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ICAR Technical Series no. 25

CIRCULAR FARMING AND ITS IMPACT ON ANIMAL GENETICS, ANIMAL RECORDING OF DATA AND CATTLE / HERD MANAGEMENT

Proceedings of the 44th ICAR Annual Conference
virtually held from Leeuwarden, NL,
26-30 April 2021

Editors: G. de Jong, K. de Koning, H. van den Bijgaart.
M. Burke and C. Mosconi

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September 2021

Preface

Various international governmental and non-governmental organisations agree that nourishing the world with sustainable diets in the years to come poses challenges to the world..

The Dutch agricultural system is a highly efficient production system in a densely populated country, with a significant contribution to the value of exported goods. Such results come with an environmental burden, such as high releases of nitrogen, phosphate and methane from the involved production systems.

In the aim to create a more sustainable balance, the Dutch are working towards a circular agriculture system—also known as closed-loop agriculture. Key success factors are a focus on soil fertility in agriculture and adopting opportune practices in animal production, this to find a better balance between production and emissions to the environment. Animal production is to be based on optimized animal rations, reducing the waste, balancing the use of high inputs and recycling the by-products from primary production and from processing.

For closing the cycle, a local level action is essential in terms of greenhouse gas emissions like methane. ICAR members have an important role in such a transformation, through innovation and development, by following appropriate guidelines, through exchange about the local experiences and by sharing adopted solutions.

Sustainable agriculture can help achieve several sustainable development goals, such as reducing hunger, improve peoples' diets and living conditions and raise incomes.

These themes were addressed during the ICAR Annual Conference 2021. Specific sessions have been dedicated to "Supporting circular economy and how it affects the breeding goals", to the "Creation of additional value from milk analysis", to the "Data analysis and how the new analyses techniques bring better farm results".

With more than more than 500 remote participants, the ICAR Annual Conference was very meaningful in creating awareness of the role ICAR has to play in creating more sustainable food systems and in safeguarding food security for current and future generations.

ICAR Secretariat

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Development of a tail scoring as health indicator for dairy cows

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Abstract

Research investigating necrotic tail tips in dairy cows resulting in necrotic tissues is scarce. However, there is evidence that in dairy cattle tail tip necroses exist with high prevalence. In piglets, the latest research described tail and ear necroses not because of tail and ear biting only, but because of swine inflammation and necrosis syndrome (SINS). Besides tails and ears, SINS includes inflammation of claws, heels, and teats. In cattle, tail tip necroses are described mainly in fattening bulls. As known so far, these findings are often discussed related to slatted flooring,intensive housing systems and management strategies. However, an association with sub-acute rumen acidosis and laminitis is described.

In order to investigate what kind of and how often tail tip alterations appear in dairy cattle, data of 87 German Holstein dairy cows were collected over a period of 12 months. All cows were evaluated for tail tip alterations, body condition score (BCS), and locomotion score (LMS) every two weeks. In addition, milk yield data resulting from performance testing were included. Thermographic images of the tails were taken once. Firstly, all kind of tail tip alterations were described and collected. After 6 months, we categorized the observed alterations and developed a tail scoring system. The scoring for each specified trait (tail tip, tail ring) ranged from 0 to 4.

The overall prevalence for tail alterations was 94%. Especially tail tip alterations had a constantly high prevalence of 56%. Cows affected by an increased average tail tip score showed higher locomotion scores compared to others ($P = 0.02$). The prevalence of ring-like tail alterations increased from first to second lactation cows from 9 to 46%. Regarding the BCS, lighter cows showed higher scores due to ring-like alterations than heavier cows ($P = 0.054$).The most often occurring anomalies of the tail were sports or scurf (21.6%), followed by verruca-like mass (10.2%), swelling (8.4%), and thinning (4.3%).

The results and especially the scoring system can serve as a template for further studies considering larger samples sizes, to investigate prevalence for tail necroses and other tail anomalies in different herds and management systems. It was hypothesized, that an inflammatory condition in dairy cows showing up in altered/necrotic tail tips or rings exists, which is in relationship with claw disorders indicated by lameness. If so, the tail score of a cow could be used as health indicator to evaluate the health status in dairy production systems.

Keywords: Tail tip necrosis, tail ring constriction, tail tip ring, tail anomalies, dairy cattle production diseases.

Introduction

Tail alterations are described mainly in feedlot cattle and fattening bulls (Drolia *et al.*, 1991; Schrader *et al.*, 2001; Heers *et al.*, 2017). The prevalence of tail tip alterations ranges from 2.5% (Hoedemaker, 2014) up to more than 80% (Kordowitzki, 2015). Findings were often related to slatted flooring, (sub) acute rumen acidosis and laminitis (Kordowitzki, 2015).

In other species, tail tip necroses and tail ring constrictions are already known. They are related to heat stress in buffalo (Barakat *et al.*, 1960) or to chronic inflammation in pigs caused by the Swine Inflammation and Necrosis Syndrome (SINS) (Reiner *et al.*, 2019).

Knowledge regarding tail tip alterations in dairy cows is scarce. *In-vivo* investigations often suffer from small sample sizes (Ural *et al.*, 2007). Scorings to investigate the severeness of tail alterations were developed on cows' carcasses (Freitag *et al.*, 2017; Heers *et al.*, 2017), for feedlot cattle (Drolia *et al.*, 1991) as well as for fattening bulls (Kordowitzki, 2015).

However, there is evidence, that dairy cows dealing with negative energy balance, (sub) acute rumen acidosis and lameness (Cook *et al.*, 2004) because of multifactorial risk factors during lactation also show high prevalence of tail tip alterations. Therefore, our aims were to

1. Identify which kind of tail alterations occur in dairy cattle,
2. Calculate the prevalence of all kind of tail alterations,
3. Develop a tail scoring system, that can be used *in-vivo*,
4. Figure out which traits are in association with the severeness of tail alterations in dairy cattle.

Material and methods

In total, 87 German Holstein cows during their first to seventh lactation were included into the study. They were housed under field conditions in a loose housing system on a German dairy farm. The average milk yield was 10,149 kg containing 4.10% fat and 3.55% protein.

