various published FA models from 8 different countries (Belgium, USA, Netherlands, Italy, Australia, Canada, France and China) are analysed using the R square mean-centred cross validation ranking method (Grelet *et al.*, 2021), also taking into account the reference sample size. The variations in accuracy of the Fatty Acid prediction models, specifically for the individual FA C12:0, ranged from 0.92 in the best examples to 0.71 in the poorest examples. This suggests that models analysed are highly imprecise and only for use in detecting extreme values. This knowledge will be combined with the various practical applications of ExtraMIR analysis in the field, where FA data is fed back into the agricultural industry, to aid and benefit farmers in on-farm management and future proofing milk production. Typically, the main constraint between application of FA models across different countries arises from the variability within farming systems. This is due to differences in climate, nutrition and breed. With research visits to Belgium, New Zealand and Canada not only were these constraints clear to witness but also the demands within the agricultural markets were different. These differences in demand generated research focuses specific to the needs of the national agricultural industries which further deviated from the aspirations of the international agricultural focus areas. This differing of objectives can also give rise to innovation within research areas and dairy consultancy organisations. Given that the benefits of milk FA's can be used as an indicator of ration quality of cattle feed, animal health and welfare concerns, environmental footprint of milk production and the technological properties of milk. This will contribute to closing the gap between

### Abstract

the existing extensive research and the application in the field from advisory service providers.

## Introduction

Milk MIR spectra can be used to quantify the fatty acid (FA) composition in dairy milk. The applications in the agricultural Research into milk FA prediction offers potential benefits to the dairy industry, including at the farm level, through analysis of the nutritional value of milk. However, the application of this technology at the farm level is currently limited.

The purpose of this study is to investigate the variability and reliability of reference data sets for statistical FA modelling to predict the milk FA composition from MIR spectra. The application potential is analysed using the R square mean-centred cross validation ranking method published by Grelet *et al.* (2021).

## Material and methods

The reliability of data sets used in 6 different published research papers, in 6 different countries, focused on statistical FA modelling predictions, was analysed using a classification table developed for mean-centred cross-validation of RPD, relative RMSE and  $R^2$ . For the purpose of analysis the data sets were converted to  $R^2$ , allowing for comparison between the studies. The ranking method of Grelet *et al.* (2021) was used to analyse the models. Phenotypes including fine milk components, blood components, status of dairy cows and technological properties of milk were used in order to perform a non-supervised K-means Near Neighbour (KNN) clustering of models, with seven clusters, following 3 parameters: their mean-centred cross-validation RPD, relative RMSE and  $R^2$ .

## Results and discussion

The seven groups of models are recorded below in Table 1, with the range of their performance indicators and their interpretation for potential applications.

### Model Analysis 1

The interpretation and cluster groupings from Table 1 have been applied to a study conducted by Grelet *et al.* (2021) in Belgium which looked into large scale phenotyping in the dairy sector using milk MIR spectra. Table 2 contains the fatty acid and the subsequent interpretation of the reference material.

When applying the interpretations from the classification groupings to the  $R^2$  values given in Table 2, the quality of the reference data suggests that the grouped fatty acids have a strong correlation, falling within the any allocation and quality control classification and that the applications in the field would be well received and factually accurate. However, C18:2c9t11 has ranked poorly and should not be used as part of any research other than to detect extreme values.

Table 1. Characteristics of the 7 K-mean clusters resulting from the classification of 57 milk MIR models following their mean-centred cross-validation RPD, relative RMSE and R<sup>2</sup> (adapted from Grelet *et al.*, 2021).

Cluster	RPD <sub>cv</sub>	Relative RMSE <sub>cv</sub>	R <sup>2</sup> <sub>cv</sub>	Interpretation for application
1	> 6	<5%	> 0.97	Any application
2	4.2 - 6	<10%	0.94 - 0.97	Quality control Quantitative screening
3	3 - 4.2	<10%	0.89 - 0.94	Quantitative screening
4	2 - 3	<25%	0.74 - 0.89	Rough screening Allows to compare groups, discriminate high or low values
5	1.5 - 2	<25%	0.55 - 0.74	Highly imprecise, can be used to detect extreme values
6	1.5 - 2	>25%	0.55 - 0.74	Not recommended
7	< 1.5	-	< 0.55	Not recommended

The interpretation and cluster groupings have Table 1 have also been applied to a study conducted by Rutten *et al.* (2009) in the Netherlands, which looked into prediction bovine milk fat composition using infrared spectroscopy based on milk samples collected in winter and summer. Table 3 contains the summer and winter milk analysis correlations alongside the cluster group ranking.

Table 3 shows that the reference data used in this study ranked very low again the cluster group ranking scores and averages around group 4 which would suggest that the data should only be used as a method of rough screening. There are some higher scores in the groupings of 2 and 3 which would then be useable as quality control and quantitative screenings.

### Model Analysis 2

The interpretation and cluster groupings have Table 1 have also been applied to a study conducted by Ferrand-Calmels *et al.* (2014) in France which investigated the prediction of fatty acid profiles in cow milk by mid-infrared spectrometry. Table 4 contains the comparison of methods used to develop calibration equations on the MilkoScan FT6000 analyser data for FA cow milk (g/100 mL of milk) on the validation set.

### Model Analysis 3

Table 2. Details of the 7 K-mean clusters resulting from the classification of 57 milk MIR models following their normalised cross-validation RPD, relative RMSE and R2 (Grelet et al., 2021).

Phenotype (Fatty Acid)	R <sup>2</sup> cv	Cluster Group	
		Ranking	Interpretation
SAT FA (g/dL)	0.99	1	Any application
C18:1cis9 (g/dL)	0.95	2	Quality control
LCFA (g/dL)	0.95	2	Quality control
MCFA (g/dL)	0.97	2	Quality control
MONO FA (g/dL)	0.97	2	Quality control
Tot18:1cis (g/dL)	0.95	2	Quality control
Total_C18:1 (g/dL)	0.96	2	Quality control
UNSAT (g/dL)	0.97	2	Quality control
C10 (g/dL)	0.91	3	Quantitative screening
C12 (g/dL)	0.92	3	Quantitative screening
C14 (g/dL)	0.93	3	Quantitative screening
C16 (g/dL)	0.94	3	Quantitative screening
C4 (g/dL)	0.93	3	Quantitative screening
C6 (g/dL)	0.91	3	Quantitative screening
C8 (g/dL)	0.91	3	Quantitative screening
SCFA (g/dL)	0.93	3	Quantitative screening
C17 (g/dL)	0.80	4	Rough screening
C18 (g/dL)	0.84	4	Rough screening
Odd Fatty Acids (g/dL)	0.83	4	Rough screening
PUFA (g/dL)	0.77	4	Rough screening
Total Trans (g/dL)	0.80	4	Rough screening
18:1 trans (g/dL)	0.79	4	Rough screening
C14:1 (g/dL)	0.68	5	Allows to compare groups, discriminate high or low values
C16:1c (g/dL)	0.73	5	Allows to compare groups, discriminate high or low values
C18:2c9c12 (g/dL)	0.72	5	Allows to compare groups, discriminate high or low values
C18:3c9c12c15 (g/dL)	0.68	5	Allows to compare groups, discriminate high or low values
FA isoanteiso (g/dL)	0.75	5	Allows to compare groups, discriminate high or low values
Omega3 (g/dL)	0.66	5	Allows to compare groups, discriminate high or low values
Omega6 (g/dL)	0.72	5	Allows to compare groups, discriminate high or low values
Tot18:2 (g/dL)	0.69	5	Allows to compare groups, discriminate high or low values
C18:2c9t11 (g/dL)	0.74	6	Highly imprecise, can be used to detect extreme values



Table 3. Validation coefficients of determination ( $r^2$ ) for individual and groups of fatty acids expressed on the basis of milk and fat for all scenarios (Rutten *et al.*, 2009).

Trait	Fatty Acids - Milk (g/dL)											
	AA	AA Cluster Group Ranking	WW	WW Cluster Group Ranking	WS	WS Cluster Group Ranking	SS	SS Cluster Group Ranking	SW	SW Cluster Group Ranking	SW	SW Cluster Group Ranking
C40	0.91	3	0.83	4	0.78	4	0.77	4	0.82	4	0.82	4
C60	0.96	2	0.89	3	0.89	4	0.89	3	0.90	3	0.90	3
C80	0.94	2	0.85	4	0.84	4	0.84	4	0.85	4	0.85	4
C100	0.92	3	0.75	4	0.81	4	0.81	4	0.76	4	0.76	4
C120	0.85	4	0.61	5	0.71	5	0.73	5	0.62	5	0.62	5
C140	0.94	3	0.81	4	0.84	4	0.85	4	0.82	4	0.82	4
C160	0.94	2	0.85	4	0.75	4	0.79	4	0.83	4	0.83	4
C18:0	0.82	4	0.58	5	0.64	5	0.70	5	0.59	6	0.59	6
C18:1 cis-9	0.92	3	0.69	5	0.79	4	0.81	4	0.68	6	0.68	6
C18:1 cis-11	0.27	7	0.18	7	0.15	7	0.23	7	0.12	7	0.12	7
C18:1 trans-4-8	0.48	7	0.23	7	0.13	7	0.20	7	0.15	7	0.15	7
C18:1 trans-9	0.53	7	0.32	7	0.27	7	0.29	7	0.27	7	0.27	7
C18:1 trans-11	0.63	6	0.26	7	0.09	7	0.17	7	0.18	7	0.18	7
C18:2 cis-9,12	0.36	7	0.19	7	0.14	7	0.17	7	0.15	7	0.15	7
C18:3 cis-9,12,15	0.58	3	0.27	7	0.14	7	0.20	7	0.13	7	0.13	7
C6-C12	0.95	2	0.81	4	0.87	4	0.87	4	0.82	4	0.82	4
C14-C16	0.97	2	0.90	3	0.81	4	0.85	4	0.89	3	0.89	3
C18u	0.94	2	0.69	5	0.77	4	0.80	4	0.68	5	0.68	5
Ratio SFA:IJA	0.91	3	0.43	7	0.65	5	0.59	5	0.38	7	0.38	7

- AA - Calibration in half of all data and validation in the other half of all data.
- WW - Calibration in half on the winter data and validation in the other half of the winter data.
- WS - Validation of the model from scenario WW in all summer data.
- SS - Calibration in half of the summer data and validation in the other half of the summer data.
- SW - Validation of the model scenario SS in all winter data.

Table 4. Comparison of the methods used to develop calibration equations on the MilkoScan FT6000 analyser data for FA in cow milk (g/100mL) on the validation set (Ferrand-Calmels et al., 2014).

Fatty Acid	Comparison of the methods used to develop calibration equations on the MilkoScan FT6000 Analyser (Food Electric + AS Filtered, Denmark) data for FA in cows milk															
	PLS R <sup>2</sup>	Cluster Group Ranking	AGPLSR R <sup>2</sup>	Cluster Group Ranking	AGPLSR R <sup>2</sup>	Cluster Group Ranking	Electric net R <sup>2</sup>	Cluster Group Ranking	LASSO R <sup>2</sup>	Cluster Group Ranking	Ridge Regression R <sup>2</sup>	Cluster Group Ranking	First Derivative + PLS R <sup>2</sup>	Cluster Group Ranking	Wavelet + PLS R <sup>2</sup>	Cluster Group Ranking
C40	0.93	2	0.93	2	0.93	3	0.87	4	0.90	3	0.84	4	0.92	3	0.94	2
C60	0.96	2	0.96	2	0.97	1	0.93	3	0.93	3	0.84	4	0.96	2	0.96	2
C80	0.97	1	0.98	1	0.97	1	0.89	3	0.90	3	0.75	4	0.97	1	0.97	1
C100	0.95	2	0.95	2	0.96	2	0.84	4	0.85	4	0.62	5	0.96	2	0.96	2
C120	0.96	2	0.96	2	0.96	2	0.82	4	0.84	4	0.48	7	0.97	1	0.96	2
C140	0.95	2	0.95	2	0.95	2	0.84	4	0.86	4	0.74	5	0.96	2	0.95	2
C160	0.94	2	0.92	3	0.93	3	0.86	4	0.87	4	0.71	5	0.93	3	0.92	3
C180	0.85	4	0.89	3	0.85	4	0.76	4	0.77	4	0.46	7	0.85	4	0.87	4
Totaltrans 18:1	0.85	4	0.83	4	0.83	4	0.71	5	0.71	5	0.30	7	0.88	4	0.87	4
cis-9 C18:1	0.97	1	0.97	1	0.97	1	0.85	4	0.90	3	0.38	7	0.98	1	0.96	2
Totalcis C18:1	0.97	1	0.97	1	0.96	2	0.84	4	0.90	3	0.37	7	0.98	1	0.95	2
Total C18:1	0.97	1	0.97	1	0.96	2	0.84	4	0.88	4	0.38	7	0.98	1	0.96	2
cis-9, cis-12 C18:2 (linoleic acid)	0.78	4	0.76	4	0.75	4	0.58	5	0.61	5	0.45	7	0.80	5	0.80	4
cis-9, trans-11 C18:2 (conjugated linoleic acid)	0.83	4	0.83	4	0.83	4	0.58	6	0.71	5	0.21	7	0.87	4	0.78	4
C18:3n-3 (linolenic acid)	0.86	4	0.69	5	0.25	7	0.54	7	0.58	6	0.30	7	0.82	5	0.85	4
SFA	1.00	1	1.00	1	0.99	1	0.96	2	0.96	2	0.91	3	1.00	1	0.99	1
MUFA	0.98	1	0.98	1	0.97	1	0.84	4	0.89	3	0.41	7	0.99	1	0.97	1
PUFA	0.78	4	0.81	4	0.81	4	0.71	5	0.73	5	0.53	7	0.87	4	0.82	4
trans FA	0.86	4	0.86	4	0.86	4	0.74	5	0.74	4	0.26	7	0.90	3	0.88	4
n-3	0.84	4	0.79	4	0.81	4	0.67	5	0.65	5	0.26	7	0.86	4	0.84	4

Table 4 highlights the variability of different coefficient models when applied to the same reference data set. The Ridge Regression method generally ranks very low across all fatty acid types with the average cluster group being 7, suggesting this would not be a good method to use for application in the field and further research studies. The first derivative + PLS R<sup>2</sup> shows great variability in ranking scores, with multiple fatty acids groupings in the any application interpretation but also some scores are within group 5 which are less reliable and suitable for comparing groups and discriminating

Table 5. Fitting statistics of each prediction equation estimating fatty acid concentrations using the model development data sets expressed as g/100g of milk (Fleming et al., 2017)

Individual Fatty Acid	R <sup>2</sup>	Cluster Group Ranking	Interpretation
C4:0	0.66	5	Allows to compare groups, discriminate high or low values
C6:0	0.38	7	Not recommended
C8:0	0.37	7	Not recommended
C10:0	0.66	5	Allows to compare groups, discriminate high or low values
C11:0	0.21	7	Not recommended
C12:0	0.71	5	Allows to compare groups, discriminate high or low values
C13:0	0.19	7	Not recommended
C14:0	0.80	4	Rough screening
C14:1	0.61	5	Allows to compare groups, discriminate high or low values
C15:0	0.61	5	Allows to compare groups, discriminate high or low values
C16:0	0.86	4	Rough screening
C16:1	0.62	6	Highly imprecise, can be used to detect extreme values
C17:0	0.53	7	Not recommended
C17:1	0.31	7	Not recommended
C18:0	0.73	6	Highly imprecise, can be used to detect extreme values
C18:1 in-9 trans	0.60	5	Allows to compare groups, discriminate high or low values
C18:1 in-9 cis	0.79	4	Rough screening
C18:2n-6 trans	0.17	7	Not recommended
C18:2n-6 cis	0.62	5	Allows to compare groups, discriminate high or low values
C18:3n-3	0.58	6	Highly imprecise, can be used to detect extreme values
C18:2 cis-9,cis-12	0.65	5	Allows to compare groups, discriminate high or low values
C22:6n-3	0.22	7	Not recommended
SFA	0.94	2	Quality control
MUFA	0.84	3	Quantitative screening
PUFA	0.66	5	Allows to compare groups, discriminate high or low values
UFA	0.84	4	Rough screening
Short-Chain	0.72	5	Allows to compare groups, discriminate high or low values
Medium-Chain	0.90	3	Quantitative screening
Long-Chain	0.83	4	Rough screening

high or low values. The most successful method of regression in this study was AG1 PLS  $R^2$ , scoring very well throughout on most of the individual fatty acids.

#### Model Analysis 4

The interpretation and cluster groupings have Table 1 have also been applied to a study conducted by Fleming *et al.* (2017) in Canada, which investigated predicting milk fatty acid content with mid-infrared spectroscopy in Canadian dairy cattle, using differently distributed model development sets. Table 5 contains the statistics of each prediction equation estimating fatty acid concentrations using the model development data sets expressed as g/100g of milk.

The data in Table 5 shows high variability in the classification grouping, with no fatty acids falling into the number 1 grouping. There are also several fatty acids that fall into the category of not recommended, which would suggest that the reference data set used in this study does not have a good fit in to the regression model used.

#### Model Analysis 5

The interpretation and cluster groupings in Table 1 have also been applied to a study conducted by Wang *et al.* (2017) in Australia, which investigated the use of mid-infrared spectrometry to predict milk fatty acid, energy balance and methane emissions. Table 6 contains the Pearson correlations between milk fatty acids and energy balance derived using individual cow data and the prediction accuracy using MIR data on the fatty acids compared with the cluster group rankings.

Table 6 contains no classification groups 1, 2 or 3 which means that the categories of any application for quality control and quantitative screening have been removed from the analysis. The fatty acids groupings are largely focused around 4 and 5 which would fall into the rough screening and group comparison and discrimination high or low value categories. This study therefore would not be accurate enough to use in the field but would be of use as a general screening method based on the reference data set that was used.

#### Model Analysis 6

The interpretation and cluster groupings in Table 1 have also been applied to a study conducted by Zhao *et al.* (2022) in China based on the prediction of milk fatty acid content by mid-infrared spectroscopy in Chinese Holstein cows. Table 7 contains the best prediction accuracy of prediction models for each fatty acid expressed as g/100g of milk.

*Table 6. Pearson correlations between milk fatty acids and energy balance derived using individual cow data and the prediction accuracy using MIR data on the fatty acids.*

Individual Fatty Acid	R <sup>2</sup>	Cluster Group Ranking	Interpretation
Un-identified	0.54	7	Not recommended
C4:0	0.73	5	Allows to compare groups, discriminate high or low values
C6:0	0.78	4	Rough screening
C8:0	0.76	4	Rough screening
C10:0	0.72	5	Allows to compare groups, discriminate high or low values
C10:1	0.61	5	Allows to compare groups, discriminate high or low values
C12:0	0.72	5	Allows to compare groups, discriminate high or low values
C14 iso	0.68	6	Highly imprecise, can be used to detect extreme values
C14:0	0.73	5	Allows to compare groups, discriminate high or low values
C14:1	0.56	6	Highly imprecise, can be used to detect extreme values
C15 iso	0.68	5	Allows to compare groups, discriminate high or low values
C15 anteiso	0.55	6	Highly imprecise, can be used to detect extreme values
C15:0	0.72	5	Allows to compare groups, discriminate high or low values
C16 iso	0.69	5	Allows to compare groups, discriminate high or low values
C16:0	0.74	4	Rough screening
C16:1	0.62	5	Allows to compare groups, discriminate high or low values
C17 iso	0.53	7	Not recommended
C17 anteiso	0.49	7	Not recommended
C17:0	0.61	6	Highly imprecise, can be used to detect extreme values
C17:1	0.52	7	Not recommended
C18:0	0.80	4	Rough screening
C18:1 t9	0.65	5	Allows to compare groups, discriminate high or low values
C18:1 t10	0.59	6	Highly imprecise, can be used to detect extreme values
C18:1 t11	0.58	6	Highly imprecise, can be used to detect extreme values
C18:1 cis	0.63	5	Allows to compare groups, discriminate high or low values
C18:1 c9	0.51	7	Not recommended
C18:1 c11	0.65	5	Allows to compare groups, discriminate high or low values
C18:2 n6	0.56	6	Highly imprecise, can be used to detect extreme values
C18:3 n3	0.57	6	Highly imprecise, can be used to detect extreme values
C20:0	0.79	4	Rough screening
C20:1 c11	0.68	5	Allows to compare groups, discriminate high or low values
CLA	0.65	6	Highly imprecise, can be used to detect extreme values

Table 7. Best prediction accuracy of different prediction models for each fatty acid expressed as g/100g of milk (Zhao et al., 2022).