The region of the tail tassel was shaved and investigated every two weeks. Tail alterations were recorded over a period of 12 months from December 2019 to November 2020. First of all, tail alterations were described and collected. After 6 months, we categorized the observed alterations and developed a tail scoring system. The scoring for each specified trait ranged from

- 0 (physiological),
- 1 (hairloss),
- 2 (scab/constriction),
- 3 (bloody lesions/constriction),
- 4 (necrotic tissue, part loss).

It was applied on the tail tip including ring-like alterations. Additionally, the body condition score (BCS) ranging from 1 to 5 (Edmonson *et al.*, 1989) and locomotion score (LMS) ranging from 1 to 5 (Sprecher *et al.*, 1997) were recorded at the same time. Milk yield recording data of the same period were added; thermal images of the tail tips (FLIR® T1030) and urine density data were taken once.

Data analyses were performed using R (R Core Team, 2020). Prevalence for all six groups of tail alterations were calculated by dividing the number of affected cows by the total number of cows under investigation and given in percent. Means between two groups were compared using a Wilcoxon test.

Results

All different kind of tail alterations were categorized using the following descriptions: tail tip alteration, ring-like alteration, swelling, thinning, scurf, and verruca-like mass. Prevalence for all tail alterations during this study was 94%; only five cows were unaffected. The prevalence for all groups of tail alterations were: tail tip alteration 56%,ring-like alteration 38%,scurf 21.6%, followed by verruca-like mass 10.2%, swelling 8.4%, and thinning 4.3%.

The group of tail tip alterations had a high prevalence during all lactations (Figure 1). Ring-like alterations were on a low level during the first lactation (9%) and increased to the second (46%) and ≥third lactation (52%). On average, verruca-like mass showed low prevalence, but increased from first (4%) to ≥third lactation (14%). Scurf showed a decrease from the first lactation (27%) to ≥third lactation (19%).

The tail scoring system ranging from 0 to 4 was used to describe the severeness of tail tip alterations and ring-like alterations. The scoring for tail tip alterations increased by higher LMS (*P* = 0.015, Figure 2). Cows with a LMS of 1 (normal walk) had a mean tail tip score of 0.9, which was suggestively different to cows with LMS of 2 (mildly lame) with a mean tail tip score of 1.2 (*P* = 0.092) and significantly different to cows with a LMS of 3 (moderately lame) and a mean tail tip score of 1.5 (*P* = 0.012). LMS 4 (lame, n = 8) and 5 (severely lame, n = 2) were not significantly different.

Cows affected by ring-like alterations were grouped into lighter and heavier cows with an average BCS of ≤ 3 and ≥ 3 during the whole lactation period, respectively. The lighter cows showed suggestively (*P* = 0.054) higher ring-like alterations (tail ring score mean = 1.9) compared to heavier cows (mean = 1.1).

Discussion

The prevalence for all kind of tail alterations in dairy cows was high. Especially tail tip alterations were found showing a higher prevalence (56%) compared to other studies (2.5% to 37%; Hoedemaker, 2014; Freitag *et al*., 2017; Heers *et al*., 2017). This could be due to the use of a different scoring system and to the preparation of shaved tail

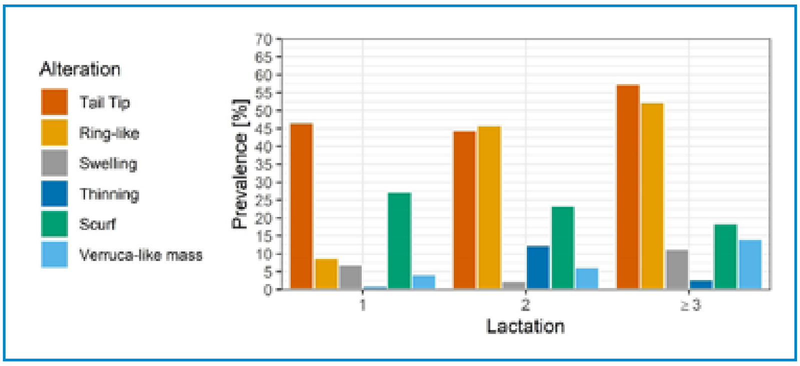

Figure 1. Prevalence grouped as tail tip alteration, ring-like alteration, swelling, thinning, scurf, and verruca-like mass per lactation (1st, 2nd, ≥3rd).

tassels during our study, which allows a better investigation of the tail tip compared to unshaved tail tassels.

Tail tip alterations were evaluated using the new scoring system ranging from 0 to 4. The locomotion score was collected using the scoring of Sprecher *et al.*, 1997. Data showed that cows affected from mildly lameness (LMS 2, mean = 1.2, $P = 0.031$) and moderate lameness (LMS 3; mean 1.5; P=0.004) were also affected from higher tail scores. Lameness is caused by painful conditions of the limb or claw, which can also result from systemic disorders, e.g. laminitis, which is amongst others caused by sub-acute rumen acidosis. According to literature, sub-acute rumen acidosis influences both, laminitis and tail tip alterations (Cook *et al.*, 2004; Kordowitzki, 2015). Especially high yielding dairy cows are at risk of (sub-) acute rumen acidosis because of high concentrate amounts. This could eventually explain the high prevalence of tail tip alterations during this study (øherd milk yield >10,000 kg), however the feed ration was not evaluated here.

Interestingly, ring-like alterations were on a lower level during the first lactation (9%) and increased to the second (46%) and ≥third lactation (52%). As mentioned before, the average BCS tended to be related to the severeness of ring-like tail alterations. This effect could eventually be explained by the metabolic stress of cows, especially caused by high milk yield contemporaneous to low feed intake during the early lactation period (Römer, 2011).