Fatty Acid	R <sup>2</sup>	Cluster Group Ranking	Interpretation
C8:0	0.75	4	Rough screening
C10:0	0.61	5	Allows to compare groups, discriminate high or low values
C11:0	0.57	6	Highly imprecise, can be used to detect extreme values
C12:0	0.79	4	Rough screening
C13:0	0.24	7	Not recommended
C14:0	0.66	5	Allows to compare groups, discriminate high or low values
C15:0	0.45	7	Not recommended
C16:0	0.64	6	Highly imprecise, can be used to detect extreme values
C17:0	0.65	5	Allows to compare groups, discriminate high or low values
C18:0	0.66	5	Allows to compare groups, discriminate high or low values
C20:0	0.52	5	Allows to compare groups, discriminate high or low values
C22:0	0.70	5	Allows to compare groups, discriminate high or low values
C24:0	0.64	5	Allows to compare groups, discriminate high or low values
C14:1	0.63	5	Allows to compare groups, discriminate high or low values
C16:1	0.54	7	Not recommended
C18:1n9c	0.60	6	Highly imprecise, can be used to detect extreme values
C20:1	0.54	7	Not recommended
C22:1n9	0.51	7	Not recommended
C18:2n6c	0.59	6	Highly imprecise, can be used to detect extreme values
C18:3n3	0.60	6	Highly imprecise, can be used to detect extreme values
C18:3n6	0.18	7	Not recommended
C20:3n6	0.50	7	Not recommended
C20:4n6	0.44	7	Not recommended
C20:5n3	0.33	7	Not recommended
LCFA	0.68	5	Allows to compare groups, discriminate high or low values
MCFA	0.64	5	Allows to compare groups, discriminate high or low values
MUFA	0.61	6	Highly imprecise, can be used to detect extreme values
PUFA	0.71	5	Allows to compare groups, discriminate high or low values
SCFA	0.66	5	Allows to compare groups, discriminate high or low values
SFA	0.66	5	Allows to compare groups, discriminate high or low values
UFA	0.62	6	Highly imprecise, can be used to detect extreme values

The data in Table 7 again shows no data falling into groupings 1, 2 or 3, which again limits the application for this of this study based on the reference data set. The common grouping for this data set is around 5, which places a lot of the individual fatty acid values in the comparison group and for use in discriminating high and low values. There are also 9 instances where the grouping falls into the not recommended category. This would suggest that the reference data set used has very limited applications in the field.

This study has highlighted that there is in some cases a lack in application value of the reference material. This has long been an issue in the industry where there is a disconnect between the amount of research that is being done and the actual application in the dairy industry. The general low scores using the cluster grouping method would suggest that there are some strengths in the research and that some studies are very positive for individual fatty acids or grouped fatty acids although few are good for both.

The variability between research studies in each country also reinforces the industry opinion that it is difficult to replicate the work of others between countries. Reference data sets are often unique to each country with nutrition, climate and milk system all leading to discrepancies between the milk quality observed in each country.

## Conclusions

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## References





## GreenFeed and Sniffer Standard Operating Procedure (SOP) in dairy and beef cattle

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Enteric methane emissions from ruminants are a major contributor to atmospheric greenhouse gas accumulation. Accurate measurement of methane production in ruminants is crucial to not only develop reliable national greenhouse gas emission inventories, but also evaluate mitigation strategies for methane emissions. Measuring actual enteric emissions in livestock is complex, expensive and time consuming. Many different research and industry bodies globally are investigating the feasibility and accuracy of a range of different techniques for recording enteric methane emissions. Amongst the techniques available, GreenFeed (*C-Lock Inc. Rapid City, SD, USA*) and sniffer systems are the most common. The objective of this study is to describe standard operating procedures for GreenFeed and sniffers in measuring enteric methane emissions in dairy and beef cattle leveraging the expertise and experience of those operating the equipment in a range of different settings; the procedures were shared and discussed through meetings organized by the ICAR Feed and Gas Working Group. Standard operating procedure items of interest include, amongst others, animal training protocols and adaptation period length, number of animals per machine, equipment troubleshooting and upgrades. Experiences collected will be part of the ICAR Methane Emission Recording Guidelines update.

### Abstract

*Keywords: greenhouse gas emissions, data-collection, GreenFeed, sniffer.*

Enteric methane emissions from ruminants are a major contributor to atmospheric greenhouse gas accumulation. Accurate measurement of methane production in ruminants is crucial to not only develop reliable national greenhouse gas emission inventories, but also evaluate mitigation strategies for methane emissions. Measuring actual enteric emissions in livestock is complex, expensive and time consuming. Many different research and industry bodies globally are investigating the feasibility and

### Introduction

accuracy of a range of different techniques for recording enteric methane emissions. Amongst the techniques available, GreenFeed (*C-Lock Inc. Rapid City, SD, USA*) and sniffer systems are the most common.

## Material and methods

Enteric methane ( $\text{CH}_4$ ) production and carbon dioxide ( $\text{CO}_2$ ) production can be measured non-invasively using a GreenFeed emission monitoring system (*C-lock Inc. Rapid City, SD, USA*). GreenFeed is an adapted feeding station that continuously measures both  $\text{CH}_4$  and  $\text{CO}_2$  concentration and the quantitative airflow in order to generate individual gaseous production. GreenFeed is equipped with a fan that pulls air from around the head of the cow with 30-40 L/s. The airflow is measured continuously by a hot-film anemometer that needs to be calibrated monthly. In the air collection pipe continuous subsamples are drawn to analyse the  $\text{CH}_4$  and  $\text{CO}_2$  concentration by nondispersive infrared sensors, which are automatically calibrated on a daily basis. Additionally, head positioning is registered by an infrared sensor. Temperature and radiofrequency identification tags specific to each cow for individual recognition are recorded as well. All variables are logged at a 1 s interval. GreenFeed measures continuously, even if there are no animals present, to correct for background emissions in the barn. The periods within a visit where the head position of the animal is correct are used for measuring gaseous emissions. Quantitative concentrations in g/d were calculated at a 1 s interval, which were then averaged per visit (minimum of 2 min).

Over years other instruments and techniques have been developed and used for research purposes. Between all instruments and techniques available, Sniffer method has assumed significance. Sniffer has been developed to measure and collect data about methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ) emissions in the breath of ruminants during milking and/or feeding. The feed bin might be in an automatic milking system (AMS) or in an automatic feeding system (AFS). Before considering all features, advantages and disadvantages of the system, it is important to remember that they have been originally designed to detect dangerous gas leaks. Breath-sampling methods are non-invasive because, once installed, animals are unaware of the equipment and animals are in their normal environment. Animals follow their normal routine, which includes milking and feeding, so no training of animals, handling or change in diet are required. Equipment is relatively cheap and running costs are negligible. In Sniffer method, gases are continuously sampled into a sampling tube installed in the feed bin or through. The other end of the sampling tube is connected to an infrared  $\text{CH}_4$  and  $\text{CO}_2$  concentration analyser. Data are collected every 1-5 seconds by the Sniffer.

This document is the report obtained from presentations, talks, chats and discussions of the Zoom meetings on 5<sup>th</sup>, 7<sup>th</sup> December 2022 and 23<sup>rd</sup> February 2023. In all meetings an introduction was made about the *ICAR Feed and Gas Working Group*, the objectives of the Working Group, including the revision of the guidelines (*Section 20 - Recording Dairy Cattle Methane Emission for Genetic Evaluation*) published two years ago. Moreover, the aim of the meeting was to share knowledge on GreenFeed and Sniffer standard operating procedure including tips, tricks and trouble-shooting data recording.

## Results

This document is the report obtained from presentations, talks, chats and discussions of the Zoom meetings on 5<sup>th</sup>, 7<sup>th</sup> December 2022 and 23<sup>rd</sup> February 2023. In all meetings an introduction was made about the *ICAR Feed and Gas Working Group*, the objectives of the Working Group, including the revision of the guidelines (*Section 20 - Recording Dairy Cattle Methane Emission for Genetic Evaluation*) published two years ago.

Moreover, the aim of the meeting was to share knowledge on GreenFeed and Sniffer standard operating procedure including tips, tricks and trouble-shooting data recording.

1. Discussion is organized in four different paragraphs as follow: Equipment description;
2. Experimental protocols;
3. Training and adaptation period;
4. Problems faced and upgrades;

The correct placement of the GreenFeed unit inside the barn is crucial.

Indoor, it is advisable to place it on solid, non-slatted floor, in a location with good ventilation. Irregular ventilation or background emissions from manure may negatively affect the estimates of methane and carbon dioxide emissions.

Outdoor, the GreenFeed have to be located in a solid place (not in the mud), with a good phone (internet) connection and a good sunlight (if equipped with solar panel). The GreenFeed unit and the grazing site have to be nearby. The grazing area should not be so large as it could impact the number of visits. It may be appropriate to install an electric fence to protect the GreenFeed unit from animals' damage, but also to avoid animals to enter by side of the GreenFeed; this was not always undertaken and warrants further investigation. A plastic and waterproof cover can be installed on the unit and a weather station on its top. Together with the GreenFeed, the trailer can also be purchased from C-Lock. This trailer is suitable for short distance moving and it is essential for placement in pasture (i.e. the trailer contains a spot where the unit can be attached, since it has to be standing solid to prevent it from getting damaged by cows, where the gas cylinders can stand and contain power supply (solar panel or batteries). If it is necessary to move the GreenFeed unit for longer distances it is essential to have a more solid trailer available and should be compatible with the legal requirements for road vehicles in the respective country.

All users agree that wooden side shieldings provided by C-Lock are not enough to contain the animal and to reduce the influence of other animals' emissions. According to user experience to reduce influence of gaseous emissions from cows to the side, a good side shielding must be 0.80-0.90 m wide and adjustable, at least 2.5 m long, both indoor and outdoor.

Some advanced users have created a "L-port gate" that closes behind the animal, so it cannot be pushed out of the unit by other cows. Cows that use this "L-port gate" are calmer (once they are used to it).

All users consider positive the ventilation noise of the GreenFeed fan. This ventilation noise is easily associated by the animals with the supply of feed, so the animals adapt more quickly.

As regards the feed to be used in GreenFeed, most users use a pellet feed with a diameter of less than 7 mm as suggested by C-Lock. Few users use feed blocks.

## Discussion

### *GreenFeed equipment description*

### Sniffer equipment description

On the market several suppliers of this product are available, but all systems share the same basic structure consisting of: gas meter, pressure inlet, flow meter, pressure outlet and a tube to suction with filter. It is essential to have a filter at level of the suction tube to avoid clogging of the cylinder with dust, saliva or feed. In the tube between the cylinder and the gas meter, whose diameter is 2-4 mm, one or more filters can be inserted which ensure the removal of impurities and debris from the animal's breath. For the correct functioning of the system and for the collection of good quality data, the aspiration pump must work with an aspiration volume of 0.5-1.5 L/s. In more complex systems and to deal with particular needs (e.g., high environmental humidity) a dryer tube can also be inserted. Generally, Sniffer systems are provided of at least two gas meters: one for the methane (CH<sub>4</sub>) and one for carbon dioxide (CO<sub>2</sub>); further gas meters could be available, e.g., oxygen (O<sub>2</sub>) or nitrous oxide (N<sub>2</sub>O). Gas cards used to estimate CO<sub>2</sub> and CH<sub>4</sub> concentrations have different sensitivities: 0-10.000 ppm for CH<sub>4</sub> and 0-50.000 ppm for CO<sub>2</sub>. Internet connection is guaranteed through a 5G modem or through a Wi-Fi router. Internet connection allows data storage or download. Connections problems can occur; therefore, a hard disk can be provided to ensure data still collected when connection is lost.

It is important to install the Sniffer sampling tube in a position that is not so visible to the animal to avoid frequent damages.

If there are power cuts, the system restarts automatically when power is restored.

### GreenFeed experimental protocols

The overall duration of the trial varies, from a minimum of 7 days up to a year, both indoor and outdoor, according to the purpose of the experimental trial. Feeding settings can vary dependent on the preferred feed gift and preferred number of measurements per day. There are two important principles that should be met: the time of a feeding period (and thus gaseous measurement) should be between 2 and 5 minutes (at least 2 minutes, preferably 3-4 minutes), and the number of visits per day should be between 2 and 8, and divided over the day. The number of cows that can visit multiplied by the number of visits per cow per day multiplied by the time per visit should never exceed 24 hours, but preferably not exceed 12 hours (which means occupation of the GreenFeed 50% of the time).

The wide variability of the experimental protocol variables can be summarized as follows:

Table 1. Indoor and outdoor protocols.

	Indoor	Outdoor
Drop dispense interval	10 - 60 seconds	10 - 30 seconds
Min. time between feeding periods	7.200 - 21.600 seconds (2 - 6 hours)	3.600 - 14.400 seconds (1 - 4 hours)
Max. drops per feeding time	4 - 25	4 - 25
Max. feeding periods	4 - 12	4 - 12

No particular experimental protocols are applied. Once installed, the system runs continuously.

Change in diet are not required.

### ***Sniffer experimental protocols***

The overall duration of the training and adaptation period last from 7 to 20 days, both indoor and outdoor. Animals that are already used to feeding boxes in the barn are generally easier to train to use the GreenFeed.

For all the adaptation period it is essential to locate the GreenFeed within the eyesight of the animals.

During training the “drop dispense interval” can be slightly adjusted, for example reduced to 10 seconds in first days of adaptation, then increased to 20 seconds and then it is possible to use the experimental protocol. Outdoor could be useful to provide a small amount of feed around the GreenFeed unit or near to the GreenFeed chute.

Using a camera installed on the top of the unit can be useful for training, so the user can drop feed when animals are near to the GreenFeed unit.

If using side shieldings, it is important to set the funnel at largest size and reduced regularly day by day up to the end of the training period and the start of the experimental trial.

In some cases, there might be animals that are more difficult to train. In this case it is good practice to guide them into the GreenFeed and evaluate their behaviour. If they are reluctant, it is advisable not to involve them in the experimental trial. Generally, 50% of the animals adapt autonomously, 25% of the animals need an intervention, and the remaining 25% are more reluctant. A good training and adaptation period provides for the success at least 70% of indoor animals and at least 50% of grazing animals.

Having non-users located beside users of machines has proven to be helpful when later training those non-users.

Spread concentrates in the chute or using salt licks may help entice animals to use the machine

When based on grazing, it is important that the machine is moved to the edge of the fresh pasture once strip wire is moved – animals should not have to move far to reach the machine as this will impact number of visits.

### ***GreenFeed training and adaptation period***

Training, handling or adaptation period are not required.

### ***Sniffer training and adaptation period***

Table 2 reports all the problems faced including solution and possible home-made upgrades found by the several institutions.

### ***Greenfeed problems faced and upgrades***

Table 2. tips and tricks.

Problem	Solution	Suggestions/upgrades
Feed clogging in the bin	Unclog the feed bin and try a more solid pellet. Prevent the feed bin from moist.	Extra molasses in feed pellet.
Motor problem	Replaced using C-Lock spare parts and assistance.	
Airflow problems	Replaced using C-Lock spare parts and assistance.	
Connectivity problem	Replaced using C-Lock spare parts and assistance.	In alternative, purchase an external Wi-Fi router or SIM-card router.
Power supply problem	Replaced using C-Lock spare parts and assistance.	
Batteries under solar panel are getting low and smart solar controllers decide battery is too flat.	Moving from lead acid to lithium iron phosphate (LiFePO <sub>4</sub> ) batteries has largely eliminated the problem. This is because LiFePO <sub>4</sub> give a constant output voltage.	
RFID identification problems	Caused by either low power supply (check and replace power supply if needed) or antenna problems (follow instructions from C-lock to find the cause and replace if needed).	
Leaky CO <sub>2</sub> cylinder and lower CO <sub>2</sub> release	Replaced CO <sub>2</sub> cylinder by C-Lock.	Make sure to never hold the CO <sub>2</sub> tool upside down when attached to a CO <sub>2</sub> cylinder.
Concentration sensor	Replaced using C-Lock spare parts and assistance.	
Shipping time and shipping issues in some parts of the world (e.g., Europe and Oceania)		List of “must have spare parts” to create a personal warehouse.  European or Oceanian dealer?
Feed bin brush chewed by a rodent	Tack on feed bin chute.	Internal component more sealed.

Despite the various problems encountered, all users consider the assistance provided by the C-Lock very good (video tutorials, operating instructions...). E-mail reply is quickly (few hours considering time zone).

It is recommended to keep spare parts in stock.

### Device waterproof

Sniffer devices are not always completely waterproof. Considering that there is electrical wiring inside and that in some cases the intervention of a technician is necessary, it is important to choose an IP65 box or to equip the Sniffer box with a waterproof coating.

### *Sniffer problems faced and upgrades*

### Clogging problems

To avoid clogging of the pipes, ideally a venting hose is provided. This pipe is connected to the compressed air outlet pipe of the AMS. When the gate of the milking robot opens, allowing the animal to exit, compressed air is blown into the venting hose. This air flow in the direction of the cylinder helps to avoid clogging.

### Animals' identification

Antenna for ID reader is not always successful. It is suggested to use AMS data identification and merge them later on.

### Calibration procedure

In many cases problems of calibration drift can occur. It is necessary to standardize zero and span calibrating procedure.

### Personnel in charge of the trial

It is essential to have a good technician who monitors Sniffer's activities on daily basis and who also knows the AMS and AFS system. Among the activities that the technician must carry out: setting up, moving equipment, daily data monitoring and download, checking up on equipment at irregularities, fixing problems, downloading AMS data and ordering spare parts. If a good technician is not available, it is essential to have an alert system (sms, e-mail) of anomalies.

### Filter replaces

Filter obturations by dust or condensation can occur. It is possible to prevent these problems changing external filter every 15 days and change internal filter every 6 months.

GreenFeed and Sniffer are two different systems, but to date they are the most reliable. The definition of SOP and ICAR Guidelines update are only first steps. Further steps will be data editing, trait definition, phenotypic and genetic analysis.

### Conclusion





## Genetic parameters and single step evaluation of sexual precocity traits in Charolais beef cattle

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Breeding a cow is an expensive process, especially due to its nonproductive period. Hence, decreasing the age at first calving may reduce the latter and improve economic efficiency of beef farms. Since 2018, a French network of 15 Charolais farmers gathered within a project called “FERTI38”, led by CHAROLAIS UNIVERS, equip their cows with neck tags (HEATIME® - MSD Animal Health Intelligence) to monitor cows behaviour. The collar collects hourly information as rumination, activity, eating time enabling detection of heat and health events. The latter are added to calving and pregnancy diagnosis information. Last, farmers are involved in validating the data collected, and recording additional information on tag replacement and cow management practices. These data were completed by insemination, gestation datasets and genotyped information. The goals of this project is to estimate genetic parameters of traits related to sexual precocity and reproductive traits in order to implement them in a single step evaluation. A dataset of 781 females with performances recorded was used. The mean age at first “Heat” was 14.8 months ranging from 8.6 months to 20.4 months. The heritability obtained for this trait is 0.36 with a standard error of 0.12. These results were implemented in a private single step evaluation to estimate EBVs and reliability using HSSGBLUP software. The EBVs were standardized to obtain a mean of 100 and 10 points corresponding to a genetic standard deviation (21.6 days). For the 25 bulls evaluated with reliability higher than 0.30, EBVs ranged from 85 and 113 and the mean reliability is 0.52 with a maximum of 0.76.

### Abstract

*Keywords: sexual precocity, Charolais beef cattle, single step evaluation.*

Non-productive periods are a loss of money for breeders, during this time, an animal is fed and raised without any possibilities to earn money. The time for a heifer to be sexually ready for insemination is one of these periods. Decreasing age at first calving could reduce a non productive period and improve economic efficiency of beef farms but also ecological impact of beef productions (Farrié *et al.*, 2008; Nguyen *et al.*, 2013).

In this study, we propose to analyse the data of a network of French beef farms in Charolais for sexual precocity traits, through genetic parameters calculations and implementation of Single Step evaluation for these traits, one of the first in France.

### Introduction

## Material and methods

### Farm network and data collection

With the project « FERTI 38 », a French network of 15 Charolais farmers are involved in since 2018. Farmers were chosen with several criteria's, around one hundred of calving per year on small reproductive periods (less than 3 months), with more than 80% of artificial insemination, collection of birth and weaning phenotypes, and age at first calving between 24 and 30 months of ages. Each farmers had to equip their cows with neck tags (HEATIME® - MSD Animal Health Intelligence) to monitor cows' behavior. The collar collects hourly information as rumination, activity, eating time enabling detection of heat and health events. The latter are added to calving and pregnancy diagnosis information. Last, farmers are involved in validating the data collected, and recording additional information on tag replacement and cow management practices. These data were completed by insemination, gestation datasets and genotyped information.

In the current study, the first trait analyzed was the "Age at first heat" for heifers with a minimum of 1 month of neck tag equipment time in order to take into account the adaptation time. The data of 781 heifers were collected and used. The corresponding pedigree with up to 4 generations contains 3,631 individuals.