Conversely, neither LMS showed an effect on ring-like tail alterations nor BCS did on tail tip alterations. This could be due to the fact, that these alterations have to be considered independently from each other, or that the sample size was too low to observe significant relations. Data from milk performance testing (milk yield, fat and

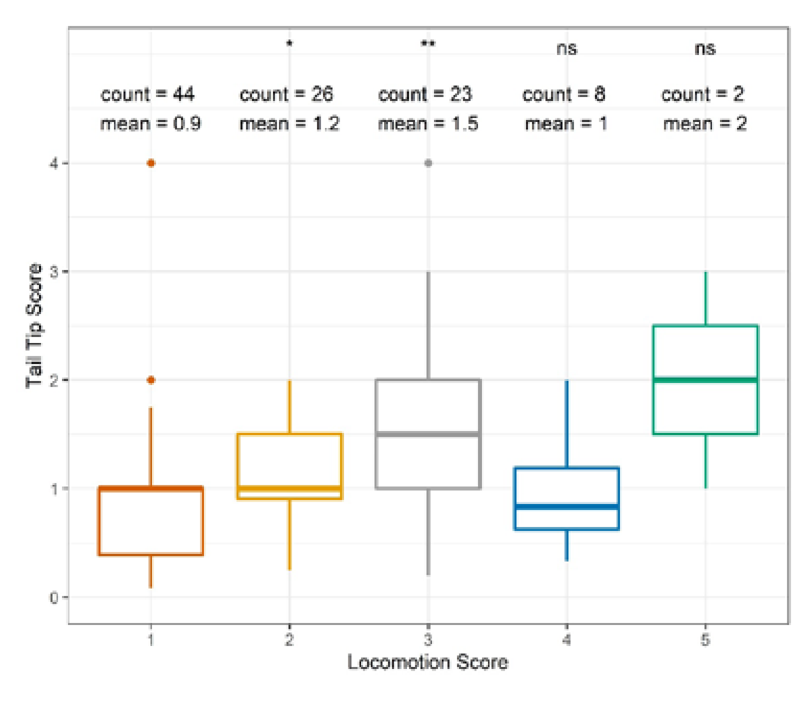

*Figure 2. Cows affected by tail tip alterations measured by the Tail Tip Score (ranging 0-4) showed higher Locomotion Scores (ranging 1-5). Number of data (count) and mean tail tip score are given above box plots besides level of significance (*P <0.05; **P<0.01, ns = not significant) from Wilcoxon test comparing each mean to the mean of LMS 1.*

protein content and ratio, somatic cell count, days in milk) also did not show an effect on our dataset.

Conclusion

There is evidence that high yielding dairy cows, amongst other performance- and feed-related diseases, show health disorders accompanied by tail tip alterations. Since 2019 (Reiner *et al.*, 2019), in pigs SINS is reported to influence tails, ears, teats, and claws, appearing as chronic inflammation and necrotic tissue. Finally, we hypothesized that there might be also health disorders and inflammatory conditions in dairy cows resulting in tail alterations, which could be part of a Bovine Inflammation and Necrosis Syndrome (BINS).Further studies are required to evaluate if the tail scoring presented here could potentially be used as an early-warning system to measure the severity of tail alterations in dairy cows.

List of references

Barakat, M. Z., Wahby, A. M., and Abdalla, A. (1960). Necrosis of the Tail in the Buffalo - A Deficiency Disease? Br. Vet. J. 116, 151–155. doi:10.1016/s0007-1935 (17) 44306-5.

Cook, N.B., Nordlund, K.V, and Oetzel, G.R. (2004). Environmental Influences on Claw Horn Lesions Associated with Laminitis and Subacute Ruminal Acidosis in Dairy Cows. J. Dairy Sci. 87, E36–E46. doi:10.3168/jds.S0022-0302 (04) 70059-4.

Drolia, H., Luescher, U.A., Meek, A.H., and Wilcock, B.P. (1991). Tail tip necrosis in Ontario beef feedlot cattle. Can. Vet. J. 32, 23–29.

Edmonson, A., Lean, I., Weaver, L., Farver, T., and Webster, G. (1989). A Body Condition Scoring Chart for Holstein Dairy Cows. J Dairy Sci 72, 68–78.

Freitag, M., Heers, P., and Beune, H. (2017). Schädigt falsche Fütterung die Schwanzspitzen? top Agrar 9, 26–28.

Heers, P., Beune, H., and Freitag, M. (2017). Schwanzspitzennekrosen - Weil sich die Mastbullen bei enger Haltung auf den Schwanz treten? Nutztierpraxis Aktuell, 84–88.

Hoedemaker, M. (2014). Beudeutung von Clostridium botulinum bei chronischem Krankheitsgeschehen. Hannover.

Kordowitzki, P. (2015). Untersuchungen zum Auftreten der Schwanzspitzennekrose bei Mastbullen.

R Core Team (2020). R: A language and environment for statistical computing. Available at: *https://www.r-project.org*/.

Reiner, G., Lechner, M., Eisenack, A., Kallenbach, K., Rau, K., Müller, S., et al. (2019). Prevalence of an inflammation and necrosis syndrome in suckling piglets. Animal 13, 2007–2017. doi:10.1017/S1751731118003403.

Römer, A. (2011). Untersuchungen zur Nutzungsdauer bei Deutschen Holstein Kühen. Zuchtungskunde 83, 8–20. Available at: *https://www.zuechtungskunde.de/Untersuchungen-zur-Nutzungsdauer-bei-Deutschen-Holstein-Kuehen,QUlEPTE5NjkwMTImTUlEPTY5MTQy.html*.

Schrader, L., Roth, H.R., Winterling, C., Brodmann, N., Langhans, W., Geyer, B., et al. (2001). The occurrence of tail tip alterations in fattening bulls kept under different husbandry conditions. Anim. Welf. 10, 119–130. doi:10.5167/uzh-113733.

Sprecher, D.J., Hostetler, D.E., and Kaneene, J.B. (1997). A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 47, 1179–1187. doi:10.1016/S0093-691X (97) 00098-8.

Ural, K., Alýç, D., Karakurum, M., Aktaþ, M., Haydardedeoðlu, A., and Cýngý, C. (2007). Tail-Tip Necrosis in Beef and Dairy Cattle: A Report of Seven Cases in Ankara. 13, 203–207.

Development and implementation of an on-farm self-monitoring tool to promote welfare in dairy cows in northern Germany

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Abstract

Following the German Animal Welfare Act from 2014, livestock keepers/stock farmers in Germany are obligated to gather und evaluate data regularly in order to monitor their animals' welfare. Since the legal basis doesn't present detailed implementations regarding the extent and method of monitoring, farmers are left alone finding a way to meet legal requirements. To challenge this problem the Operational Group "Tierwohl-Check" was initiated within the European Innovation Partnership (EIP-AGRI) to develop a management tool assisting dairy farmers to meet legal requirements and support their general herd management at the same time. A distinctive feature of EIP projects is their practical orientation. In order to create practical solutions, farmers are directly involved in the work process of the Operational Group supporting bottom-up interactive innovation. The principal objective is to assist farmers with an easy-to-use application which provides reliable indicators, reveals weak points and therefore supports herd management. At the same time the evaluation can be used to meet the legal requirements to document animal welfare. Accompanying the development process, the project's aim is to develop and implement an e-learning program for farmers and to assist multipliers such as veterinarians, consultants and others by providing training material as a train-the-trainer approach. This "Tierwohl-Check" indicator set for on-farm self-monitoring of animal welfare in dairy cattle will be presented as well as an assessment framework regarding the welfare outcomes (target and threshold values, benchmarking).

Keywords: Monitoring system, key indicators, animal welfare, animal health, self-assessment, web application, dairy sector.

Introduction

A main idea of Tierwohl-Check was to avoid re-discussing the suitability of well-known indicators, since this fundamental work has been carried out in two fundamental projects throughout Germany. While various initiatives have developed a variety of reliable indicators to effectively monitor the welfare of dairy cows, "Tierwohl-Check" directly benefited from the outcomes of the projects "Q Check" (German Association for Performance and Quality Testing (DLQ), https://infothek.q-check.org/) and "EiKoTiGer"

(Association for Technology and Structures in Agriculture (KTBL), https://www.ktbl.de/themen/tierschutzindikatoren-milchrinder). Dairy farmers in Germany can already rely on an existing strong infrastructure, which provides four existing systems of data recording. Q Check following an interdisciplinary approach, has examined potential indicators from existing data and produced a set of 14 indicators mainly relating to the health of dairy cows. This set of indicators was fully implemented into the Tierwohl-Check tool and is presented in table 1.

At the same time, a self-assessment tool should provide a good evaluation on farm using indicators scoring the animals' condition. EiKoTiGer has focused on the collection of data on farm, evaluating indicators for milking cows, calves and beef cattle. For Tierwohl-Check the animal-based indicators for body condition, cleanliness, integument alterations, tail injuries, claw condition and lameness, of dairy cows as well as adequate use of lying places, rising behaviour and water availability were selected (Brinkmann et al. 2020). For herds with horned cattle, horn injuries were added to the set of indicators (Johns et al. 2020). The combination of the two sets of indicators ensures a maximum usage of the existing data from milk recording and cattle database and enables the user to describe the animal's welfare state by generating on-farm data.

Table 1. Analyse of the existing data for the control year 2019of 2,585 dairy farms in Schleswig-Holstein, Germany (LKV SH 2020) giving the mean values of all farms (average) as well as the top +25% / +10% of farms and the low-ranking -25%/-10% of farms. Target and alarm values used in Tierwohl-Check are given in the right columns.

Indicators	-10 %	-25%	Average	+25%	+10%	Target	Alarm
Culling rate [%]	21.9	27.0	32.8	39.4	46.4	< 25	> 40
Culling rate under 100 days p.p. [%]	3.6	5.6	8.6	12.2	16.4	< 5	> 10
Productive life span (in months)	46.7	40.8	35.4	30.7	26.5	> 48	< 30
Mortality cows [%]	1.1	2.4	4.0	6.2	8.8	< 2	> 5
Cows with SCC<= 100 [%]	73.5	67.9	60.6	52.7	44.0	> 75	< 50
Cows with ZZ > 400 [%]	5.8	7.8	10.7	14.2	18.6	< 5	> 15
Healing rate during dry off [%]	72.7	64.5	55.1	44.4	33.8	> 75	< 50
Infection during lactation[%]	9.4	14.6	21.6	30.5	40.0	< 15	> 30
First lactation mastitis[%]	16.2	22.2	30.0	38.5	47.6	< 15	> 30
Chronical infection [%]	0.0	0.0	0.8	1.9	3.2	< 1	> 5
Milk fat-protein ratio ≥ 1.5(100 days p.p.) [%]	4.3	6.1	9.1	13.1	18.2	< 10	> 15
Milk fat-protein ratio < 1.0 (100 days p.p.) [%]	1.5	2.9	5.3	8.9	13.4	< 5	> 15
Dead birth rate cows [%]	0.0	1.8	4.2	6.9	9.6	< 5	> 10
Dead birth rate heifers [%]	0.0	2.0	6.1	10.7	16.2	< 5	> 10
Mortality rate calves 0-3 weeks[%]	0.0	0.0	2.1	4.4	7.6	< 3	> 5
Mortality rate 3-12 weeks[%]	0.0	0.0	2.1	4.3	7.4	< 2	> 5
Mortality rate young stock 3 - 6 months [%]	0.0	0,0	0.0	2.3	4.7	< 1	> 5

The set of indicators is completed by a short overview of the cows' husbandry, making it easier for consultants or veterinarians to support farmers with the results of their self-assessments. The set of indicators allows a precise documentation of the animal welfare situation as well as conclusions on the animals' health, husbandry and management.

The integration of practical farmers throughout the project work was a key factor of finding successful solutions. For getting these indicators into practical use for the farmers, it was the aim to provide a tool to easily analyse the animal welfare without too much effort. At the same time the farmers should gather valuable information to optimize their individual herd's management..

Material and methods

Together with the group's farmers, the application setup was carefully optimized during several practical cow-side test sessions, ensuring high usability and supporting user's focus on the important points. Thus, it was possible to take into account the point of view on animal welfare of practical dairy farmers, research institutes and consulting organisations.

By access to LKV's cattle register, the application knows all animals in the herd according to the last milk recording and offers the scoring of indicators by using representative pictures for each indicator. The number of cows recommended to be scored is automatically calculated according to the herd size.