## Genotypes

Available genotypes of animals with phenotypes or parents of these animals were included in this study. They consisted of 1,578 genotypes of purebred Charolais animals, 106 males and 1,472 females with 781 with phenotypes available. 53,498 autosomal SNP markers were retained from the Illumina 50K chips used routinely in France for genomic selection. Genotypes were imputed with FImpute (Sargolzaei *et al.*, 2014) with the pipeline used in the routine French national evaluation system.

### Genetic parameters calculation

Estimation of genetic parameters for the traits studied were performed using the WOMBAT software (Meyer, 2006) and the following animal model:

$$y_{ijk} = \mu + h_j + b_k + \alpha a_i + g_i + e_{ijk}$$

With  $y_{ijk}$  the phenotype measured on animal  $i$ ,  $\mu$  a general mean,  $j$   $h_j$  fixed effect of the Herd  $j$  in which animal  $i$  obtained his phenotype,  $b_k$  fixed effect of the birth year  $k$  of the animal  $i$ ,  $\alpha$  linear regression of the age  $a_i$  of animal  $i$  at neck tag equipment,  $g_i$  random genetic effect of animal  $i$  and  $e_{ijk}$  the residual of the model.

### Single Step evaluation

Evaluation of animals were performed using a Single Step Evaluation (SSTEP) approach, using the HSSGBLUP developed by Tribout *et al.* (2020) in France. This software used the Hybrid Single Step model proposed by Fernando *et al.* (2016). All animals with phenotypes and the corresponding pedigree were included in the evaluation (781 heifers with phenotypes). All the relevant genotypes (1,578) were included in order to maximize genetic relationships between animals. The corresponding model of genetic parameters calculation was used for the SSTEP evaluation.

The mean age at first heat in our dataset is 14.6 months of age with a standard deviation of 2.2 months. The minimum and maximum values were 8.6 months and 20.4 months, respectively. Genetic parameters estimation showed an heritability of 0.36 for the trait age at first heat with a standard error of estimation of 0.12, in relation to the medium number of animals with phenotypes taken into account. The corresponding genetic variance of the trait is 0.50 month. These results were in accordance with the results of Mialon *et al.* (1998) with a value of 0.34 (0.09). Phocas and Sapa (2004) found a heritability of 0.15(0.03) for the trait first heat at 15 months (0/1) in accordance with our results of 0.26 (0.12) for this trait.

The SSTEP evaluation provided Estimated breeding values (EBVs) for the bulls of Charolais Univers linked to the animals with phenotypes. EBVs were standardized to obtain a mean of 100, corresponding to 14.8 months of ages, and 10 points corresponding to a genetic standard deviations (21.6 days). For the 25 bulls with a reliability higher than 0.30, EBVs ranged from 85 to 113, reliability ranged to 0.30 to 0.76 with a mean of 0.52.

In this study, we report results from one of the first applications of SSTEP evaluation in France in beef breeds on sexual precocity traits. This evaluation is permitted by the implication of farmers that collect regularly phenotypes since 6 years and continue.

The first steps are focused on more synthetic traits like age at first heat but the idea is to go further and to study traits describing HeatIndex curves from a genetic perspective in order to implement genetic selection on them.

## Results and discussion

### Genetic parameters calculation

### Single Step Evaluation

## Conclusions

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## Exploring opportunities to evaluate genomically MIR-predicted residual CH<sub>4</sub> exploiting correlations to MACE traits

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Promising strategy to reduce methane (CH<sub>4</sub>) emissions in dairy cows can be based on breeding. However, at least two issues remain open. First, many trait definitions were suggested and currently there is no consensus which one to use. Also, establishing effective genetic evaluation systems remain a significant issue, especially given the sparsity of data. The objective of this study was to demonstrate an optimized one-step computational genomic model framework exploiting correlations with traits assessed in an international context by INTERBULL via Multiple Cross-Country Assessment (MACE) allowing to obtain efficiency related GEBV for residual CH<sub>4</sub>.

### Abstract

Keywords: CH<sub>4</sub> mitigation, efficiency, trait definition.

Breeding has been identified as a promising strategy to reduce methane (CH<sub>4</sub>) emissions in dairy cows. However, trait definitions and setup of efficient genetic evaluation systems remain important issues despite availability of genomic data. The aim of this study was to demonstrate an optimized computational single step genomic model setting, hereafter called ssGBLUP, exploiting correlations to traits evaluated in an international context by INTERBULL through Multiple Across Country Evaluation (MACE).

### Introduction

Objective was to generate genomically enhanced estimated breeding values (GEBV) for the trait “Residual CH<sub>4</sub>” (RCH<sub>4</sub>) defined on a genetic level as the deviation of “MIR-predicted CH<sub>4</sub>” (MCH<sub>4</sub>) from “Expected CH<sub>4</sub>” (ECH<sub>4</sub>) obtained from traits available internationally through MACE. The required milk mid infrared (MIR) spectral data was obtained from the Walloon Breeders Association through the Futurospectre consortium. Multiple Across Country (MACE) EBV were provided by INTERBULL as a service to the Walloon genetic evaluation system.

### Material and methods

The trait MCH<sub>4</sub> was obtained as a prediction from MIR spectra with a model based on 1,089 reference values showing a R<sup>2</sup> and a standard error of calibration of about

0.73 and 53g/d respectively. Genetic parameters and GEBV for MCH4 were estimated using test-day MCH4 records on 229,465 first-, 151,726 second-, and 90,484 third-parity Walloon Holstein cows, a ssGBLUP model and Gibbs sampling. GEBV for 305-d MCH4 of each lactation (and average of first three lactations) were regressed on published EBV of MACE evaluated milk, fat and protein yields, derived fat and protein percentages, and linear type traits. The used (G)EBV of ECH4 were defined as predictions combining the MACE evaluated traits using the obtained regression equations.

## Results

Mean (SD) MCH4 yields were 327 (68), 356 (70), and 358 (72) g/day and mean (SD)  $h^2$  estimates for daily MCH4 were 0.12 (0.04), 0.14 (0.05), and 0.13 (0.05), for the first three lactations, respectively. For 1,129 bulls with at least 30 daughters (727 genotyped), GEBV for 305-d MCH4 of each lactation (and average of first three lactations) were regressed on published EBV of MACE evaluated milk, fat and protein yields, derived fat and protein percentages, and linear type traits. The used (G)EBV of ECH4 were defined as predictions combining the MACE evaluated traits using the obtained regression equations. Correlations between (G)EBV of 305-d MCH4 and ECH4 ranged from 0.57 to 0.61 in the first three lactations and was 0.60 for the averaged three lactations EBV of 305-d MCH4. Linear type traits including body depth, chest depth, teat placement, udder support, bone quality, and udder texture, the two later traits being non-MACE trait available locally, showed significant association to MCH4.

## Discussion

An innovative evaluation setup is possible considering direct MCH4 data and, as external information, EBV for ECH4 combined in a bivariate ssGBLUP exploiting the indirect extra information provided by correlated traits that are available for sires based on daughters found internationally. Different settings to include external information for ECH4 are currently being tested. Final (G)EBV of RCH4 could then be predicted by subtracting ssGBLUP recomputed 305-d (G)EBV for ECH4 from (G)EBV for MCH4, but alternative strategies are under review. Breeding using RCH4 could avoid complex weighting of all involved traits and simplify development of an efficiency sub-index in the setting of the Walloon genomic evaluation system.

## Beef cattle breeding in Latvia

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Today one of the major challenges for beef cattle breeders is to increase meat quality while contributing to breeding efficiency. Beef cattle breeding contributes to the supply of beef cattle in the market and involves collecting information on livestock production. To make prudent decisions on the selection of beef cattle, it is necessary to collect and process the data, as well as interpret and distribute the results. Beef production is mainly based on beef cattle breeds, involving natural insemination, rearing the calves as well as fattening the young cattle. In contrast, artificial insemination is mainly practiced in dairy farming, which also makes a significant contribution to beef production in many countries. The International Committee for Animal Recording (ICAR) has specified two main ways of beef cattle registration: the European approach and the North American approach. This could be mainly explained by differences in consumer demand, which affect the pricing system and, therefore, selection goals. There are also significant differences in production conditions, particularly in herd size. After amendments to the European Union (EU) legal framework governing animal breeding and the sale of purebred and crossbred breeding animals and their reproductive products were made in 2016, the EU Member States also adopted new legal acts and revised prerequisites for animal production. Therefore, the present research aims to examine beef cattle breeding in Latvia. The research found that at the beginning of 2023, 4536 beef cattle herds with 94418 beef cattle were registered in Latvia, of which 25% or 1113 herds with 77829 beef cattle were under performance recording.

### Abstract

*Keywords: beef, beef breeds, breeding, crossbreeds, Latvia, recording.*

Identifying cattle productivity and the unique climatic and production systems around the world is important to be able to examine the diversity of cattle productivity in beef cattle production (Kahn L. and Cottle D., 2014).

### Introduction

Despite the large amount of available information, there is still no convincing evidence in Europe that would allow us to project the quality of beef and the supply of beef breed cattle of constant quality to consumers (Przybylski C. *et al.*, 2015). Raising animals for food is associated with various controversies. Consumers often are not aware of the specifics of the industry regarding the cost of producing quality meat, animal welfare, food safety and free enterprise. It should be considered that food safety needs to be assured to explain the specifics of meat production and trade (Terence J. Centner, 2019).

One of the main possibilities for providing an adequate supply of beef cattle in the market is an animal registration system that is capable of collecting and processing

comprehensive information about the animals involved in the production process, as well as the interpretation of the results obtained (Flamant J.C., 1998). Therefore, the present research aims to examine beef cattle breeding in Latvia.

## Material and methods

The research used data for the period 2004-2023 available in the information system of the Agricultural Data Centre (hereinafter referred to as the ADC), a subordinate institution of the Ministry of Agriculture of the Republic of Latvia (RoL), on:

1. Changes in the number of herds of beef breeds and crossbreeds under performance recording.
2. Changes in the number of beef breed cattle under performance recording.
3. The distribution of beef cattle raised in Latvia by breed (incl. crossbreed) and by sex (F, M).

The research employed the comprehensive analysis and synthesis methods and performed a comprehensive analysis of ADC data, incl. collecting, interpreting and visually representing the data.

□The novelty of the research involves setting goals and objectives for beef cattle breeding and identifying opportunities for its development in Latvia, based on the information obtained after performing an analysis of the data, to increase the efficiency of farming and the demand for cattle of beef breeds in Latvia.

## Beef cattle breeding in Latvia

### Legal aspects of beef cattle breeding in Latvia

In the Republic of Latvia, beef cattle breeding traditions began in the late 1990s, in contrast to European countries such as Spain, Italy, France (Hocquette J.F. *et al.*, 2018), as well as Argentina, Uruguay, the USA and Australia, where the beef cattle industry has been highly developed for a long time (Scholtz M.M. *et al.*, 2011).

□In 1998 in Latvia, a purebred animal producer organization – the JSC Beef Cattle Breeding Association – was founded with the aim of designing a programme for raising beef cattle and contributing to beef cattle breeding. Even before joining the EU, a number of cooperation projects with foreign countries on the creation and enhancement of the beef cattle breeding system was implemented in Latvia, e.g. a joint project of Latvia and Denmark for making legal acts on methods for identifying a pedigree value and creating a breeding book system, as well as a joint project of Latvia and Switzerland for creating a system for high-quality beef cattle production in Latvia (Annual Report by the Latvian Beef Cattle Breeding Association, 2002). In 2004 when Latvia joined the EU, Cabinet regulation No. 275 Beef Cattle Monitoring Procedure, which governed the monitoring procedure for cattle of beef breeds and other cattle produced for meat in Latvia, was revised. Based on the legal acts, the ADC developed software for processing monitoring data on beef breed cattle, and in 2004 the recording of quality performance data on beef breed cattle began in Latvia. The data were sent to cattle breeders for further herd enhancement and management (Annual Report, 2005).

In 2014, Latvia joined INTERBEEF, which was a working group of the International Committee for Animal Recording (ICAR), for comparing the data on beef breed cattle to create a unified genetic evaluation method that would facilitate cattle breeding in the future and give the owners of cattle an opportunity to select the most genetically valuable cattle. The first official Estimated Breeding Values (EBVs) for Latvia were



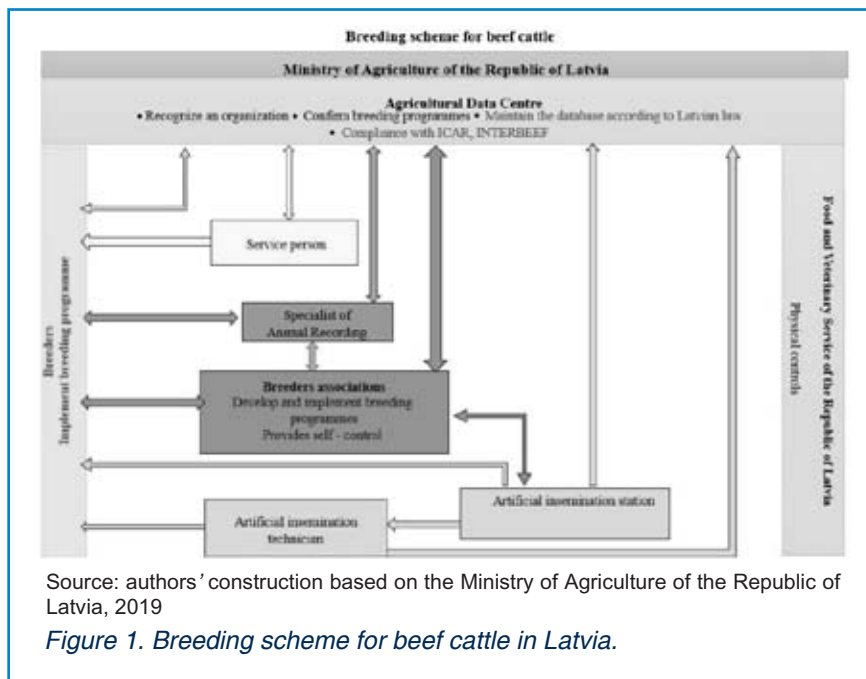
published by INTERBEEF in 2020, indicating the weaning weight for Charolais and Limousin cattle.

The participation in INTERBEEF gave an opportunity to compare the data on beef breed cattle raised in Latvia with international data. This was an important achievement to have access to the results of evaluations of breeding beef cattle, gain the experience of other countries in beef cattle production and develop beef cattle breeding and this industry (Agricultural Data Centre, 2023).

In 2016, Regulation (EU) 2016/1012 of the European Parliament and of the Council was adopted, which aimed to ensure uniform application of EU regulations in the Member States on breeding animals and trade in breeding animals and the reproductive products, as well as to govern the trade in breeding animals and the reproductive products in the EU (EU regulation, 2016). Therefore, the Law on Animal Production and Breeding was adopted in Latvia in 2018. In accordance with this law, Cabinet Regulation No. 796 Procedure for Recognition of the Purebred Agricultural Animal Producer Association, and the Crossbred Pig Producer Organization, as well as the Procedure for Approving the Breeding Programme was adopted. The purpose of the regulation was to set eligibility criteria and recognition procedures, as well as procedures for approving breeding programmes.

In 2019, Cabinet Regulation No. 227 Procedure for Beef Cattle Monitoring and Performance Testing with the aim of establishing a uniform monitoring and performance testing procedure in Latvia was adopted.

Figure 1 shows the breeding scheme for beef cattle, which represents the procedure for beef cattle monitoring and breeding activities in Latvia. In 2019, the Latvian Beef Cattle Breeding Association designed a new breeding programme for beef cattle, the purpose of which was to increase the genetic quality of beef cattle. In Latvia, the four most popular beef cattle breeds were Charolais, Hereford, Limousin and Aberdin Angus, followed by Simmental, Highland and Galloway that were gradually approaching them (Beef Cattle Breeding Programme, 2019).



Source: authors' construction based on the Ministry of Agriculture of the Republic of Latvia, 2019

Figure 1. Breeding scheme for beef cattle in Latvia.

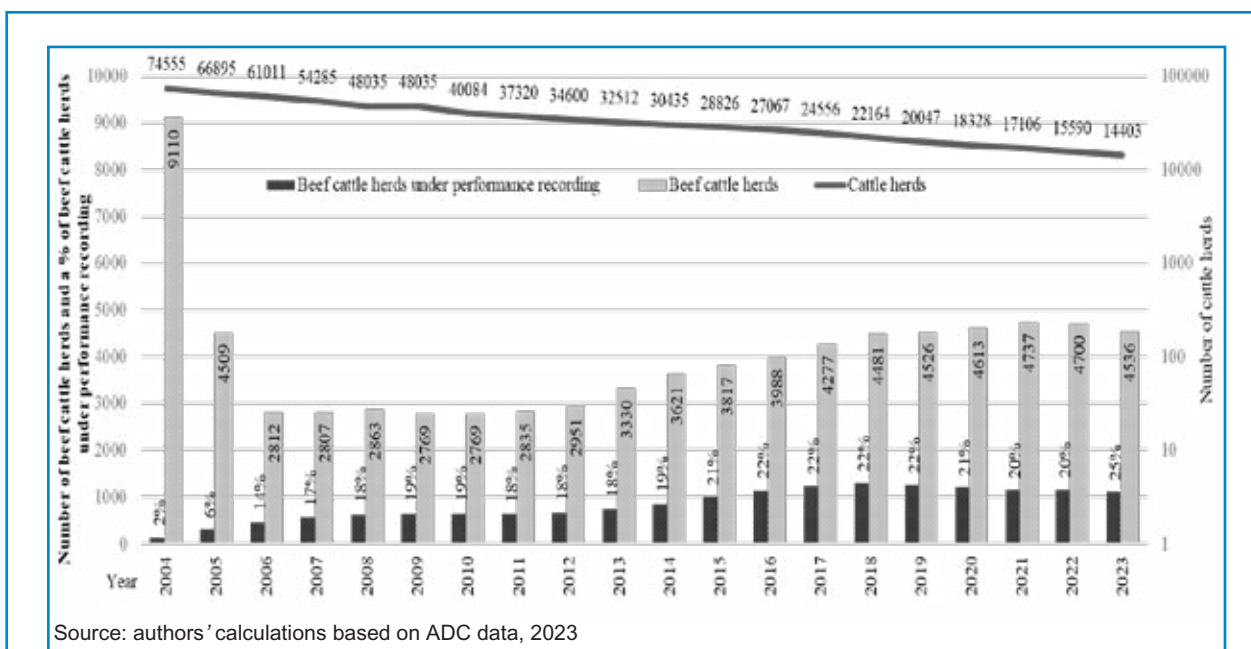
In 2019, the ADC introduced a Web-based application program – the beef monitoring data recording system CILDA, which aimed to provide centralized administration of the monitoring data in accordance with the current national and international legislation, with a special focus on the International Agreement on Monitoring Procedure and the ICAR and INTERBEEF guidelines to assist producers in beef cattle monitoring planning (Agricultural Data Centre, 2017).

**Performance analysis of beef cattle breeding in Latvia**

On 1 January 2004, 74555 cattle herds were registered in Latvia, of which 9110 or 12% were specialized in beef cattle breeding, and only 139 herds or 2% of the total number of registered beef cattle herds performed performance recording. From 2004 to 2023, the performance recording of beef cattle herds had progressed steadily, i.e., by 1 to 2% of the total number of beef cattle herds a year.

In 2023, 25% or 1113 beef cattle herds were under performance recording (totally 4536) (Figure 2).

After granting support for breeding activities to the beef industry in the country, the herds under performance recording began to focus on identifying the genetic quality and implementing evaluation programmes (Law on Animal Production and Breeding, 2018) when starting the breeding activities. However, it should be noted that performance recording was only a process for the implementation of a cattle breeding programme that provided quantitative and qualitative data on beef cattle, their productivity and appearance. Breeding is considered successful if 50% of the animals produced are sold for further breeding. It sets special requirements for the farm, the basic principles of animal diets and also the management of the farm (Averbeks F., 2013).