When optimising the order of indicators presented in the app, the testing was expanded onto more dairy farms in Schleswig-Holstein. Therefore, a group of 20 farmers trained to correctly score the animal-based indicators and get used to the app interface. The training was carried out in in one-day training events in the stable and due to an online training. All participants carried out a full data collection afterwards with their own herd, using the recommended sample size. The herd size of the farms involved varied from 60 to over 300 cows and included farms with all types of milking systems, with sample sizes from 35 to a maximum of 80 cows to be scored. Feedback of the farmers involved was collected after the data collection by a short survey.

In addition to the testing carried out by farmers, further data was collect on 13 farms by a project member to identify all remaining technical issues. After a short introduction to the herd, the data collection was all carried out by the project staff and the results were discussed with the farmer directly after the evaluation.

For each indicator a target and an alarm value was defined in the previous projects. The indicators from Q Check were checked against the existing data from the Landeskontrollverband Schleswig-Holstein e.V. (LKV SH) for the control year 2019. The results are presented in table 1(LKV SH 2020).

To meet legal requirements, Tierwohl-Check provides documentation and evaluation of the collected data. The app offers a report to easily analyse the animal welfare situation of the herd and detects weak points. Results are presented and stored directly in the app and in addition can be printed or send out as a pdf file. In addition, a benchmark will be implemented in the future. When the users notice weak points in their report, the results can be used to share information with their consultants or veterinarians to focus on problems specific for their herd or to keep track of developments over the time.

To help farmers getting used to animal based indicators and offer a training opportunity before scoring cows in the stable, an e-learning tool will provide further information and assistance. Also broader live trainings and seminars shall be enabled during the project and beyond.

Results and discussion

The chosen indicators and usability of the app were well accepted by the farmers involved. All participants were asked to rate the usability of the app, the amount of time used for the data collection, the readability and the quality of the pictures presented, technical issues and the relevance of the chosen indicators. While the content-related points and the overall usability gained positive feedback, remaining technical problems were addressed more distinct and could be optimised in the following time. The succeeding data collection by the project member to detect the described technical problems was a valuable approach and sensibly complemented the tests by the farmers.

When analysing the status-quo data of the LKV SH the given target and alarm values showed a very good suitability. The data of 2,585 dairy herds were analysed for the period of one year, representing 85.8 % of the dairy cows in Schleswig-Holstein.

Table 1 shows the average value reached by the herds, as well as the better 25/10 % and the less successful herds 25/ 10 %. The outcome was then compared to target and alarm values given by Q Check. For all indicators, except the first lactation mastitis, the average of the herds was able to achieve values between the target and alarm value or even better. For the first lactation mastitis, only the better 25% of the herds were better than the alarm value, but didn't achieve the target value.

Comparing the data base for the indicators of existing data and the results of the data collection on farm, the existing data was more representative for dairy farms in northern Germany. For the selection of farmers for the data collection on farm, the requirements were rather to involve motivated participants to gain a high-quality feedback of the app performance, than to gather representative amount of data for the evaluation of animal welfare in Schleswig-Holstein.

Conclusion

For a successful implementation into practical farming, self-assessments may not only be carried out to follow legal requirements, but have to create a real added value for the farmers and their livestock. The attempt to offer a digital tool with reliable recommendations on how self-assessments can be carried out, found broad acceptance by the farmers involved.

A high acceptance of the app in future mainly depends on good usability and the realized benefits for animal health and welfare following from reliable data analysis and consequent management measures.

Therefore the process of improving the app performance requires further consistent efforts.The execution of regular self-assessments remains to the farmers themselves. For making thebest possible use of the documentation, evaluation and benchmark given out, consultants and veterinarians ought to be involved to implement effective measures.

In the end, the implementation of a self-assessment tool based on scientifically well-developed indicators and consequently designed in cooperation with farmers is the opportunity to be one step ahead of further legal regulation and tosustainably improve the animal welfare situation of dairy cows.

List of references

Brinkmann, J., Cimer, K., March, S., Ivemeyer, S., Pelzer, A., Schultheiß, U., Zapf, R. and C. Winckler. 2020; Tierschutzindikatoren: Leitfaden für die Praxis – Rind. Vorschläge für die Produktionsrichtungen Milchkuh, Aufzuchtkalb, Mastrind. ISBN 978-3-945088-75-3

Deutscher Verband für Leistungs- und Qualitätsprüfungen e.V. 2020; DLQ-Richtlinie 2.0, Definitionen und Berechnungenvon Indikatoren und zugehörigen Vergleichswerten zur Durchführung der betrieblichen Eigenkontrolle und des NationalenTierwohlmonitorings. *https://q-check.org/wp-content/uploads/2020/05/DLQ-Richtlinie2.0.pd*f, Zugriff am 22.04.2021

Johns, J., Mück, U., Sixt, D., Kremer, H.J., Poddey, E. and U. Knierim. 2019; Werkzeugkasten für die Haltunghorntragender Milchküheim Laufstall. www.uni-kassel.de/go/werkzeugkasten, Zugriff am 22.04.2021

LandeskontrollverbandSchleswig-Holstein e.V. 2020; Jahresbericht 2019. *https://www.lkv-sh.de/downloads*, Zugriff am 22.04.2021

The intersection of on-farm animal welfare evaluation and technology integration as the future of animal-based indicators for animal welfare measurement: an example from the United States

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Abstract

More than a decade ago, the U.S. dairy industry recognized that meeting the nutritional needs of a growing and diversified population requires a sustainable food system with transparent production practices and proof points. To address these needs, the U.S. dairy industry formed the National Dairy Famers Assuring Responsible Management (FARM) Program. This program commits U.S. dairy farmers to high-quality, on-farm management practices in animal care, antibiotic stewardship, biosecurity, environmental stewardship and workforce development. FARM's transparency provides consumers with confidence that their dairy products are produced in keeping with the highest level of science-based metrics and best practices. The FARM Animal Care program is comprised of three components: best management practice manuals, second-party farm evaluations and third-party verification. A key component to evaluating animal care through the second-party farm evaluations includes scoring animal-based indicators for animal welfare. Those indicators scored include: body condition, hygiene, hock and knee lesions, locomotion and broken tails. With the advancement of precision technology available to U.S. dairy herds, FARM is working to overlay the data these technologies provide with the animal-based indicator scoring systems. In turn, program implementation burden on dairy farmers, cooperatives and processors will be eased while providing more objective, animal-based data to support demonstrating U.S. dairy's commitment to animal welfare.