Source: authors' calculations based on ADC data, 2023

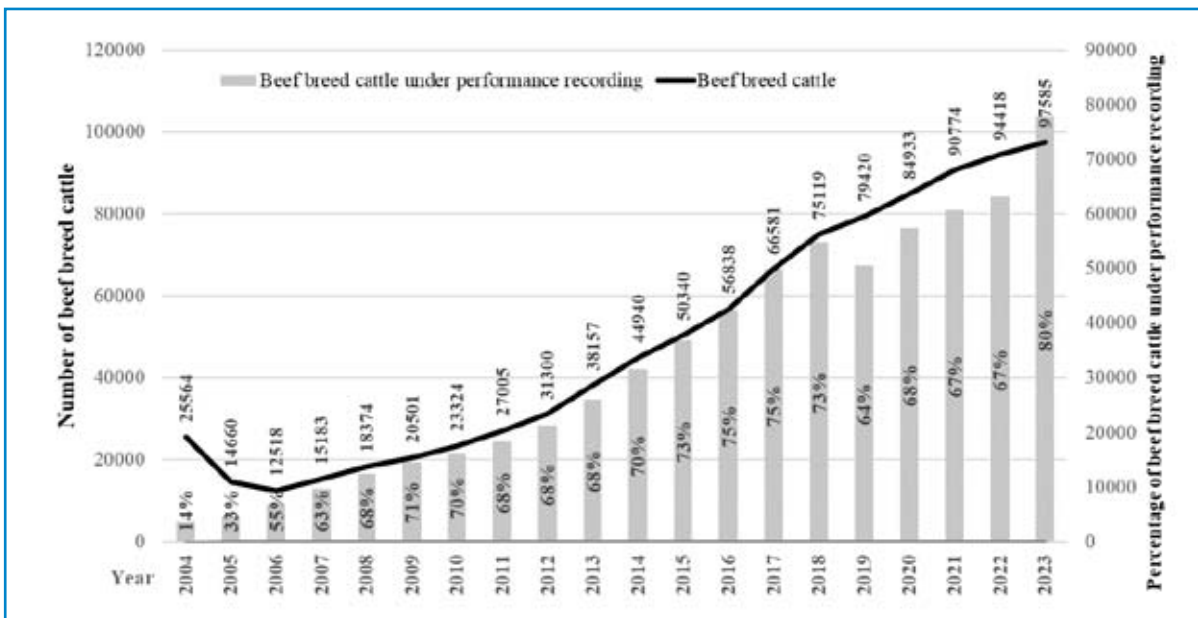
Figure 2. Changes in the numbers of cattle herds, beef cattle herds and herds under performance recording expressed as a percentage of the total, in Latvia in 2004-2023.

In 2004, according to the ADC (Figure 3), 25564 beef breeds, incl. beef crossbreeds, were registered in Latvia. An analysis of the data for the period 2004-2023 revealed that the number of cattle had increased 3.8-fold, which was the result of national support and EU Common Agricultural Policy (CAP) support payments, especially direct payments, including voluntary coupled support for cattle paid from 2015 (Pilvere *et al.*, 2022b). The increase in the number of beef cattle could be expected because without direct payments, cattle farms would operate at a loss. Support is therefore particularly important for economic, social, or environmental reasons (Vinci C., 2022).

In the period of analysis, the number of beef cattle has increased since 2004; however, as a result of meeting the requirements of European Parliament and Council Regulation (EU) 2016/1012 as well as the beef cattle breeding programme adopted in Latvia in 2019, the number of beef cattle involved in breeding decreased by 8%, yet a 13% increase was reported at the beginning of 2023 compared with the previous year (Figure 3). The data indicate that the total number of beef breed cattle and the number of breeding cattle have gradually increased in Latvia, reaching 77829 or 80% of the total (Figure 3). Therefore, in Latvia the total number of beef breed cattle gradually increased, as the cattle farms focused more on the role of cattle breeding and growth in the beef industry.

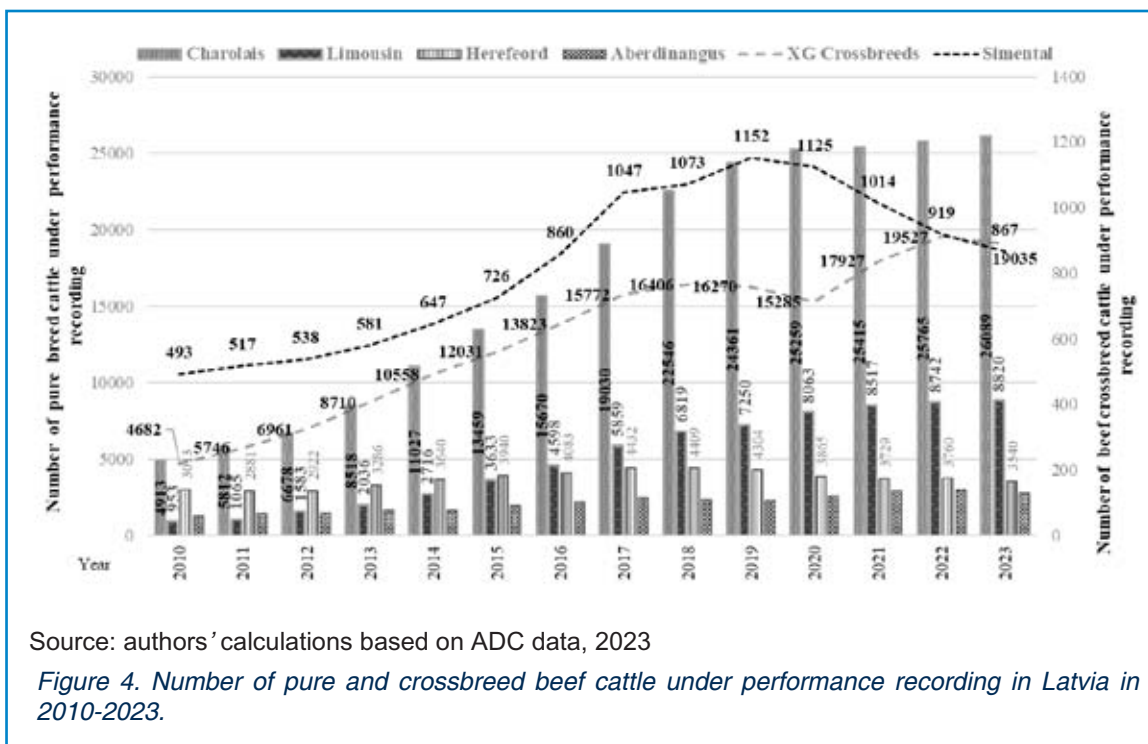
□\* Source: authors' calculations based on ADC data, 2023

In Latvia, four beef cattle breeds – Charolais, Limousin, Hereford and Aberdin Angus – made up the highest proportion in the total number of cattle, while the Simmental



Source: authors' calculations based on ADC data, 2023

Figure 3. Number of beef breed cattle, including beef breed cattle under performance recording, %, in Latvia in 2004-2023.



Source: authors' calculations based on ADC data, 2023

Figure 4. Number of pure and crossbred beef cattle under performance recording in Latvia in 2010-2023.

breed as well as crossbreeds were also gaining popularity (Figure 4). These were the most important breeds for the beef cattle breeding programme in Latvia. In Latvia, the number of Charolais and Limousin cattle, as well as the number of beef crossbreeds tended to increase every year. As regards dual-purpose beef-dairy breed cattle, Simmental were the most popular breed in Latvia. An analysis of the data presented in Figure 4 revealed that in the period 2010-2023, Charolais cattle represented the most popular breed in Latvia, as their number increased five-fold or by 21176 cattle in 2023 compared with 2010. The number of cattle of XG (beef breed) crosses increased 3-fold or by 14353 cattle, the number of Limousin breed cattle increased 9-fold or by 7867 cattle, the number of Simmental breed cattle increased almost 2-fold, while the increase in the number of Aberdinangus breed cattle was 2.3-fold in the period 2010-2023. The number of Hereford cattle had increased only 1.2-fold. Therefore, it could be concluded that, when implementing the beef cattle breeding programme, beef breed cattle producers obtained offspring by continuing crossing their cattle with breeding cattle of the same breed that could be recorded in the supplementary and basic parts of the breeding book. To achieve the goals of breeding, it was necessary to involve as many beef cattle farms as possible in the implementation of the beef cattle breeding programme in order to improve the genetic potential of the breeds, develop the production of high-quality, competitive products (breeding material, meat) for the domestic and foreign markets (Beef Cattle Breeding Programme, 2019).

In the period 2010-2022, as shown in Table 1, the largest increase in the number of cattle of beef breeds, incl. crossbreeds (suckler cows, heifers <15m and ≥ 15m, bulls <15m and ≥ 15m and breeders) was found for Charolais cattle – 5.2-fold and for XG cattle – 4-fold. This could be explained by the fact that Charolais bulls were used for crossing with other beef and dairy breeds (Lujane B. *et al.*, 2013). The increase in the number of XG cattle was due to the restructuring of dairy farms to beef production. Overall, it could be concluded that the breeding activities in beef cattle herds have been successful in Latvia, as the number of beef cattle continued to increase, as well as their productivity performance improved. It is important to be aware that in beef cattle production, many possible combinations of animal genetics, production settings

Table 1. Breakdown of the number of animals in beef breeding herds by breed, including crossbreeds (cows, heifers <15 m and ≥ 15 m, bulls <15 m and ≥ 15 m and breeders) in Latvia for the period 2010;2022

Breeds	01/01/2010							01/01/2022						
	Total	Suckler cows	Heifer <15 m.	Heifer ≥15m	Bull <15 m.	Bull ≥15m	Sires	Total	Suckler cows	Heifer <15 m.	Heifer ≥15 m.	Bull <15 m.	Bull ≥15 m.	Sires
<b>Charolais</b>	<b>4913</b>	2106	1003	902	644	258	169	<b>25765</b>	12808	4460	4775	2654	1068	722
<b>Beef Crossbreed</b>	<b>4682</b>	1214	1387	951	1001	129	5	<b>19527</b>	8213	4926	2782	3313	293	0
<b>Hereford</b>	<b>3013</b>	1526	487	495	367	138	43	<b>3760</b>	2131	514	580	339	196	73
<b>Aberdin Angus</b>	<b>1288</b>	641	193	218	193	43	19	<b>3019</b>	1399	531	445	388	256	66
<b>Limousin</b>	<b>953</b>	344	190	195	137	87	63	<b>8742</b>	4012	1445	1789	967	529	388
Simmental	493	276	54	83	40	40	35	919	562	122	113	79	43	29
Highland Cattle	410	153	58	76	53	70	14	638	263	71	90	92	122	21
Galloway	291	106	39	61	39	46	8	794	319	90	144	91	150	21
Saler	71	28	19	11	10	3	3	43	27	5	2	8	1	0
Tyrol Grey	70	30	12	11	13	4	2	6	6	0	0	0	0	0
Belgian Blue	19	9	6	0	1	3	3	10	9	0	1	0	0	0
Brown Swiss	10	9	0	1	0	0	0	0	0	0	0	0	0	0
Dexter	7	2	1	1	0	3	2	0	0	0	0	0	0	0
Blonde d'Aquitaine	1	0	0	0	0	1	1	61	34	4	6	7	10	2

Source: authors' calculations based on ADC data, 2023

and market expectations can produce favourable or unfavourable outcomes (Herring A.D., 2014). According to the basic principles of sustainable development, in order for meat of beef cattle to be recognized as produced in a sustainable way, the meat must meet the highest quality standards and its production must be economically feasible for beef cattle farms, thereby benefiting not only the farms themselves but also the population (Jamieson A., 2013).

In Latvia, the legal framework for beef cattle breeding is shaped by EU and national legal acts, as well as producer association documents, e.g., the Animal Breeding Programme, as well as documents of international organizations (ICAR, INTERBEEF).

□ In Latvia, the ADC has introduced a Web-based application program – the beef monitoring data recording system CILDA –, which aimed to provide centralized administration of the monitoring data in accordance with the current national and international legislation, with a special focus on the International Agreement on Monitoring Procedure and the ICAR and INTERBEEF guidelines.

At the beginning of 2023, 4536 herds of beef cattle with 94418 beef cattle were registered in Latvia. Of the total, 25% or 1113 herds were included in the breeding programme, which covered 77829 cattle. The most popular breeds in Latvia were Charolais with 26089 cattle, Limousin (8820), Hereford (3540) and Aberdin Angus (2812), making up 44% of the total beef cattle.

The number of dual-purpose beef-dairy breed (Simmental) cattle under performance recording tended to decrease, reaching 867 (25% decrease) in 2023 compared with 2019, while the number of crossbreed beef cattle reached 19035 or 15% of the total cattle involved in breeding in 2023. It follows that that the breeding work expanded, with a focus on pure breed cattle, whereas the number of crossbreed beef cattle involved in breeding tended to decrease.□

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## Application of ssGBLUP in Murrah buffaloes reared under small holder conditions in India

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Buffaloes contribute more than 50% of milk in India. Among 13 recognized buffalo breeds, Murrah is the most popular buffalo breed in India. Application of Genomic Selection (Meuwissen *et al.*, 2001) is expected to double the rate genetic progress (Schaeffer, 2006) in dairy cattle. Similarly, Genomic Selection can help increasing rate of genetic progress in Murrah buffaloes covered under AI.

Female reference populations are now gaining in popularity, especially for novel traits, such as feed efficiency, methane emissions, and detailed reproductive measures, the traits which are expensive to measure and are therefore available for only some animals in the population (Pryce *et al.* 2012). As the population of buffaloes is restricted to mostly South East Asia, and these buffaloes are mostly kept under small holder conditions, large scale progeny testing programs are not implemented for buffaloes. Use of genomic selection based on mixed reference population, where both AI bulls and recorded females are genotyped, can be a practical solution in such areas.

Considering above, a study was conducted to demonstrate practicability of implementing Genomic Selection for young bull selection and its superiority over traditional methods like dam yield based selection as well as selection based on pedigree based breeding values. A single step GBLUP model was used to estimate Genomic Breeding Values.

National Dairy Development Board, Anand, India is involved in implementing progeny testing projects for various cattle and buffalo breeds. The projects collect performance records of cattle and buffaloes reared by mostly small and marginal farmers under small holder conditions. Each farmer rears one to three milking animals. Data used in present study are collected by milk recorders engaged by milk cooperative unions of Gujarat state of India. Village level inseminators engaged by cooperatives provide AI services to farmers in villages and record pedigree information of all the daughters born by test insemination. The daughters when calve for 1<sup>st</sup> lactation, their yield is recorded for 10 monthly test days both morning and evening. All the information is recorded in Information Network for Animal Productivity and Health (INAPH- (Nayee *et al.* 2016)).

68,808 first lactation test day milk yield records of daughters born to Murrah Sires were extracted from INAPH. Records from animals that were recorded for first time within 5 to 35 days post calving, having sire IDs against them and have minimum 3 test day records were used for present study. Daily milk yields between 1 to 25 Kg was only used as valid records. Total 61321 test day records belonging to 6811 daughters of 190 Murrah sires were used for final analysis.

### Introduction

### Material and methods

#### Phenotype records

### Genotypes

A custom made SNP genotyping array on Illumina platform, BUFFCHIP, was used to genotype 3087 buffaloes and 138 bulls. 40748 SNPs that were having Minor allele Frequency (MAF) of >0.1 and genotyping rate of >90% were used for present study.

$$y_{thijkl} = A_h + HYMR_i + YS_j + OWN_k + \sum_{m=0}^3 \phi_{ltm} \beta_m + \sum_{m=0}^3 \phi_{ltm} u_{lm} + \sum_{m=0}^3 \phi_{ltm} p e_{lm} + e_{thijkl}$$

### Statistical Models for breeding value estimation

Similarly genotype information for all 3225 individuals that have >90% SNPs genotyped out of the QC passed SNPs was used for present study.

Breeding values were estimated using a conventional test day model and ssGBLUP model. The two models were described below.

- Conventional test day random regression model with 3<sup>rd</sup> order Legendre polynomials for both fixed and random regression.

where  $y_{thijkl}$  is the test-day milk yield of cow  $l$  produced - within the  $h^{th}$  A (age at calving class, fixed effect), the  $i^{th}$  HYMR (herd x year of recording x month of recording, random effect) and  $j^{th}$  YS (year x season of calving, fixed effect); with the  $k^{th}$  OWN (Owner, random effect)  $\beta_m$  is the fixed regression coefficient of the  $t^{th}$  test day record on the  $m^{th}$  order of Legendre polynomial;  $u_{lm}$  and  $p e_{lm}$  are random regression coefficients of the test day record on the  $m^{th}$  order of Legendre polynomial for animal additive genetic and permanent environmental effects for animal  $k$ ;  $\phi_{ltm}$  is the  $m^{th}$  Legendre polynomial of the  $t^{th}$  days in milk for cow  $k$ .

Considering that individual farmers only have a few cows and farmers in the same village have more common management practices compared with farmers in different villages, a herd was defined as all animals in the same village. Owner of the animal was considered as random effect. Age at first calving ranged from 18 months to 72 months. Age class was defined by combining animals less than 2 years of age at first calving in to a single class. Animals in every 12 month age increment at first calving were put in separate age class groups till 5 years. Animals above five years of age at first calving were grouped in a single class.

Variance components obtained by AIREML using pedigree information were used for both BLUP and ssGBLUP breeding value estimation. The variance components and breeding values obtained under random regression model were represented in terms of 305 day EBV as per the procedure described by Mrode and Thompson (2005).

### ssGBLUP model

The ssGBLUP (Christensen et. al. 2012) had the same structure and effects as the conventional model. The only difference was that the ssGBLUP used a combined relationship matrix (**H**) instead of pedigree-based relationship matrix (**A**). The inverse of the **H** matrix is

$$\mathbf{H}^{-1} = \begin{bmatrix} \mathbf{G}_w^{-1} - \mathbf{A}_1^{-1} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} \end{bmatrix} + \mathbf{A}^{-1}$$

Here  $\mathbf{G}_w$  was the adjusted  $\mathbf{G}$  matrix,  $\mathbf{G}_w = 0.8 * \mathbf{G} + 0.2 * \mathbf{A}$ , in which  $\mathbf{G}$  was the genomic relationship matrix and  $\mathbf{A}$  was pedigree based relationship matrix. To get  $\mathbf{G}$  in the same scale as  $\mathbf{A}$ , the scale of  $\mathbf{G}$  was adjusted so that the average of diagonals and average of off-diagonals were the same as those in  $\mathbf{A}$  (Christensen *et al.*, 2012).

Purpose of genomic evaluation is to predict breeding value of individual especially bulls with high accuracy. In other words, we want to test how breeding value (or selection criteria) of a bull is correlated with average of sire's daughter's corrected yield. To evaluate predictive ability of selection criteria viz. bull's dam yield (DY), traditional BLUP model based on pedigree and ssGBLUP model additionally using genotype information, a 5 fold cross-validation method was adopted.

**Estimation of predictive ability of various selection criteria for bull selection**

In each validation dataset, records of all the daughters of 20% randomly selected bulls (paternal half-sib groups) were set missing and then EBF or GBV for the bull were estimated using traditional BLUP or ssGBLUP. The GBVs were compared with average of corrected phenotype of the daughters of the bulls in validation set. Only sires with more than 15 daughter records were considered for them to be included in validation process.

The corrected phenotype for a buffalo (Yc) was obtained by correcting observed phenotype for all other effects in the model except for animal genetics and permanent environment effects obtained by traditional BLUP model using full data.

The EBV or GBV obtained only based on pedigree or genotype for a bull were compared for their ability to predict corrected daughter average. This ability was calculated by comparing Pearson correlation coefficients of EBV/GBV with that of average Yc of daughters for a bull.

Of the total 190 sires having daughter records, 118 sires had  $\geq 15$  daughter records. Thus in each validation data set, at random around 23 sire's daughter observations were dropped. However care was taken to keep similar number of observations in each validation data set to avoid bias.

The regression coefficient of EBV with GEBV were calculated to see bias in estimation of Genomic Breeding Values.

Table 1. Predictive ability of selection criteria.

Validation set No.	No. of bulls	Correlation with Daughter's corrected yield			% increase in correlation for GBV	
		DY	EBV	GBV	Over DY	Over EBV
1	22	-0.03	-0.02	0.49	1733%	2550%
2	22	0.23	0.35	0.42	83%	20%
3	24	0.16	0.33	0.42	163%	27%
4	25	0.42	0.25	0.17	-60%	-32%
5	25	-0.08	0.17	0.43	638%	153%
<b>Overall</b>	<b>118</b>	<b>0.15</b>	<b>0.21</b>	<b>0.37</b>	<b>147%</b>	<b>76%</b>

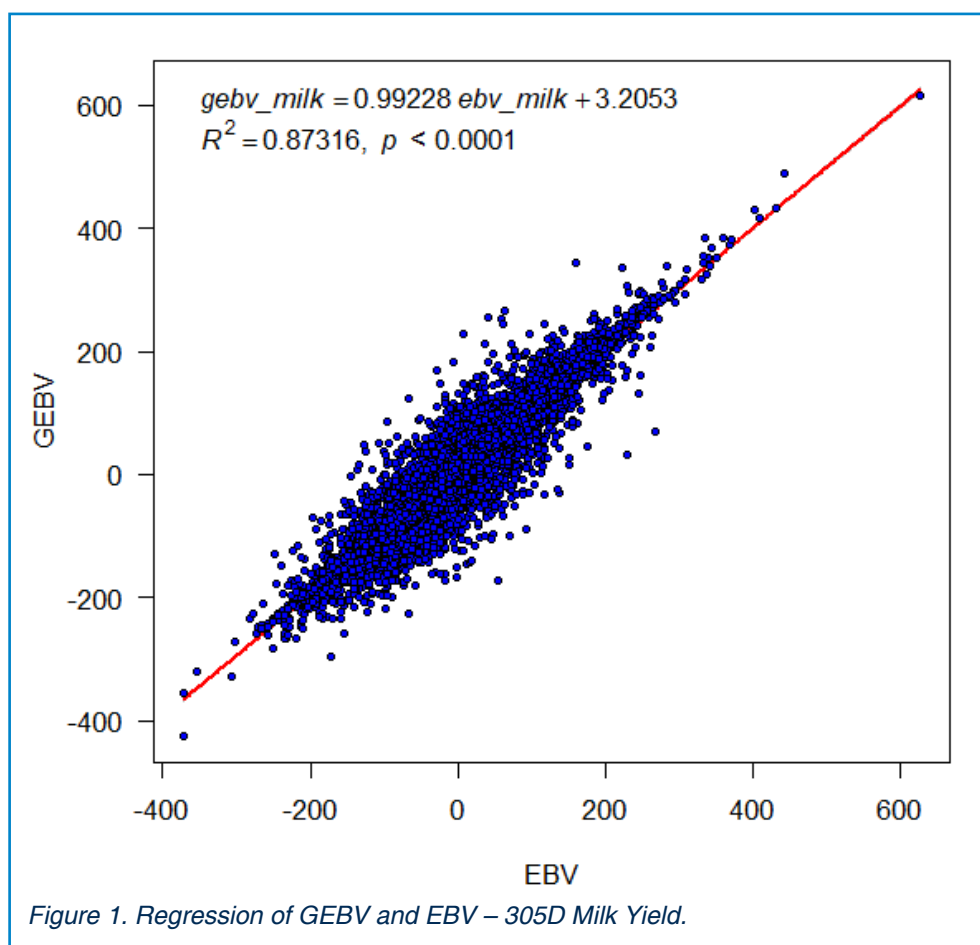
## Results and discussions

Average first lactation test day yield of the daughters of Murrah bulls used for AI in the project area was 5.85 Kg per day which translates to 1785 Kg per lactation (305 day std. lactation yield). The heritability estimate for milk yield was 0.199, total phenotypic variance was 158788 and total genetic variance was 31556 Kg<sup>2</sup> in this population.

The correlation between GBV and Average of daughter's corrected Yield (Yc) of individual animal is compared with the correlations obtained between DY and Yc and pedigree based EBV and Yc in table 1 for each validation data set and overall for all validation bulls.

As seen in the table, overall, the correlation between DY and Yc over all validation datasets considered together is 0.15, the correlation between EBV and Yc is 0.21 whereas correlation between GBV and Yc is 0.37. Thus there is 0.16 (147%) rise in correlation coefficient if GBV is considered for selection in place of DY. Also, the GBVs are 76% correlated to daughter production compared to pedigree based EBVs.

The comparison mimics real world scenario. Here we are interested in selecting bulls that should increase average yield of their daughters. When we select bulls, we will not have their daughter's production information. However we will be knowing bull's pedigree information and also information on their parent's performance (sire EBV and dam's Milk Yield as well as EBV). The results here indicates that the bulls can be selected more accurately if we add genomic information and use ssGBLUP over and above the traditional information while selecting a bull for semen production.



Tsuruta et.al (2019) in a simulation study demonstrated that, when males had no daughters and no siblings with phenotypes, regression coefficient of TBV with GBV was  $< 0.9$  as GEBV for males represented inflation. This is termed as “Bias” in estimation of GEBV. To see whether there is any bias while calculating genomic breeding values compared to traditional breeding values, the GEBV were plotted against EBV and regression coefficient was calculated. Figure 1 below shows the scatter of EBV against GEBV and regression equation for the same.

Above figure clearly shows that there is no bias while estimation GEBVs through ssGBLUP as compared to traditional breeding values (EBV). Vitezica et.al. (2011) in a simulation study concluded that predictions by the multiple-step method were biased. Similarly Ma et.al. (2015) has showed that the use of ssGBLUP has reduced the bias in genomic prediction in Danish Holstein population. Current results also shows that ssGBLUP estimates are unbiased.

Use of genomic information for estimation of breeding value with test day milk yield records was attempted for Murrah buffaloes performing under small holder conditions of India. Average daughter corrected phenotype was considered as reference point while comparing accuracy of DY, EBV or GBV. The GBVs obtained for validation animal with missing record were having higher correlation with corrected phenotype of the individual. The correlations were 147% higher for bulls for GBV compared to DY which was traditionally used for selection of bulls in India. The present study encourages bull selection based on GBVs obtained through ssGBLUP method to get higher genetic progress in small holder conditions.

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## Regression of EBV on GEBV

## Conclusion

## Acknowledgement



## Genomics improves the reliability of Breeding Value Prediction of morphological and reproductive traits in the Pura Raza Español Horse: Preliminary results

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This study aimed to compare reliabilities for some conformational and reproductive traits between classical approach with pedigree-based REML and single-step genomic REML in PRE horse. Measurements from 5 zoometric traits were analysed: scapular-ischial length (SiL), length of back (LB), dorso-sternal diameter (DsD), thoracic perimeter (TP), and perimeter of anterior cannon bone (PACB). The following seven traits were considered in this study as measures of mare's fertility: age at first foaling (AFF), age at last foaling (ALF), average interval between foaling (AIF), total number of foalings (NF), interval between first and second foaling (IF12), productive life (PL) and reproductive efficiency (RE). The datasets consisted of 7152 conformation and 11,798 reproductive records and the pedigree included 41,888 animals. A total of 2916 animals were genotyped and 61,271 SNPs were included in the analysis. All analyses were performed using a multivariate model, separately for conformational and reproductive traits. The estimates of heritabilities were similar in both methodologies (0.34 to 0.64 for morphological traits, and 0.02 to 0.23 for the reproductive ones). Genetic correlations between EBVs and GEBVs varied from 0.59 (DsD) to 0.98 (SiL) for conformational traits and from 0.96 (IMPm) to 0.99 (PPm and EUPm) for reproductive traits.

### Abstract

A significant gain in reliabilities for ssGREML over REML evaluations has been observed in all conformational traits with an overall increase oscillating between 7.74% (LB) and 27.83% (DsD), being greater in genotyped animals (14.97% to 41.0%) compared to non-genotyped (6.84% to 26.13%). In general, when the reliability of the animal was previously very low, a greater gain in reliability has been observed, especially in the ungenotyped subpopulation. Similarly, considering only stallions with less than 40 controlled foals, this increase was much greater (7.92% to 26.43%). In the case of reproductive traits, a noticeable smaller increase was obtained for all traits, ranging from 1.99% for PL and RE to 4.29% for IF12. This work demonstrate the effectiveness of the genomic approach for the routine genetic evaluation of conformation and reproduction traits in the PRE breed..

*Keywords: single-step, estimated breeding value, reliability, PRE.*

## Introduction

The Pura Raza Española (PRE) horse is a native Spanish equine breed that has been officially recognized since the 15<sup>th</sup> century. Actually, the PRE is the most popular equine breed in Spain, representing the 70% of all registered equids. The total PRE population, 275,018 horses, are mainly located in Spain but also in other 67 countries (MAPA, 2023). In 2003, the genetic breeding program was approved to enhance the breed's conformation, functionality and reproductive traits, reduce inbreeding, and preserve its genetic heritage. More recently, the PRE breeding program has been updated in 2020 (MAPA, 2023). In order to ensure the PRE genetic integrity, various molecular tools such as blood groups, biochemical polymorphisms, and microsatellite markers have been used to establish paternity controls since the early 1980s. This has led to over 40 years of verified parental information, providing an accurate and reliable basis for genetic improvement efforts.

Conformation traits are very important to this breed because of its relationship with the functionality since PRE horses are mainly used for dressage competitions (Sánchez-Guerrero *et al.*, 2016). So, a good conformation will determine the horse's final price. In the same sense, reproductive traits are a critical factor for the profitability of equine stud. To maintain a healthy and profitable equine operation, successful breeding and reproduction are crucial. The birth of a foal not only represents the continuation of the bloodline but also signifies a considerable investment of time and resources. For all these reasons, obtaining reliable genetic parameters and high accuracy of estimated breeding values is essential in genetic improvement programs, especially for traits with low heritabilities, such as the reproductive ones.

Historically, in animal populations, estimated breeding values have been calculated from phenotype and pedigree information and extended literature exists, however, genomic estimates are expected to be more accurate because they do not depend on the quality and completeness of the pedigree. The single-step genomic best linear unbiased prediction (ssGREML) method allows the inclusion of information from genotyped and non-genotyped relatives in the analysis, along with phenotypes, improving the accuracy of breeding values estimation (Lourenco *et al.*, 2020).

Then, this study aimed to compare reliabilities for some conformational and reproductive traits between classical approach with pedigree-based REML and single-step genomic REML in PRE horse breed.

## Material and methods

### Pedigree, phenotypic and genotypic data

The datasets used in this study were provided by the Royal National Association of Spanish Horse Breeders (ANCCE). Data consisted on 7152 conformation and 11,798 reproductive records. The total number of animals in the pedigree used for the genetic and genomic evaluations was 41,888. A total of 2,916 individuals were genotyped with medium density GGP Equine Array (NEOGEN), including over 70,000 evenly distributed SNPs. The raw genotype data was filtered using PLINK software v1.9: SNPs with call-rate > 0.95 were retained. The final genomic data included 61,271 SNPs located on autosomal and X chromosomes. Measurements from five conformation zoometric traits were analysed: scapular-ischial length (SiL), length of back (LB), dorso-sternal diameter (DsD), thoracic perimeter (TP), and perimeter of anterior cannon bone (PACB). Also, the following seven traits were considered as measures of mare's fertility: age at first foaling (AFF), age at last foaling (ALF), average interval between foaling (AIF), total number of foalings (NF), interval between first and second foaling (IF12), productive life (PL) and reproductive efficiency (RE).



The following animal model was fitted to analyze conformation and reproductive traits separately:

$$y = Xb + Za + e$$

where **y** is the vector of observations of conformation and reproductive traits; **b** is the vector of fixed effects (for conformation traits: sex, age, coat colour and geographical area; for reproductive traits: classical inbreeding coefficient and age at last foaling as linear covariables, coat colour, geographical area and birth stud size); **a** is the random additive genetic effect, and **e** is the random residual effect. **X** and **Z** are incidence matrices relating observations to fixed and random additive genetic effects, respectively.

The additive genetic effect was modeled using two kinds of genetic covariance structures: for the classical evaluation, a matrix **A** that denotes the pedigree-based additive genetic relationship was used, and, for ssGREML, the **A** matrix was replaced by **H**, the pedigree-genomic relationship matrix. **H** was derived by blending **A** with the genomic relationship matrix **G** calculated using (VanRaden, 2008) as follows::

$$G = 0.95 \frac{SS'}{2 \sum_{i=1}^n p_i(1-p_i)} + 0.05A$$

where, *n* is the number of SNP markers and *p<sub>i</sub>* is the allele frequency of marker *i*, **A** is the pedigree relationship matrix, and **S** is a centred incidence matrix of SNP markers.

Variance components, estimated breeding values (EBVs) and genomic estimated breeding values (GEBVs) were estimated applying a restricted maximum likelihood (REML) and ssGREML approaches for conventional and genomic evaluations, respectively. Analyses were performed with the HiBlup v1.3.1 (Yin *et al.*, 2023) and the BLUPF90+ (Lourenco *et al.*, 2022) programs for conformation and reproduction traits, respectively..

This study has established, for the first time, a genomic evaluation using a single-step approach with a combined relationship matrix for conformation and female fertility in PRE horse.

Heritabilities were similar in both methodologies being high for conformation and low for reproductive traits. Their estimates ranged between 0.34 to 0.64 (DsD and SiL, respectively) for conformation traits and 0.02 to 0.23 (IF12 and AFF-RE, respectively) for the reproductive ones. In the literature, high heritability estimates were reported for conformation traits in horse breeds (Solé *et al.*, 2014 in Menorca horse population; Gómez *et al.*, 2021 in PRE horse breed; Vosgerau *et al.*, 2022 in German Warmblood horses). However, for fertility, lower heritability values were observed (Mantovani *et al.*, 2020; Perdomo-González *et al.*, 2021).

The gain in reliability (R<sup>2</sup>) between REML and ssGREML estimates of conformation and reproductive traits is shown in Table 1. Our results indicated a gain for ssGREML over REML evaluations and that gain was even greater in the case of conformation traits. The overall increase (considering all the animals in the pedigree) oscillated between 7.8% and 27.83% for conformation, and between 1.99% and 4.29% for reproductive traits.

Moreover, for conformation traits, more detailed information about the gain for ssGREML over REML using some criteria (sex, number of foals per sire, etc) is provided in Table 2. This gain was greater in mares (from 7.8% to 29.2%) than in stallions, and in genotyped animals (from 15% to 41%) compared to non-genotyped (from 6.8% to

### Estimation of genetic parameters, EBVs and GEBVs

### Results and discussion

Table 1. Gain in reliability ( $R^2$ ) between REML and SSGREML estimates of conformation and reproductive traits in the PRE horse.

	Trait	$R^2$ _REML	$R^2$ _SSGREML	Gain(%)
Conformation traits	SiL	0.282	0.308	9.12
	LB	0.258	0.278	7.80
	DsD	0.221	0.283	27.83
	TP	0.229	0.283	23.35
	PACB	0.229	0.259	12.83
Reproductive traits	PPm	0.381	0.389	2.04
	EUPm	0.263	0.270	2.90
	IMPm	0.101	0.105	3.81
	NP	0.264	0.272	2.86
	I12m	0.076	0.079	4.29
	VPm	0.092	0.094	1.99
	ER	0.388	0.395	1.99

26.1%). In addition, stallions with less than 40 controlled foals or having a previously low REML reliability a greater gain was observed.

The impact of genomic breeding values (GEBVs) on the accuracy of EBVs has been analysed in previous studies (Haberland *et al.*, 2012; Vosgerau *et al.*, 2022). Haberland *et al.* (2012) observed that, for animals with a large number of progeny records available, additional gain in accuracy from GEBV is small. In the same way, in the study of Vosgerau *et al.* (2022) about withers height, the increase in reliability was greater for animals with a small number of offspring.

Figure 1 represented the plot of the estimates of reliability for single-step GREML ( $R^2$ \_SSGREML) versus reliability for REML ( $R^2$ \_REML) in the trait with the higher overall gain for conformation and fertility, DsD and IF12, respectively. It was clearly noticeable that when the reliability of the animal was previously very low, a greater gain was reached for the DsD trait (Figure 1.A). However, for IF12, the gain in reliability was even unfavorable for animals that already have low reliabilities estimates for REML (Figure 1.B).

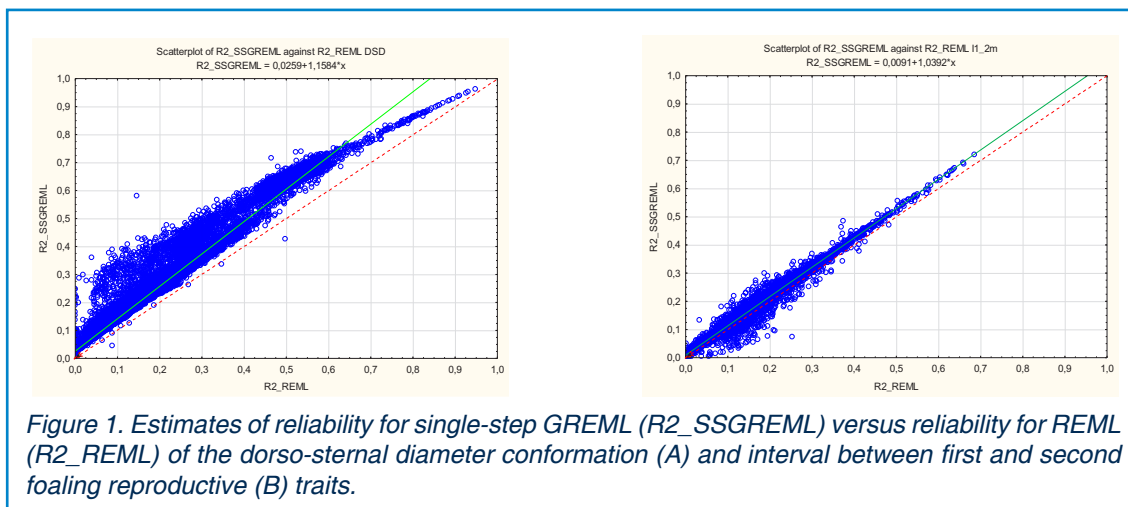
In general, this study demonstrated the effectiveness of the genomic approach for the routine genetic evaluations of conformation and reproduction traits in the PRE horse breed. The single-step method is an appealing approach for practical genomic prediction in PRE horses, because not many genotypes are available yet in this breed and animals without genotypes can by this way directly contribute to the estimation system..

Table 2. Gain in reliability ( $R^2$ ) between REML and ssGREML estimates of conformation traits based on different criteria in the PRE horse.

	Criteria	SiL	LB	DsD	TP	PACB
Sex	Stallions	9.08	7.75	26.1	22.1	12.2
	Mares	9.15	7.8	29.2	24.3	13.3
Stallion N°of foals	40 or more	3.5	2.2	15.2	13.3	4.9
	Less than 40	9.2	7.9	26.4	22.4	12.5
Genotyped	No	8.1	6.8	26.1	21.8	11.7
	Yes	16.2	15.0	41.0	35.1	21.6
Reliability	>=0.6	3.2	2.0	11.4	11.1	3.0
	<0.6	35.6	25.9	44.7	38.8	24.5

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	Less than 40	9.2	7.9	26.4	22.4	12.5
Genotyped	No	8.1	6.8	26.1	21.8	11.7
	Yes	16.2	15.0	41.0	35.1	21.6
Reliability	$\geq 0.6$	3.2	2.0	11.4	11.1	3.0
	$< 0.6$	35.6	25.9	44.7	38.8	24.5



The authors would like to thank the Royal National Association of Spanish Horse Breeders (ANCCE) for providing the data used in this study. This work has been financed with FEDER funds by the EQUIGENOM Operational Group (Ministry of Agriculture, Fisheries and Food, through the Spanish Agrarian Guarantee Fund, FEGA).

This study has established, for the first time, a genomic evaluation using a single-step approach with a combined relationship matrix for conformation and female fertility in PRE horse. Our results demonstrated the effectiveness of the genomic approach for the routine genetic evaluation of conformation and reproduction traits in the PRE breed.

## Conclusion

The authors would like to thank the Royal National Association of Spanish Horse Breeders (ANCCE) for providing the data used in this study. This work has been financed with FEDER funds by the EQUIGENOM Operational Group (Ministry of Agriculture, Fisheries and Food, through the Spanish Agrarian Guarantee Fund, FEGA).

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## Validation of US genomic prediction for milk and fat yield for Holstein cattle in subtropical Taiwan

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Taiwan is located in the subtropical zone, with high temperature and high humidity and small temperature difference between day and night. It is not an ideal place to raise Holstein dairy cows. It is worth wondering how efficacies for US genome-enhanced genetic predictions for actual milk and fat production traits of Holstein cows in subtropical Taiwan. The correlation between genomically-enhanced predicted milk and fat transmitting ability (gPTAM and gPTAF) and the first lactation milk and fat yield (305, 2X, ME) were conducted and 733 dairy cows from 19 farms were used as experimental animals. The correlation coefficient is 0.36 and 0.29 for milk and fat yield, respectively. The correlation coefficient for milk and fat yield of the 19 herds ranged from -0.02 to 0.70 and -0.01 to 0.90, respectively. gPTAM and gPTAF were used to rank and assign animals to quartiles (genetic groups: worst 25%, 26–50%, 51–75%, and best 25%). The average first milk yield performance of the above four herds was 8,410 ( $\pm 1,499$ ), 8,916 ( $\pm 1,653$ ), 9,324 ( $\pm 1,539$ ) and 9,993 ( $\pm 1,679$ ) kg, respectively. The average first fat yield performance of the above four herds was 370 ( $\pm 72$ ), 394 ( $\pm 73$ ), 402 ( $\pm 77$ ) and 428 ( $\pm 83$ ) kg, respectively. Significant differences were observed between quartiles for milk and fat production ( $P < 0.05$ ). According to Taiwan average raw milk price (\$1.17 dollar per kg), the profit differences between the top and bottom quartiles were \$1,852 dollar per cow. It showed that the gPTA of genetic testing can be used as a breeding tool for selecting superior dairy cows. With proper management, it will help increase the profitability of the herd in subtropical zone.