Keywords: Social responsibility, animal welfare, animal care, precision technology.

Introduction

In 2009, the National Milk Producers Federation with support from the U.S. levy program Dairy Management Inc.developed the National Dairy Farmers Assuring Responsible Management (FARM) Program[1]. FARM is open to all U.S. dairy farmers, cooperatives and processors. The Innovation Center for U.S. Dairy coordinated alignment behind the FARM Program as the industry-wide social responsibility program for on-farm production. The goal is to assure dairy consumers and customers that dairy farmers care for the animals, workforce and land in a humane and ethical manner.

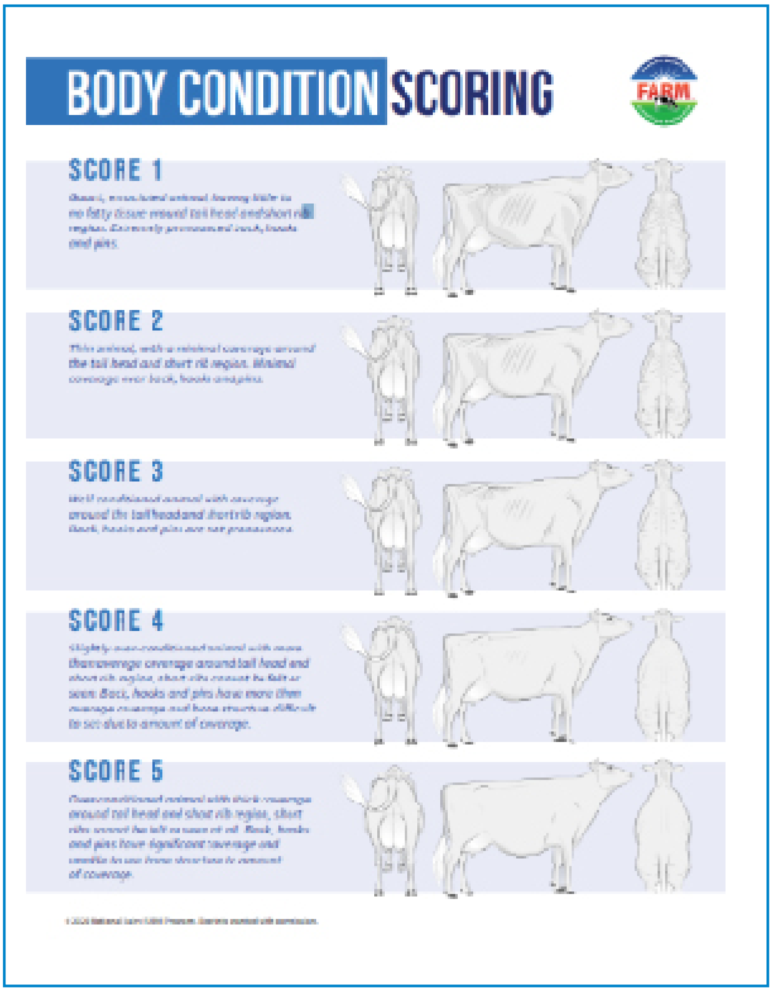
Through FARM Animal Care, the U.S. dairy industry has embraced on-farm evaluations to assess animal welfare through various science-driven standards and best practices, instilling a commitment tocontinuous improvement. It also identifies areas of risk and liability to the industry in terms of consumer perception.

Material and methods

As the world’s first Animal Care program to meet the International Organization for Standardization Technical Specification requirements for the World Organization for Animal Health dairy cattle welfare standards, FARM Animal Care provides assurances throughout the supply chain regarding on-farm animal welfare through three primary components: best management practice manuals, second-party farm evaluations conducted by trained and certified evaluators and independent third-party verification to demonstrate the program’s integrity.

The best practices and standards that make up the FARM Program are required to have a scientific and technical basis. These standards are reviewed every three years to ensure that the program is keeping up with the latest science-based recommendations. Best management practice manuals, including the Animal Care Reference Manual[2] and the Milk and Dairy Beef Drug Residue Manual[3], provide a comprehensive set of expectations and are educational tools and resources for farms as they develop on-farm best management practices.

The second-party farm evaluation follows a standardized protocol and evaluation rubric based upon current FARM Program standards and best practices. Trained and certified evaluators conduct triennial on-farm evaluations include interviews with farm owners and employees, review of content and implementation of written protocols, and evaluation of animal-based indicators for animal welfare.



BODY CONDITION SCORING

SCORE 1

SCORE 2

SCORE 3

SCORE 4

SCORE 5

Figure 1. Body Condition Scoring Guide.

The animal-based indicators for animal welfare, each having a unique scoring system with industry benchmark, are conducted through observation of individual animals. These include:

- Body Condition Score – an indicator for nutritionally adequacy.
- Hygiene Score – an indicator for sanitation and cleanliness.
- Locomotion Score – an indicator for hoof and leg health.
- Hock and Knee Lesion Score – an indicator for resting area conditions.
- Broken Tail – an indicator for stockmanship.

In order to ensure consistency in the evaluation of these animal-based indicators, a number of evaluator scoring guides have been developed (Image 1 and 2) that serve as a reference during second-party evaluation animal-based observation.

Newer on-farm technology data streams, ranging from in-line milk quality readers to animal activity monitoring, will be incorporated into FARM Animal Care as an overlay for key animal welfare indicators augmenting existing on-farm evaluations.

The third component of FARM Animal Care is a third-party verification process. This process helps to demonstrate program integrity by objectively evaluating the consistency and accuracy of farm evaluators and the program itself. This process, too, would be greatly enhanced by the integration of on-farm technology data streams.

Results

Robust adoption has allowed the U.S. dairy industry a unified approach to animal welfare. In its fourth iteration, FARM Animal Care participation includes 99% of U.S. milk production from dairy farmers in 49 states – 31,000+ dairy farm participants from 130+ dairy cooperatives and processors.