### Abstract

*Key words: Holstein cattle; Genetic testing; Genomically-enhanced predicted transmitting ability.*

A technology based on the detection of single nucleotide polymorphisms (SNP) is a major revolution in animal breeding, as a tool termed genomic selection (Meuwissen *et al.*, 2001), combined with phenotype and pedigree data to comprehensively detect the allelic variances that affect these phenotypes have the advantages of increasing accuracy of selection, selection intensity, and reducing generation interval to accelerate genetic gain (Seidel, 2010). Widespread use of DNA markers have significantly impacted breeding program strategies and reduce breeding costs. After Illumina launched the first genome-wide SNP chip (BovineSNP50 BeadChip) in 2007, it first published genomic estimated breeding value (GEBV) of American Holstein cattle in 2009. Since then, dairy-advanced countries have established genomic evaluation of Holstein cattle and other dairy cattle breeds on a routine basis (Cole, 2014; Lund *et al.*, 2011; De Haas *et al.*, 2012; Durr and Philipsson, 2012). Norman *et al.* (2014) reported that the implementation of genomic evaluation has changed the breeding program

### Introduction

in the United States. The number of genotyped US bulls and cows has increased steadily since 2007. Over 6.5 million dairy animals have been genetically evaluated. Genomically-enhanced predicted transmitting abilities (gPTA) and reliabilities was calculated for each trait to assess how well or bad it is.

The correlation assessment between genetic predictions and the observed performance of tested animals in externally validated populations or even foreign populations is helpful in evaluating the accuracy of any genetic evaluation or prediction algorithm. McNeel *et al.*, (2017) and Wijma *et al.*, (2022) have evaluated the validity of genetic prediction of wellness traits and abortion in commercial herds of American Holstein dairy cattle, respectively. The tested herds did not provide any phenotypic information and were not involved in genetic evaluation. Fessenden *et al.* (2020) also assessed that a specific selection index comprising gPTAs was able to predict successfully the observed lifetime profits in American Holstein animals. It is worth wondering how efficacies for such prediction apply to herds in other regions. Therefore, a validation study was performed to assess the efficacy of genome-enhanced genetic predictions for milk and fat production traits in Holstein cows located outside the United States, such as subtropical Taiwan.

## Material and methods

The hair follicles or blood sampling approach was similar to previous report by our group (Chao *et al.*, 2022). Briefly, samples were collected from 733 random cows in 19 herds (herds from Northern, Central, Southern, and Eastern Taiwan and Kinmen Islands, respectively). The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Taiwan Livestock Research Institute (LRI-IACUC108-1 and LRI-IACUC109-1). The data, including the date of birth, sire and dam of the tested cow and sample collection card were mailed to the Neogen Genomics Lab in Lincoln, NE, United States, and the GGP bovine 50K SNP chips that use Illumina Infinium technology (Illumina, 2017) were used for genotyping. The CDCB-certified laboratory at Neogen uses the Illumina Infinium XT genotyping assay platform (Illumina, 2017).

The milk production traits analyzed in this study include milk yield and fat yield. Taking the milk yield of the full lactation period as an example, it refers to the accumulated milk yield of a cow after adjusting the lactation days of the first parity to 305 days after parturition. The data editing procedure included the following steps: (1) Extracted the “Monthly Lactation Record Data” of the DHI database as the data source. (2) Screened more than 7 monthly milk sampling times with lactation days between 235 and 315 days, and added them to the data set. (3) For lactation records with less than 305 days of lactation, used the linear regression method to calculate the milk yield on the day without milk sampling records (Robert *et al.*, 1997).

## Results and discussion

The aim of this study was to evaluate the associations between gPTAs of genomic evaluation and observed milk and fat performance in Taiwan cattle, and to dissect whether the accuracy of genomic prediction analysis is different due to environments. A total of 733 dairy cattle were verified the accuracy of genomic prediction. The correlation between the gPTAM, gPTAF and the first lactation milk, fat yield is 0.36 and 0.29, respectively (Figure 1 and 2). Further comparison of the data among 19 herds showed that the correlation of the gPTAM and the first lactation milk yield were different, and the value of the correlation ranged from -0.02 to 0.70 and -0.01 to 0.90, respectively. The higher correlation between gPTAM and the first lactation milk yield indicated that

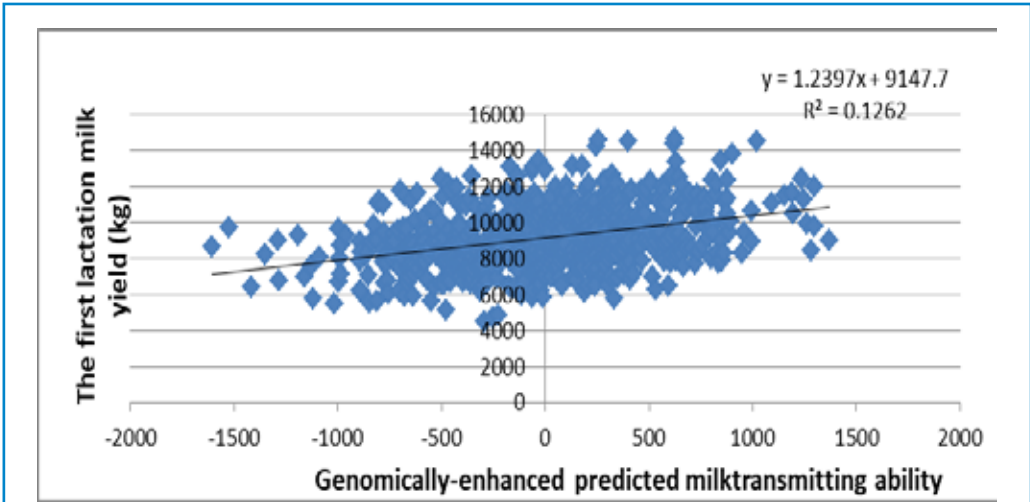


Figure 1. The correlation between the gPTAM and the first lactation milk yield.

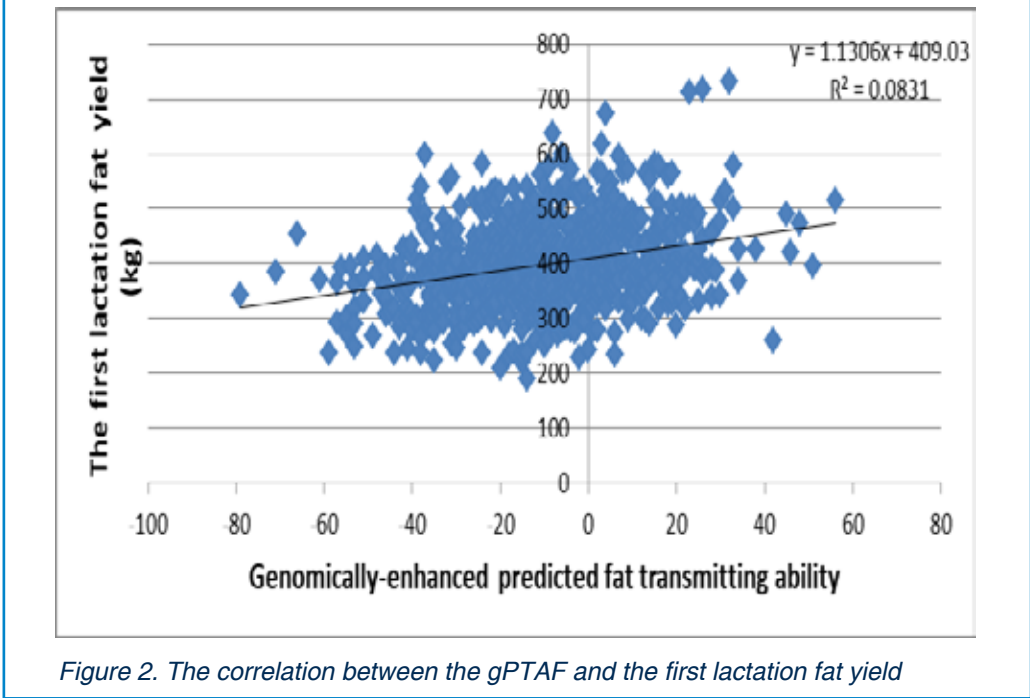


Figure 2. The correlation between the gPTAF and the first lactation fat yield

superior cattle could be exerted their genetic potential outstandingly in well-managed herds. On the other hand, even if poorly managed herds own superior cattle that could only be achieved moderate production performance, and moreover, even poor management makes the performance of superior cattle even worse. It can be seen that the genetic composition of cattle is the core for its production performance, and it needs to cooperate with management practice to achieve the genetic effect. These tests can also inform dairy producers on the genetic makeup and actual performance of the cattle on the farm to adjust management strategies.

Differences in milk and fat production were statistically significant between the genetic groups. gPTAM and gPTAF were used to rank and assign animals to quartiles (genetic groups: worst 25%, 26–50%, 51–75%, and best 25%). The average first

milk yield performance of the above four herds was 8,410 ( $\pm 1,499$ ), 8,916 ( $\pm 1,653$ ), 9,324 ( $\pm 1,539$ ) and 9,993 ( $\pm 1,679$ ) kg, respectively (Figure 3). The average first fat yield performance of the above four herds was 370 ( $\pm 72$ ), 394 ( $\pm 73$ ), 402 ( $\pm 77$ ) and 428 ( $\pm 83$ ) kg, respectively (Figure 4). Significant differences were observed between quartiles for milk and fat production ( $P < 0.05$ ).

As shown in Figure 3 and 4, the differences between the top and bottom quartiles were 1,583 kg for milk production and 58 kg for fat production. According to Taiwan average raw milk price (\$1.17 dollar per kg), the profit differences between the top and bottom quartiles were \$ 1,852 dollar per cow. This indicated that animals with higher value

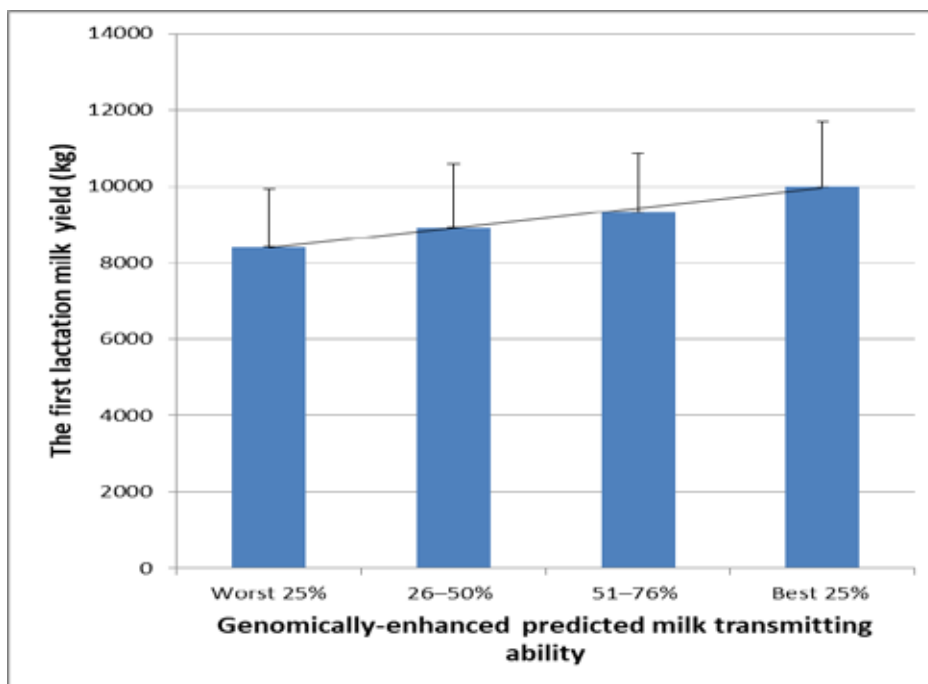


Figure 3. Differences in milk production between the genetic groups.

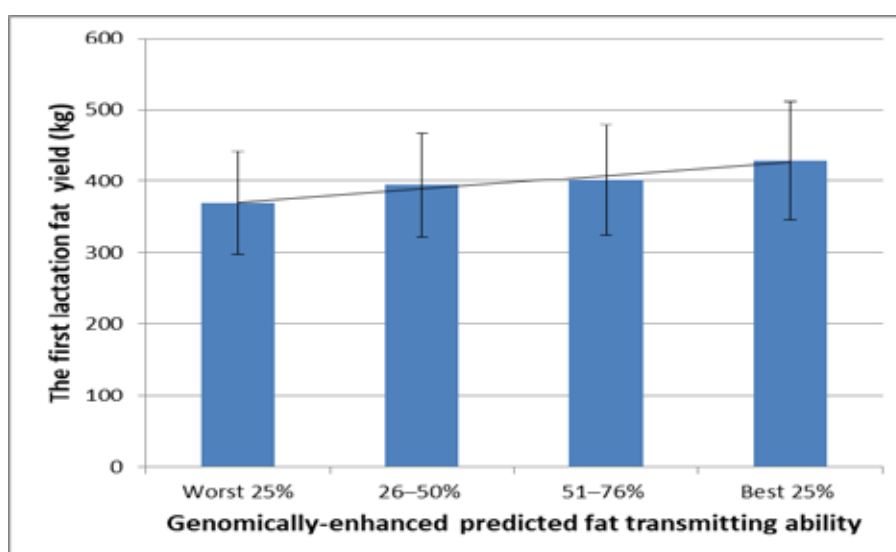


Figure 4. Differences in fat production between the genetic groups.



predictions had higher observed profit than animals with lower value predictions. The observed results demonstrate the ability of production trait predictions to accurately predict production performance.

Our results showed that it is very suitable to use the gPTA values of tested heifers for making culling and breeding decisions. In the future, it can be expanded to milk protein, somatic cell counts and other traits for such association testing. It will move towards a balanced selection that takes into account all economic performances and use the gNM\$ and productive life to select healthier, fertility and longevity cattle.

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## Valuing the Certificate of Quality at Holstein UK

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The Holstein UK and British Friesian Cattle Herdbook has registered animals since 1909. The modern Holstein UK continues to operate the Herdbook, as well as owning a milk recording company (CIS) and operating a classification and data company (NBDC). NBDC provides Herdbook registration and database services to 7 other dairy Herdbooks in the UK and classification services to 20 dairy and beef Herdbooks.

ICAR certification for animal ancestry and phenotype recording provides a modern guarantee of quality for customers of Holstein UK. Data customers of Holstein UK include farmers, genetic evaluations services, auctioneers, AI companies, government, and other commercial companies. All these rely on the Herdbook to provide up to date and accurate information for use in genetic and genomic evaluations, sales, traceability and even breed conservation. Our core customers are farmers, who face greater demands on time than ever before. They need the Herdbook to operate accurately and efficiently when recording their animals' data, to continue adding value to their businesses into the future.

Historically challenges to the accuracy of the Herdbook were limited. In 2022, about 20% of calves registered in the Herdbook were genomic tested – a figure that is growing each year. Most of the genomic testing in the UK is done by multinational companies, whose commercial goals do include accurate parentage recording. About 5% of Herdbook females are declared to have a different genomic parent after they have been pedigree registered. This is a challenge for both Holstein UK and ICAR animal recording.

The Holstein UK Herdbook has been in existence since 1909 and incorporates two breeds, Holstein and British Friesian. In 2022, 185,000 female calves were registered in the Herdbook, a similar level to that seen in recent years (see Figure 1). Conversely, the number of male calves registered has fallen dramatically in recent years (Figure 2), probably due to the increase in use of sexed semen by breeders.

Holstein UK is also the parent company for CIS and NBDC. The former provides milk recording and health testing to UK farmers. NBDC provides Herdbook services to seven other dairy breed Herdbooks and conformation scoring (classification) to twenty dairy and beef cattle Herdbooks in the UK.

### Abstract

### Introduction

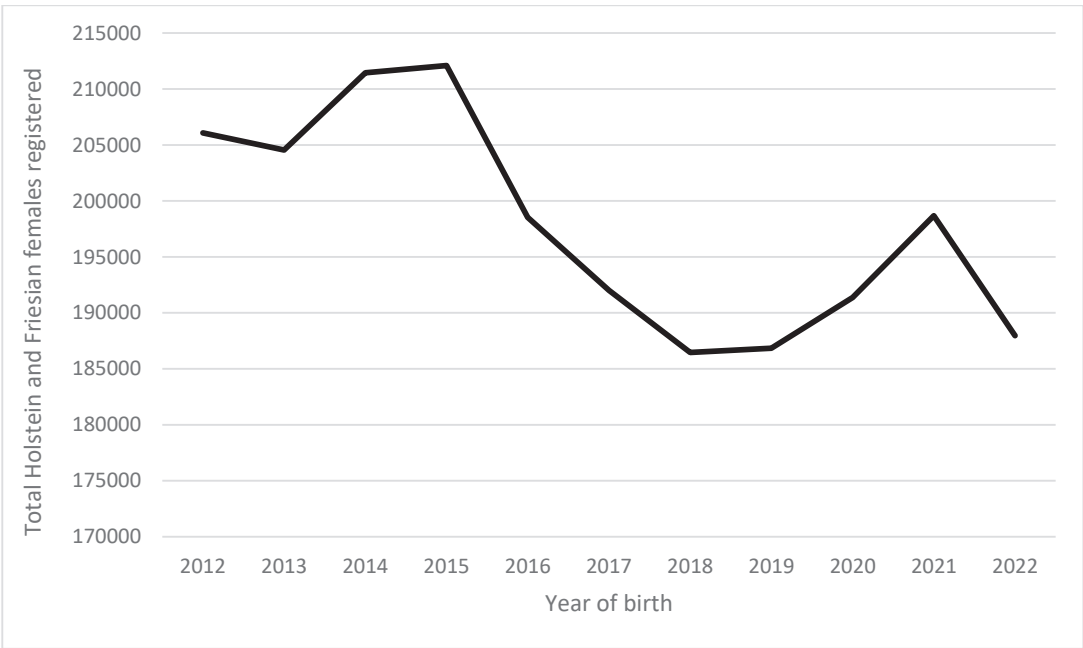


Figure 1. Total annual registrations of females in the Holstein UK Herdbook.

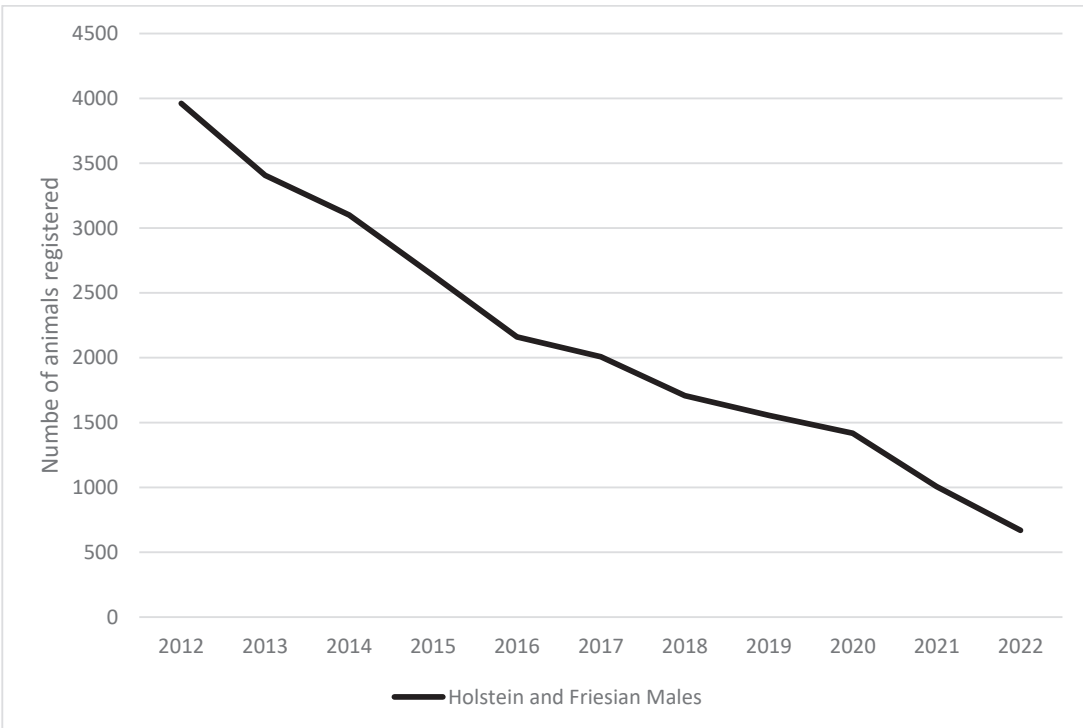


Figure 2. Total annual registrations of male calves (excluding AI bulls) in the Holstein UK Herdbook.