In Version 4.0 of FARM Animal Care, benchmarks for animal-based observations have been set based upon previous program version data collection and scientific literature review. The benchmarks[4] are outlined in Image 3 and designates which age classes these respective measures are evaluated. Some age classes have been found to be at lesser risk for locomotion, hock and knee lesions and broken tail incidence and therefore, are not evaluated. Animal-based observations scores and benchmark achievement is determined within each respective age classes i.e. 90% benchmark for hygiene is a goal for lactating cows, pre-weaned calves, post-weaned heifers and dry cows respectively instead of 90% cumulatively in all age classes.

In Version 3.0 of the program, FARM data demonstrated the animal observation benchmark standards were met on the majority of all herds evaluated. On 33,000 facilities evaluated throughout the entire program cycle (2017-2019) are listed in table 1.

Version 4.0 of FARM Animal Care, that has been in place since January 1, 2020, demonstrates similar trends in the majority of facilities evaluated meeting animal observation benchmarks in all age classes for specific animal-based measures. On 3,100 facilities evaluated to date, the results are reported in table 2.

Recognizing the risk of subjectivity that exists when individual evaluators are assessing these animal-based measures, the integration of precision on-farm technology data streams for animal-based measures, will enhance the objectivity of these data points. This will further support the overall consistency in the assessment of animal welfare providing stronger, unified assurances for the supply chain.

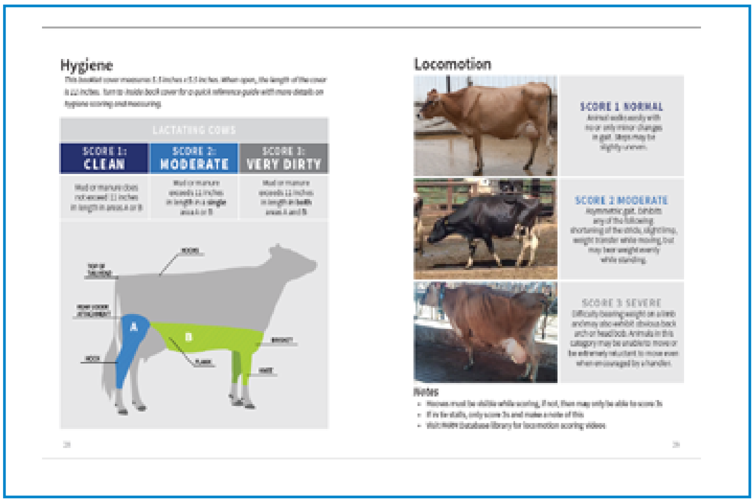
Table 1. Version 3.0 Animal-Based Observation Standard Compliance at time of a facility evaluation.

Benchmark	Standard Met	Standard Not Met
Hygiene: ≥ 90% all animals score 2 or less	69.3%	30.7%
Locomotion: ≥ 95% lactating and dry cows score 2 or less	98.0%	2.0%
Body condition: ≥ 99% all animals score 2 or higher	98.3%	1.7%
Hock and Knee Lesion: ≥ 95% lactating and dry cows score 2 or less	95.8%	4.2%

Table 2. Version 4.0 Animal-Based Observation Standard Compliance at time of a facility evaluation.

	Hygiene: ≥ 90% score 2 or less		
	Standard Met	**Standard Not Met**	**N/A**
Pre-weaned calves	74.1%	3.4%	22.4%
Post-weaned heifers	59.7%	24.8%	15.5%
Pre-fresh heifers/dry cows	79.0%	10.0%	11.0%
Lactating Cows	89.7%	9.6%	0.7%
	Body Condition: ≥ 99% score 2 or higher		
	Standard Met	**Standard Not Met**	**N/A**
Pre-weaned calves	77.0%	0.5%	22.5%
Post-weaned heifers	83.6%	0.9%	15.5%
Lactating cows	97.7%	1.5%	0.8%
	Locomotion: ≥ 95% score 2 or less		
	Standard Met	**Standard Not Met**	**N/A**
Lactating cows	96.9%	1.9%	1.2%
	Hock Lesion: ≥ 95% score 2 or less		
	Standard Met	Standard Not Met	N/A
Lactating cows	94.5%	4.7%	0.8%
	Knee Lesion: ≥ 95% score 2 or less		
	Standard Met	**Standard Not Met**	**N/A**
Lactating cows	98.5%	0.6%	0.9%
	Broken Tails: ≥ 95% do not have broken tails		
	Standard Met	**Standard Not Met**	**N/A**
Lactating Cows	80.0%	17.8%	2.2%

*N/A indicates age class is housed off-site or all animals in age class were unable to be scored



Hygiene

LACTATING COWS

SCORE 1: CLEAN

SCORE 2: MODERATE

SCORE 3: VERY DIRTY

Locomotion

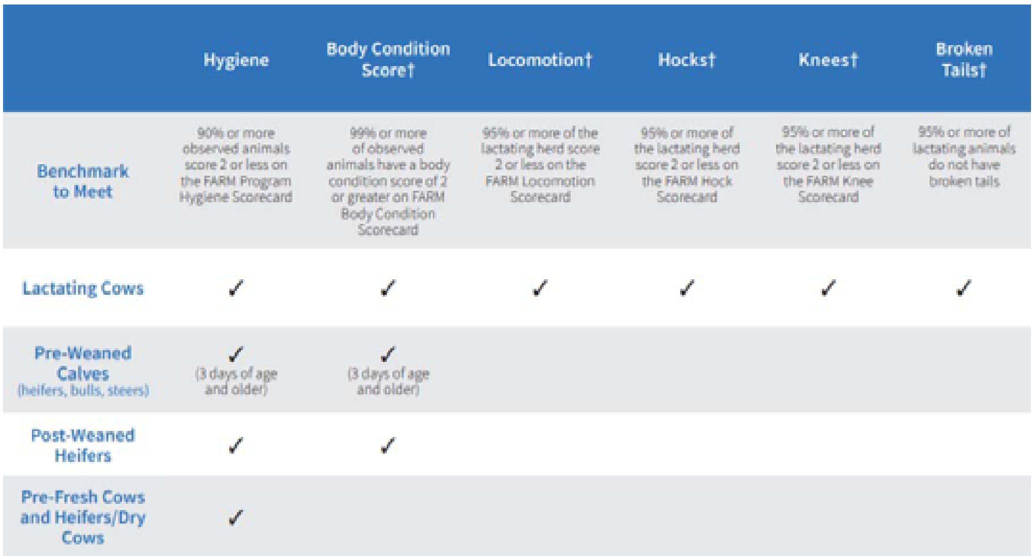
SCORE 1 NORMAL

SCORE 2 MODERATE

SCORE 3 SEVERE

Notes

Figure 2. Evaluator Pocket Guide.