As well as the core function of Herdbook registration, Holstein UK also engages in promotional (including Herdbook shows), social and educational activities for breeders.

As well as providing paper and electronic registration certificates for breeders, online data (including Ancestry, lactation, classification and genetic evaluation results) are freely published for animals registered in the last fifty years. Data is also provided to other industry partners including genetic evaluations services, auctioneers, AI companies, government, and other commercial companies. Where data is packaged (collated) for commercial companies, a fee is payable to the Herdbook.

Holstein UK is certified by ICAR for Herdbook functions involving animal identification, Herdbook recording, conformation recording and data processing. As such Holstein UK's customers (breeders and industry partners) should expect the Herdbook to accurately record and process data.

Awareness of ICAR among breeders is probably low. However, they likely have an expectation that the Herdbook will operate to high standards and assume that it will be certified by an appropriate organisation (such as ICAR).

Value derived directly from CoQ is more likely gained from (collated) data sold to industry partners, among which awareness of ICAR is higher. Although the financial reward for this is relatively small, about 0.5% of total Holstein UK group revenue, it is sufficient by itself to cover the cost of CoQ many times over.

A current challenge to the accuracy of Herdbook data comes from genomic parentage conflicts. These can arise when Herdbook registered animals are genomic tested, commonly by an outside company. The genotype for UK born animals is usually sent to (either or both) the national UK and North American genetic evaluations, operated by AHDB and CDCB respectively. Their processes may discover that one or both parents is incompatible with the genotype attributed to the animal.

About 20% of females, registered in the Holstein UK Herdbook and born in 2022, were also genomic tested. Only a quarter of those were tested via the Herdbook, with the remainder tested via mainly large international companies. It is not uncommon for a conflicting ancestry of a Herdbook female to be published online. Genomic selection is a fast-moving world and breeders adopting this tool value quick delivery of results, often via the use of online applications. Keeping up with this pace is both a challenge and an opportunity for Herdbooks and by extension to ICAR certification standards.

## Herdbook functions

## Value of CoQ

## Challenge



## Ewes' Milk Urea Concentration methods' optimization, by difference in pH and Mid-Infrared Spectroscopy (MIRS)

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### Abstract

Currently, in France, analytical methods used for ewes' Milk Urea Concentration (MUC) rely on cow's methods. However, both matrix are different, as fat and protein content are up to twice higher in ewe milk compared to cow milk. Moreover, MUC variability seems to be higher in ewe milk (from 50 to 1000 mg/l) than in cow milk (from 150 to 500mg/l), hence the question about the analysis methods adequation in ewe milk.

This project aimed at optimizing and or adapting the reference and routine methods used to determine ewe's MUC, currently based and calibrated from cows milk.

The reference method was validated as such; calibration matched for cow milk as well as for sheep milk. Concerning the routine method, creating a new specific predictive equation based on individual and bulk tank ewes' milks neatly improved the performance compared to the existing cows' milk predictive MUC equation.

*Keywords: Milk Urea Content, ewes, analytical methods, MIRS, predictive equation.*

Milk Urea Concentration (MUC) is a simple indicator reflecting ruminants diet balance, protein concentration and energy in the sheep diet (Bocquier et Caja, 1999, Cannas *et al.*, 1998, Gholi Ramin *et al.*, 2010). MUC also varies according to the fibers or non-fibers carbohydrate sources provided (Giovanetti *et al.*, 2019). Its monitoring helps optimizing the feed cost by limiting waste, hence the economical stake, knowing that the feeding expenses represent from 62% to 72% of the operational expenses in the Atlantic-Pyrenees and North Occitany French sheep dairy areas (Inosys Réseaux d'élevage, 2021). Furthermore, MUC excess exerts negative impacts on reproductive efficiency (Giovanetti *et al.*, 2019). Lastly, according to the type of dairy products, MUC excess might be associated to weaker milk technological ability and final products defects. Thus, MUC is a composite, costless indicator that breeders can easily use in herd management.

Methods currently used to measure Milk Urea Concentration (MUC) in ewe's milk rely on cow's milk methods. However the dairy matrices between those species differ. Thus, this project aimed at:

### Introduction

1. Verifying the reference method adequation (enzymatic method using difference in pH ISO 14637 / IDF 195:2004),
2. Verifying the routine method, based on a prediction from cow milk Mid Infra-Red Spectra (MIRS) to determine ewe's milk urea concentration,
3. Optimising, the routine method by developing a specific ewe's milk predictive SMIR equation for MUC.

## Material and methods

Concerning the reference method, the repeatability, reproducibility and accuracy were evaluated on 25 samples of individual ewe milk, from Lacaune and Basco-béarnaise breeds, analysed by Actalia Cécalait (Poligny – France) from December 2021 to January 2022.

Concerning the routine method, the analyses were conducted by the interprofessional lab Agrolab's (Aurillac, France), every month from January to June 2022. The data included 2 datasets:

- 260 samples from individual ewe milk (a single flock, for each area: Corse, Nouvelle-Aquitaine and Occitanie, representing around 20 animals per month and per flock),
- 401 samples from bulk tank milk (around 20 flocks respectively for each area).

This original protocol enabled to maximize the existing ewe milk variability, as recommended by De Marchi *et al.* (2014). It was meant to optimize the ewes' MUC predictive model, as the seasonal, geographical, breeds (Lacaune, Basco-béarnaise, Manech tête rousse et noire, Corse), intra and inter-flock variability were taken into account. Every Verimilk was measured by infrared on Foss electric analysers and compared to the reference method (ISO 14637 / IDF 195:2004), by Agrolab's Aurillac. Then, the specific ewe milk predictive MUC equation was established by Partial Least Square regression as described by El Jabri *et al.* (2019).

## Results

### Reference method

The reference method was validated as such; calibration matched for cow milk as well as for sheep milk, regarding the performances of repeatability, reproducibility and accuracy.

### Routin method

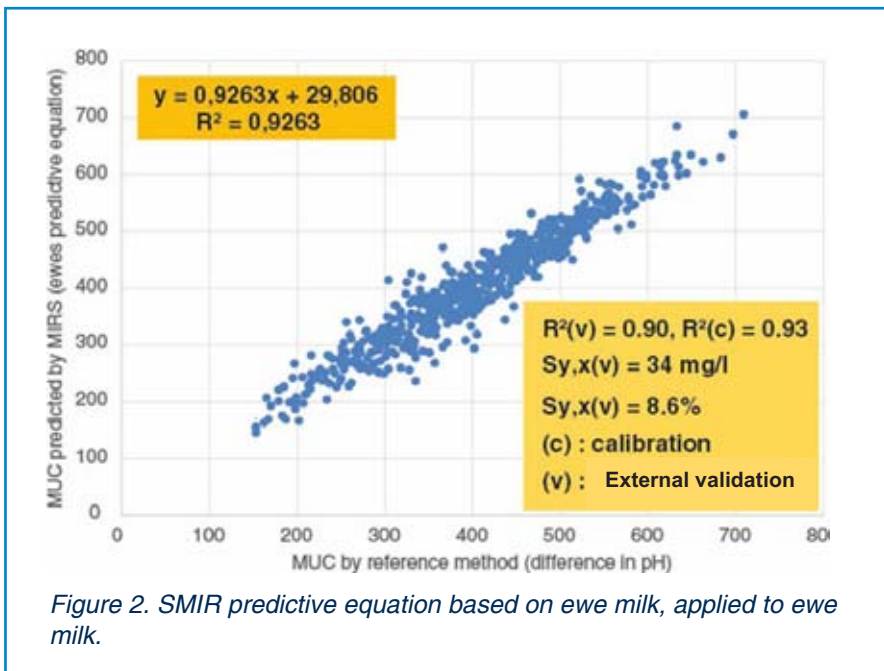
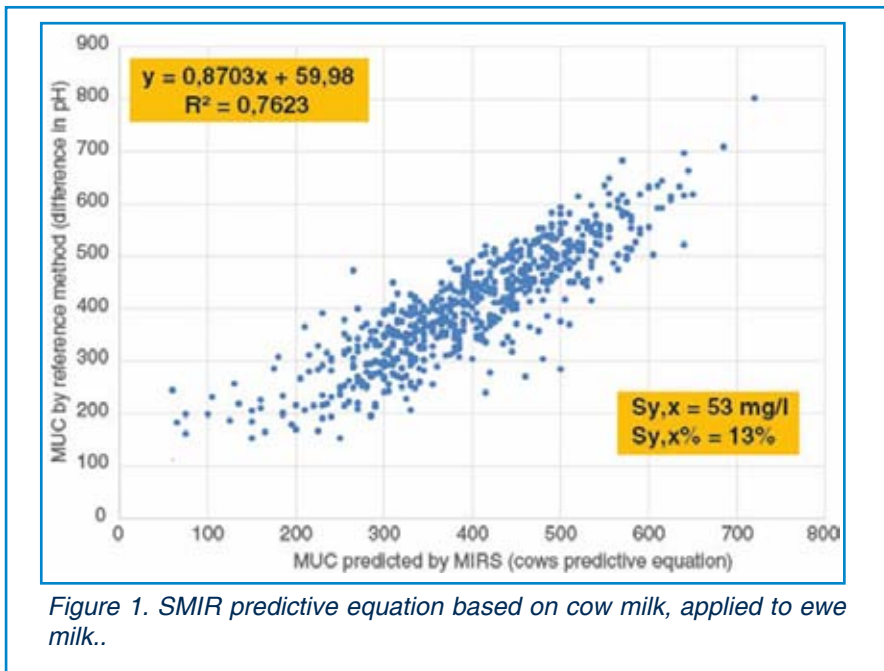
The cow's MUC predictive equation did not give a good precision for ewe's milk, as it only accounted for 76% of the ewe's milk variability (coefficient of determination,  $R^2=0.76$ ). The Residual Standard Deviation RSD ( $S_{y,x}$ ) was then of 53 mg/l MUC vs. 35 mg/l for the cow's MUC predictive equation applied to cow milk.



Table 1. Comparison of Residual Standard Deviation (RSS) on MUC, according to the predictive models and the ruminant species

Model (type of predictive SMIR equation)	Predictive equation based on cow milk	Predictive equation based on cow milk	Predictive equation based on cow milk	Predictive equation based on ewe milk
Analysed milk	cow	goat	ewe	ewe
RSD ( $S_{y,x}$ )	35 mg/l <sup>1</sup>	40 to 59 mg/l <sup>1</sup>	53 mg/l	34 mg/l

<sup>1</sup> Actalia Cécailait, Trossat P., 2014, MUC evaluation in goats' milk by MIRS method (internal report).



With the specific ewe's milk model, applied to individual and bulk tank milk samples, the performance was equivalent to the cow's milk model applied to cow milk samples. A greater variability was included in the ewe's milk predictive SMIR equation (coefficient of determination of external validation,  $R^2(v) = 0.90$ ), applied to ewe's milk samples. The RSD in external validation ( $S_{y,x(v)}$ ) was improved 34 mg/l, with the specific ewe's predictive SMIR equation vs. 53 mg/l with the cow's milk predictive equation applied to ewe's milk samples.

## Conclusion

MUC management can closely be related to ewe's feed optimization, animal health, and final dairy products quality.

Thus, developing specie-specific MUC predictive model by SMIR would neatly improve urea's precision in routine analysis for ewe's milk, may it be individual milk or bulk tank milk samples.

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## Development of a new equation based on MIR spectra to predict lipolysis in dairy goats

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France, Europe's leading producer, uses most of its goat's milk for cheese processing. The French goat industry therefore has a strong interest in producing high quality milk. The rate of lipolysis of milk is a quality criterion which must be controlled to maintain the quality of milk and dairy products. In France, a prediction equation has been calibrated using bovine mid-infrared spectra (MIR) to quantify the free fatty acids indicative of lipolysis, but to date there is no instrumental method to evaluate lipolysis in goat milk.

The present study aims to develop a prediction equation for milk lipolysis in goat milk. For this purpose, 518 milk samples were collected from 4 experimental farms. A joint analysis of lipolysis according to ISO/TS 22113 (BDI method) and MIR spectrometry was performed on each sample.

The equation, developed by partial least squares regression after square root transformation, achieved a coefficient of determination  $R^2=0.91$ , with a residual standard deviation ( $Sy,x$ ) of 0.20 meq/100 g fat. The relatively high accuracy of this equation should allow to use it to explore the genetic determinism of milk lipolysis in goats. This work offers new perspectives to deepen our knowledge of the mechanisms of lipolysis in goat milk and to improve its control on the farm.

*Keywords: lipolysis, goat, mid infrared spectroscopy.*

France is Europe's leading producer of goat's milk, with 709,510 tonnes of raw milk produced per year. A large proportion of this milk is used for cheese processing, with 97,960 tonnes of cheese produced (FAO, 2020). In France, one-sixth of goat's cheese is produced on the farm, and the country has 15 goat's cheeses with protected designations of origin (PDO). It is therefore in the interest of the French goat industry to produce high quality milk. While milk quality has traditionally focused on fat and protein contents, or bacterial and somatic cell counts, the rate of lipolysis in milk is a quality criterion that must be monitored to maintain the quality of milk and dairy products.

### Abstract

### Introduction

Lipolysis is the breakdown of milk fat by the hydrolysis of triglycerides, the main component of milk fat. This hydrolysis leads to the release of free fatty acids that may affect the flavour as well as their possible oxidation products in the milk. As a result, a high rate of lipolysis leads to a deterioration in the organoleptic (rancid taste) and technological (inability to be processed) properties of the milk.

In France, a prediction equation has been calibrated using bovine mid-infrared spectra (MIR) to quantify the free fatty acids indicative of lipolysis (Gelé *et al.*, 2022). Although the use of MIR spectra has been developed strongly in dairy cattle since the end of the 2000s to phenotype new traits on a large scale, to date, there is no instrumental method to evaluate lipolysis in goat milk.

The present study aims to develop a prediction equation for milk lipolysis for goat milk.

## Material and methods

Five hundred and eighteen goat milk samples were taken to meet the objectives. A joint analysis of lipolysis according to ISO/TS 22113|IDF/RM 204 (BDI method) and MIR spectrometry was carried out on each sample.

### Data collection

#### Milk samples collected on farms.

Five hundred and eighteen goat milk samples were collected from four experimental farms located in several regions in France, between March and October 2021. Around 40 goats were sampled on each farm three times at different periods to maximise the diversity of diets and to represent the two main dairy goat breeds found in France, Alpine and Saanen (Table 1).

During sampling, vials containing 0.02% bronopol preservative (wt/vol) were fully filled (100 mL) to avoid “churning” of the milk that could damage fat globules and thus favour lipolysis during transport.

After collection, milk samples were stored at 4°C to limit bacterial proliferation and lipase-associated activities. Milk samples were sent at 4°C to ACTALIA CECALAIT (39800 Poligny, France) for subsequent analyses.

Table 1. Distribution of samples collected between farms and sampling periods.

Experimental farm	Number of milk samples			Total
	March/April	May-June	September-October	
P3R	40	40	47	127
MoSAR	40	40	40	120
FERLUS	45	45	45	135
IE PL	48	48	40	136
Total	173	173	172	518

MIR spectra were recorded at ACTALIA CECALAIT using MilkoScan™ FT+ spectrometer (Foss, Hillerød, Denmark).

Reference values for lipolysis in milk were determined using the ISO/TS 22113|IDF/RM 204 BDI (Bureau of Dairy Industry) method which determines the titratable acidity of milk fat. This analysis was carried out by ACTALIA CECALAIT within 36 hours of sampling. Lipolysis measured by BDI method averaged 0.85 meq/100 g fat (sd=1.00 meq/100 g fat), with a median value of 0.51 meq/100g fat.

Five hundred and nine individuals were selected after removing nine outliers for the development of the goat milk lipolysis prediction equation. The lipolysis reference values obtained by the BDI method were square-root transformed. The equation was developed by partial least squares (PLS) regression using R software on a calibration set made up of 2/3 of the data. The predictions were then squared and bounded at 5 meq/100g fat. The remaining third of the data was used as a validation set.

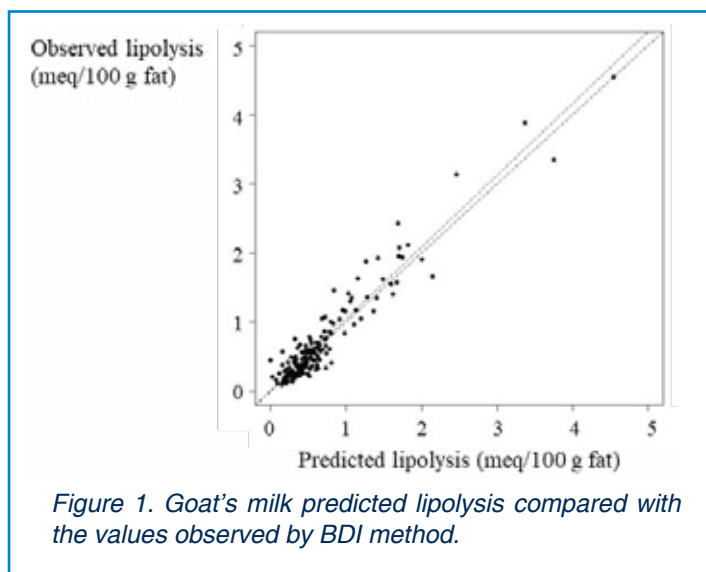
The lipolysis prediction equation developed for goat's milk has a coefficient of determination  $R^2 = 0.91$  and a residual standard deviation  $S_{y,x} = 0.20$  meq/100 g fat. Figure 1 shows the predicted results using this equation, compared with the reference values from the BDI method. The accuracy of this equation is much higher than the one developed for cow's milk under the same conditions:  $R^2 = 0.72$  and  $S_{y,x} = 0.19$  meq/100 g fat (Gelé *et al.*, 2022).

In 2011, Soyeurt *et al.* indicated that equations with an  $R^2$  greater than 0.95 could be used in milk payment systems, and that their use for genetic improvement is possible

Recording of  
MIR spectra and  
measurement of lipolysis  
in milk

Development of  
the equation for  
predicting lipolysis of  
goat's milk

## Results and discussion



from 0.75. Our equation has an  $R^2$  of 0.91, which makes it possible to envisage not only genetic exploration of the lipolysis trait, but also routine use in the field to discriminate milks according to their level of lipolysis with a satisfactory level of confidence. This equation represents a first step towards better characterising goat's milk and improving its quality on a routine basis.

## Conclusion

This work has led to the development of a new equation for predicting milk lipolysis specific to goat's milk. The high accuracy of this equation opens new prospects for increasing our knowledge of the mechanisms of lipolysis in goat milk and for improving its control on farms through finer herd management and even selection.

## Acknowledgement

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## Genomic selection in Spanish dairy sheep breeds

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This paper presents the situation of the implementation of genomic selection in Spanish dairy sheep breeds, both native and foreign. Although in all cases the methodology used is the same (ssGBLUP) and an increase in the reliability of the estimates is observed, the work shows the existing variability in the dimension of this increase (15-60 %), in the criterion of genotyping used in each of them (sex, number and type of animal) and in the characters and models used. Likewise, a reflection is made on the transfer of these results to farmers and on the expectations, contributions and methodological and organizational challenges associated with the implementation of genomic selection.

### Abstract

With the development of molecular techniques, bioinformatics and evaluation methodologies, the implementation of genomic selection in breeding programs has been widespread, although at variable rates depending on the species and breed (Ibañez-Escriche and Gonzalez-Recio, 2011; Jonas and de Koning, 2015; Meuwissen *et al.*, 2016).

### Introduction

The International Sheep Genomic Consortium (ISGC, 2002) has facilitated the development of genomic tools in ovine, among them the OvineSNP50 BeadChip (Illumina Inc., San Diego, CA, USA) was released in 2009, bringing new perspectives for genomic selection implementation in sheep breeding programs. In comparison with dairy cattle, dairy sheep breeding has several differences that should be considered. The presence of a wide range of breeds, with environmentally adapted production systems, small population sizes, very heterogeneous data recording systems and a lower economic value per individual are some intrinsic characteristics that influence the implementation of genomic selection in sheep breeding programs (Ibanez-Escriche and Simianer, 2016).

Regarding Spanish dairy sheep breeds, in 2018 studies to assess the state of GS implementation were started in four breeding programs: Assaf, Churra, Manchega and Latxa (National Institute for Agricultural and Food Research and Technology or INIA, 2020). These works focused on analysing the selection criteria to make up the genotyped population and the development and implementation of genomic schemes. Among these breeds, three of them are autochthonous, being the Manchega breed the one with the bigger population (135.000 ewes), followed by Latxa breed (70.000 ewes) and Churra breed (23.000 ewes). Meanwhile, Assaf is a foreign breed that currently has 100.000 ewes. The main objective of this work is to gather the results obtained during the last lustrum among these breeds regarding the implementation of genomic selection, such as the variability in terms of prediction reliability, the genotyping criterion used and the selection objectives considered.

## Material and methods

The genomic information available in these dairy sheep breeds has been funded by a project of the Spanish Ministry of Agriculture (2018-2022), regional projects and individual initiatives of breeder's associations. All the breeds have genotyped with the Axiom™ Ovine Genotyping Array (Thermo Fisher Scientific Inc., Waltham, MA, USA), and the Latxa breed also has some individuals genotyped with the OvineSNP50 BeadChip (Illumina Inc., San Diego, CA, USA). The Assaf breeds has the biggest genotyped population (more than 12.000 animals), followed by Manchega breed (6.000 animals), Latxa breed (3.700 animals) and Churra breed (3.000 animas). The distribution of these animals across sexes and presence/absence of phenotypic data is shown in Table 1.

Regarding the selection objectives considered by each breed, Assaf has implemented genomic selection for milk yield (120-d standardized lactation), milk quality (fat and protein), udder morphology and cell score. Latxa breed has implemented for milk yield (120-d standardized lactation), milk quality (fat and protein), and udder morphology; and Manchega for milk yield (test day) and udder morphology. Currently, the Churra breed carries on genotyping, but they has not jet implemented a genomic selection scheme. Besides, we are going to focus on the results obtained for milk yield in Assaf, Latxa and Manchega breeds.

The genomic evaluations of the three breeds are done with the single step genomic BLUP (ssGBLUP) methodology (Aguilar *et al.*, 2010; Christensen and Lund, 2010), performed using the BLUPf90 software suite (Misztal *et al.*, 2002).

## Results and discussion

Among the studies done to assess the inclusion of genomic information in genetic evaluations, prediction accuracy of classic and genomic evaluations has been considered, the results for the three dairy sheep breeds are shown in Table 2. Assaf and Latxa show higher prediction accuracies because their selection criterion is milk yield in 120 days of lactation, while Manchega breed considers test day data. Comparing the two methodologies show in all cases that genomic evaluation is more accurate, so that including genomic information increases prediction accuracy from 3 to 14 %.

Furthermore, the reliability of classic and genomic evaluations has been analysed and mean results by sex, availability of phenotypic data and genomic information, and breed can be found in Table 3. The mean values are higher for Manchega breed, as a results of the selection criterion used, as previously described. Comparing both methodologies, reliability values of genomic evaluation are in all cases or similar or higher than pedigree evaluations. This difference is noticeable when the animals are genotyped, but it is especially relevant when the genotyped animals have not progeny data (young rams) or their own phenotypic data (young ewes).

Table 1. Distribution of genotyped individuals by sex and type, for Assaf, Churra, Latxa and Manchega breeds at June 2022.

	Assaf	Churra	Latxa	Manchega
Rams with progeny	3.034	272	1.593	1.902
Ewes with lactations	3.640	2.960	1.483	988
Young rams	4.749	95	592	3.093
Young ewes	927	37	46	57
Total	12.350	3.154	3.714	6.040



Table 2. Prediction accuracy of milk yield pedigree and genomic evaluation and their difference (genomic-pedigree, G-P) for Assaf, Latxa (Cara Negra from Euskadi population) and Manchega breeds.

	Assaf	Latxa	Manchega
Pedigree	0.77	0.57	0.54
Genomic	0.79	0.65	0.59
G-P	0.02 (3 %)	0.08 (14 %)	0.05 (9 %)

Table 3. Prediction reliability of milk yield pedigree and genomic evaluation by sex and availability of phenotypic data and genomic information for Assaf, Latxa (Cara Negra from Euskadi population) and Manchega breeds.

	Assaf		Latxa		Manchega	
	Pedigree	Genomic	Pedigree	Genomic	Pedigree	Genomic
Rams with progeny	56	56	63	64	70	70
Rams with progeny + geno	62	70	77	79	87	89
Ewes with lactations	43	43	63	63	60	60
Ewes with lactations + geno	50	61	72	75	63	66
Young rams	30	30	27	41	30	30
Young rams + geno	30	48	52	60	35	48
Young ewes	30	30	23	35	30	30
Young ewes + geno	30	49	53	58	30	40

Obtaining evaluations with higher prediction reliabilities allows taking selection decisions when rams have a lower number of progeny and ewes have less lactation data. For instance, to achieve 60 % reliability based on classic evaluation a mean of 14 progeny data per ram or 5/6 lactations per ewe are needed, while with genomic evaluations rams with 5 progeny data and ewes with 2/3 lactations already achieve that reliability value. Therefore, selection decisions are taken earlier, as can be easily seen in the age of the proved rams used in 2019 and in 2022: 56 and 46 months old, respectively. This decrease in the generation interval makes possible a quicker genetic progress and a higher genetic gain. In addition, when the genomic information is included in evaluations, as the Mendelian segregation is considered, it is possible to distinguish full sibs with the same pedigree index by classic evaluation, and thus more informed breeding decisions could be taken.

Among the implementation challenges, here some of the methodological issues that have been identified:

- Mistakes in genealogy: Based on genomic information (SNPs) pedigree mistakes from microsatellites analysis could be revealed. Which should be the inconsistency threshold allowed?

### Future perspectives and challenges

- Modelling missing pedigree: Unknown parent groups work well on classic BLUP evaluations, but they are more problematic in genomic BLUP evaluations.
- Direct genomic values: Based on the pedigree index and the estimated SNP effects from genomic evaluations it is possible to estimate a direct genomic value, but how reliable are these values?
- Inflation of genomic values: It has been found especially in selection candidates, and has been already described (Harris *et al.*, 2011). The cause is not known, and currently it is being managed by the scaling of G and A matrixes (Martini *et al.*, 2018).
- Imputation from low density platforms to medium and high density, It is based on the existence of linkage disequilibrium (LD) between SNPs. In sheep breed the LD is very low, thus the imputation is highly inefficient

There are also some other points related with the profitability of the breeding program that have to be considered regarding the change to genomic selection:

- The cost of genotyping platforms could be profitable to continue genotyping at this scale?
- Do the increase in genetic gain make up for the genotyping cost?
- Low density platforms are more economical, it would be worth to genotyped some animals by this option?
- Genotyping platforms that allow unifying several analyses like filiation, scrapie and genomic evaluation could be useful to reduce costs.
- Incorporation of new characters (fertility, illness resistance, longevity, rusticity, adaptation to global warming, etc.) which improvement could make profitable the cost of genotyping platform.

Finally, the implementation of a genomic scheme implies several organizational matters that have to be reflexed to take decision from year to year:

- The maintenance of the reference population: It should be updated every year, but how much animals and what type of animals should be genotyped?
- When there are changes on the selection objectives, to select animals to enter the artificial insemination centre more animals have to be genotyped and phenotyped for the new characters?
- How would be possible to combine the genotyping for the program and for de farmer?
- It is highly relevant to organize the time, chronology and speed in obtaining genotyping results with technicians, farmers and laboratory routines.

Last but not least, it is essential to advice and explain technicians and farmers about the functioning of their genomic scheme to understand the relevance of their work and how important is the involvement of all the agents to achieve good result.

## Conclusions

The inclusion of genomic information in Spanish dairy sheep routine genetic evaluations brings an important gain in prediction accuracy and the reliability of genetic values is higher than the obtained with classic evaluations. Moreover, taking more informed

selection decisions earlier in the lives of animals gives the possibility to accelerate the genetic progress of the breeding program. There are some economic and organizations issues that have to be considered, but genomic selection is an interesting tool for Spanish dairy sheep breeds that will bring advantageous results and keep this breeds at the forefront of innovation.

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## Spanish dairy sheep breed associations were granted with the ICAR Certificate of Quality

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In 2020, FEAGAS (Spanish federation of breeding organisations) applied for the ICAR Certificate of Quality for four Spanish dairy sheep associations, under the patronage of the Ministry of Agriculture and the support of INIA-CSIC (Agricultural Spanish Research and Technology Institute). As it was the first application, a visited audit was scheduled in autumn 2020. But the Covid pandemic made necessary to cancel the visit on site at the last moment. The usual process was therefore adapted in an agile and unexpected way, in agreement with all the parties, ICAR, the breed associations members of FEAGAS, and INIA-CSIC, by replacing the visits with video conference to review the documents prepared by the associations. The visited audit was delayed to the year after, in 2021. The associations audited, members of FEAGAS, were the main four dairy sheep breed associations in Spain: AGRAMA for the Manchega breed, ANCHE for the Churra breed, ASSAFE for the Assaf breed and CONFELAC for the Latxa breed. The scope of the audit covered the 6 possible fields: animal identification, herdbook recording, milk recording, conformation recording, genetic evaluation, and data processing. The documentary audit consisted in four 3-hours-meetings in videoconference, with each of the four associations. It permitted an efficient review of the documents, bridge the gaps, answer all pending questions. This was a useful preparatory step for the in-person visits to audit scheduled the year after. In 2021, the visited audit was undertaken in each association, with one day per association, including a comprehensive review of the processes, and discussion with the breed managers and stakeholders, as well as a milk recording visit on-farm. The visits were completed by an informal meeting with INIA-CSIC and the Ministry of Agriculture to discuss the first main impressions that struck the auditor during his visits. Finally, the four associations audited were granted the ICAR Certificate of Quality for all the activities. This Certificate of Quality recognizes the high standards followed by the associations work, allowing them to produce output and services of quality for the farmers. Besides, as the aim to shift towards genomic selection is shared among the four Spanish dairy sheep breed associations, the audit was an opportunity to address this concern and to suggest that an increase in the coordination at the national level, by exchanging, harmonising, stimulating, might improve the benefits for each of the associations.

### Abstract

*Keywords: dairy sheep, Certificated of Quality, breed association, Spain.*

## Introduction

Spain has the fourth dairy sheep population among European countries (behind Romania, Greece and Italy) and its dairy sheep breeds are improved through breeding programs with large population in performance recording. The Spanish federation of breeding organisations (FEAGAS), which is the Spanish ICAR member, applied in 2020 for the ICAR Certificate of Quality (CoQ) for four dairy sheep associations (as well as for 7 beef cattle associations). The audit took place during the Covid pandemic period, that obliged the stakeholders (ICAR, the auditor and the associations themselves) to adapt the scheduling and the agenda accordingly. This paper obviously does not aim to give results of the audit. The focus is to present the background of the audited associations, the necessary adaptations required regarding the Covid restrictions and the feeling and experience of the auditor in such a situation.

## Audited organisations

- FEAGAS, which is the only Spanish ICAR member, is the Federation of the Spanish breeding organisations. FEAGAS applied for the ICAR Certificate of Quality for the first time in 2020, for dairy sheep and for beef cattle. In dairy sheep, the application concerned the four following associations:
- ASSAFE (“Asociacion Nacional de Criadores de Ganado Ovino de Raza Assaf”). ASSAFE is the Association of the Assaf breed and its head office is located in Zamora (Castilla y Leon).
- ANCHE (“Asociacion Nacional de Criadores de Ganado Ovino Selecto de Raza Churra”). ANCHE is the Association of the Churra breed and its head office is located in Palencia (Castilla y Leon).
- CONFELAC (“Confederación de Asociación de Criadores Ovinos de Raza Latxa y Carranza”). CONFELAC is the Association of the Latxa and Carranzana breeds and its head office is located in Vitoria (Euskadi).
- AGRAMA (“Asociacion Nacional de Criadores de Ganado Ovino Selecto de Raza Manchega”). AGRAMA is the Association of the Manchega breed and its head office is located in Albacete (Castilla la Mancha).

## Background on Spanish dairy sheep breeds applying for the ICAR Certificate of Quality

Among the countries with the largest populations, Spain is the first country in terms of official milk recording in sheep, before France and Italy (Table 1). 15% of the whole population of ewes are recorded (Astruc *et al.*, 2022). The audited breed associations manage some of the largest dairy sheep breeds in terms of both the recorded populations and the impact of milk recording (Table 1).

Table 1. Number of dairy sheep in official milk recording in France, Spain, Italy (from Astruc et al., 2022).

Breed	Country	Recorded population	% ewes in milk recording
Lacaune	France	192,900	17%
Assaf	Spain	141,000	14.1%
Manchega	Spain	136,000	13.6%
Sarda	Italy	115,500	3%
Manech tête rousse	France	84,300	30%
Latxa	Spain	72,000	26.3%
Churra	Spain	26,000	8.9%
	Spain	375,000	10.9%
	France	341,000	20.9%
	Italy	171,700	3.3%

This was the first application from FEAGAS to ICAR CoQ. Hence, it was due to be a visited audit. The audit concerned all the possible fields of activities: animal identification; milk recording; herdbook recording; conformation recording; data processing; genetic evaluation.

The audit was initially planned for October 2020. When the pandemic broke out in early 2020, we kept the planned schedule, which was to be held for one week, with one day per association (on site) and one day for feedback (in Madrid).

Finally, as the pandemic went on and the restrictions were strong, the decision was taken in September to cancel the visits. We then had to promptly set up a practical adaptation: we decided together with INIA, the four associations, members of FEAGAS, and ICAR to keep the dates and replace the visits with video conference to review the documents sent by the associations. Therefore, this part of the audit consisted in four 3-hours-meetings in videoconference, with each of the four associations. The visited audits were postponed to 2021.

**Running the audit:  
initial agenda  
and necessary  
reorganisation due  
to COVID**

Four 3-hours-meetings in videoconference, with each of the four associations audited were held, within the same week, to review the documents sent by the associations. The documents (regular questionnaire for ICAR audit and additional materials) had been filled out and sent to the auditor prior to the meeting. The meeting started with a presentation of the association. Then the documents were comprehensively reviewed, in relation with the ICAR guidelines (ICAR guidelines-section 16, 2022). For each association, several persons were present to deal with the various activities of the audit). This step of remote interviews generated two outputs: first, the documents were updated accordingly so that the documents were more complete and more precise for preparing the visited audit. Second, a first general report (covering all associations) was produced for FEAGAS to get first feedback. This feedback, shared with the associations, was useful during the visited audit next year. At the end of this week of meetings, a remote presentation of dairy sheep genetics and genomics in France was given by the auditor to a large audience. This was a good way to better know each other and to exchange on one of the hot topics that underpinned the ICAR CoQ application in Spanish dairy sheep.

**2020: remote  
interviews**

**2021: visited audits**

With the improvement of the COVID situation, the visited audit could take place on the first week of October 2021 (1 year after the remote audit), with the following organisation: one day par association, and a working dinner in Madrid with INIA and Ministry of Agriculture, to bring first feedback. Each association organised a field visit (Figure 1), either a participation to an on-farm milk recording (Churra, Assaf, Manchega) or a visit of an artificial insemination centre (Latxa, Assaf). In addition, around three hours were spent in the association office to review the whole process, browse the documents once again (clarification, complements, discussion), and go through questions and answers, including on pending issues following 2020 remote audit.

All the visits were very useful and informative for the auditor as there was a strong commitment of each association. It was possible to have a good insight on the process regarding the audited activities and on the practical questions such as the milk recording operations.

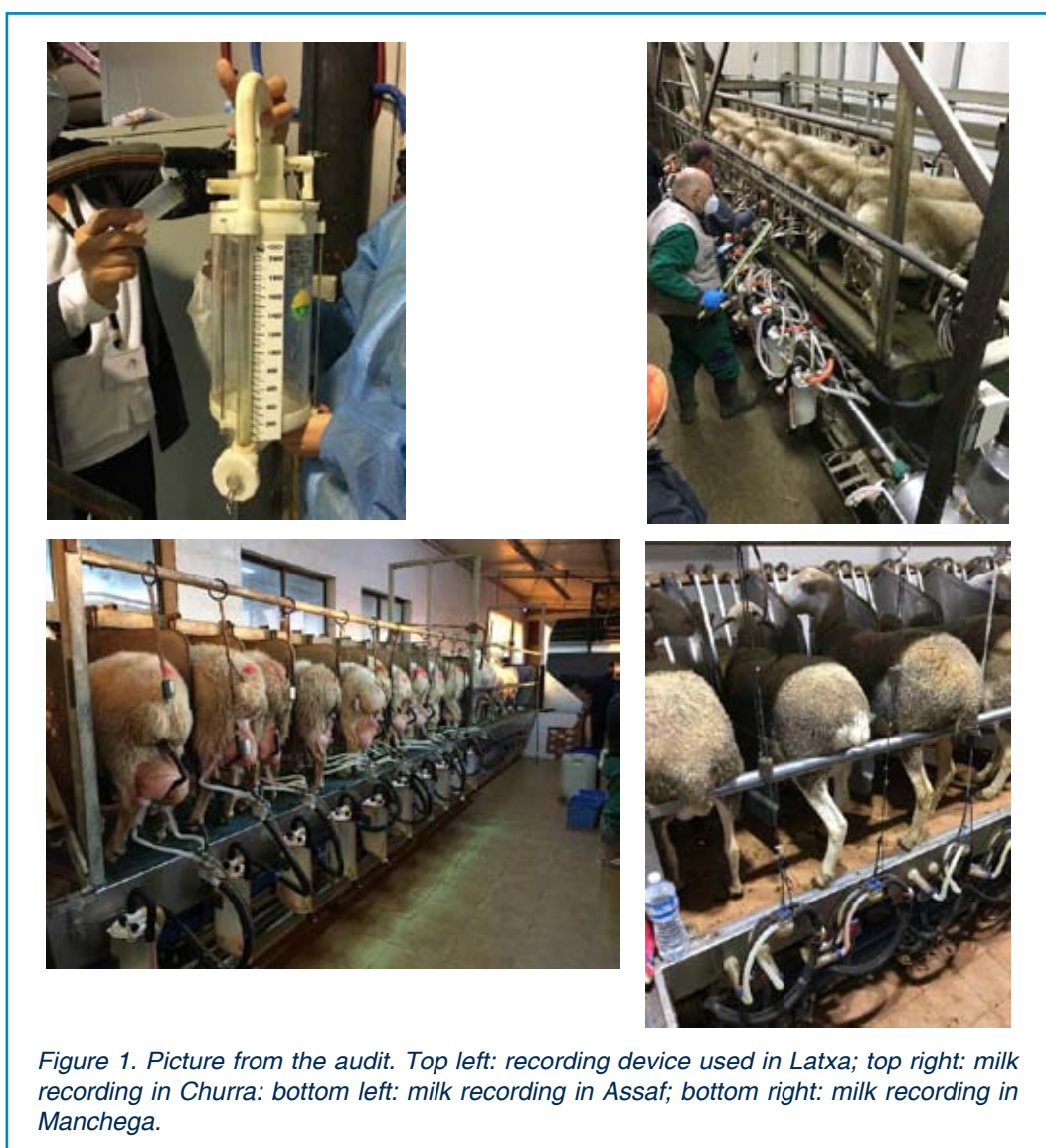


Figure 1. Picture from the audit. Top left: recording device used in Latxa; top right: milk recording in Churra; bottom left: milk recording in Assaf; bottom right: milk recording in Manchega.



After the longer-than-expected audit process due to Covid, the four associations were granted the ICAR CoQ. The reorganisation of the schedule of the visited audit did not prevent from running the audit. Unexpectedly, it even allowed a better understanding of the different processes (even if it took more time). All the possible fields of activity were audited, hence a global understanding of the selection activity.

The auditor felt the usefulness of getting at the very beginning of the audit a schematic but global vision of the ecosystem in which the breed association works. Indeed, the different associations work with different organisations (milk recording, genotypings, milk analyses laboratories, genetic evaluation), requiring a pictorial summary for a better understanding.

Beyond the obvious specific interest of the ICAR CoQ for the associations, the auditor was aware of mutual inputs: the auditor learned a lot, and at the same time, shared his experience.

Each association has its own organisation, its own material, its own scope of activities, its own ecosystem. The ICAR CoQ was given to each association and not to the Spanish umbrella. Consequently, every association was worth being audited, given its specificities.

It was a quite long process to audit four dairy sheep associations. Considering only small ruminants, if the audit had included dairy goats and meat sheep, the audit process should have been 3 times longer. That may constitute a problem for the auditor availability.

The ICAR CoQ audit was an opportunity to tackle the shift towards genomic selection in Spanish dairy sheep and to try to pursue a common thinking across breeds to share some tools (chips, management of genotypes, ...).

## Conclusions and main experiences as an auditor

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**ICAR guidelines 2022 – Section 16 Dairy Sheep and Goats.** <https://www.icar.org/Guidelines/16-Dairy-Sheep-and-Goats.pdf>

## References