	Hygiene	Body Condition Score†	Locomotion†	Hocks†	Knees†	Broken Tails†
Benchmark to Meet	90% or more observed animals score 2 or less on the FARM Program Hygiene Scorecard	99% or more of observed animals have a body condition score of 2 or greater on FARM Body Condition Scorecard	95% or more of the lactating herd score 2 or less on the FARM Locomotion Scorecard	95% or more of the lactating herd score 2 or less on the FARM Hock Scorecard	95% or more of the lactating herd score 2 or less on the FARM Knee Scorecard	95% or more of lactating animals do not have broken tails
Lactating Cows	✓	✓	✓	✓	✓	✓
Pre-Weaned Calves (heifers, bulls, steers)	✓ (3 days of age and older)	✓ (3 days of age and older)				
Post-Weaned Heifers	✓	✓				
Pre-Fresh Cows and Heifers/Dry Cows	✓					

Figure 3. FARM Outcomes-Based Animal Based Observations with benchmarks for each animal age class.

Conclusion

Animal care is a constant commitment for dairy farm owners, managers and their employees. A science-based, industry-wide social responsibility effort such as the U.S. National Dairy FARM Program creates a framework for on-farm animal care and fosters a culture of continuous improvement. The Program's adoption of precision technology data streams will further ease the implementation burden on dairy farmers and cooperatives and processors while providing more objective, animal-based data to support demonstrating U.S. dairy's commitment to animal welfare.

List of references

National Dairy FARM Program; Available from: *https://nationaldairyfarm.com/*

Animal Care Reference Manual 4; Available from: *https://nationaldairyfarm.com/farm-animal-care-version-4-0/*

Milk and Dairy Beef Drug Residue Prevention Reference Manual 2020; Available from: https://nationaldairyfarm.com/wp-content/uploads/2018/10/DRM2020-Web.pdf

FARM Animal Care Evaluation Preparation Guide Version 4.0; Available from: *https://nationaldairyfarm.com/producer-resources/farm-animal-care-evaluation-prep/*

Towards secure digital farming: security model and risks associated to machine learning

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Abstract

In digital farming, machine learning is already widely used to optimize the production using sources such as genomics, health, welfare, production, and environmental data. However, this increasing use of machine learning has led to the emergence of multiple types of confidentiality and integrity breaches targeting both the models and the data they have been trained on. Our main objective in this paper is to discuss possible security issues that could arise in digital farming due to the use of machine learning techniques and the urgency to implement innovative countermeasures to prevent them. First, we propose a security model dedicated to the specific settings and threats of the digital farming context. In this model, we identify the resources at risk, define the different classes of actors, determine the risk vectors, and propose some realistic attack scenarios. Afterwards, we use this model to put in perspective the machine learning induced risks and show how they may adversely affect digital farming. The considered attacks encompass model theft, model inversion, membership inference, data poisoning and adversarial examples. For each of these threats, we also briefly revied possible mitigation means, such as differential privacy, prediction access control and robust statistics.

Keywords: Digital farming, cyber security, security model, machine learning.

Introduction

German Agricultural Society defines digital farming as the evolution of smart farming to better emphasize that nearly all aspects of farming now heavily rely on digital means (DLG Committee for Digitization, Work Management and Process Technology *et al*., 2018). Collecting data massively from a wide variety of sources has allowed to take smart farming to a new level, leveraging big data to further improve the power of the decision-making system. To do so, various kind of data are collected such as environmental, production, health, welfare, genomics, and management. Machine learning (ML) is the core concept behind decision-making system, in which a sample of data called training dataset is used to generate a predictive model. ML is widely used across many industries and as ML techniques become, cybersecurity threats emerge (Papernot *et al*., 2016) putting data confidentiality and production system integrity at risk.

Many actors have stated that cybersecurity is a concern in agriculture. For instance, a 2019 report from the U.S. Government Accountability Office (Dodaro, 2019), has indicated that improving cyber security should be one of the main priorities for actors in the agriculture sector. Geil *et al.* (2018) presented a survey in which they show that farmers are being directly affected at large scale. Window (2019) has conducted a study that presents major issues concerning data privacy, data ownership and level of attention given to cyber security in agriculture and all those points are requirements provided by the German Agricultural Society as well in their recent position paper.

A literature review allows us to conclude that working specifically on ML induced cybersecurity risks is a missing gap in the literature. Indeed, several reports focus on networking and Internet of Things (IoT) related risks (Gupta *et al.*, 2020; West, 2018) and several others on Big Data (Sykuta, 2016; Wolfert *et al.*, 2017). However, only a few works have been produced to study risks introduced by data analysis techniques in digital farming particularly in dairy industries. The U.S. Department of Homeland Security (Champion *et al.*, 2018) has also released a report in which they mention machine learning and at the regulatory level, farm data ownership is often present in the specialized literature (Sykuta, 2016; Window, 2019; DLG Committee for Digitization, Work Management and Process Technology *et al.*, 2018).

To study ML induced threats to digital farming, we first propose a security model adapted to this context of dairy farms, particularly in Canada, before studying the data life cycle and its interactions with the different resources and actors. Secondly, we propose an adversarial model to determine realistic threat vectors to ML systems in digital farming before proceeding with the investigation of the risks associated to ML, looking at five known vulnerabilities of ML systems and three possible practical mitigation strategies. Finally, we discuss another ML related security topics that should be investigated along ML induced threats.

Security model

Data chain: resources and actors

ML applications rely mainly on two assets: the training dataset and the learnt model. We will use the CIA framework to understand the impact of potential compromises on these resources. Confidentiality of the training dataset may be critical for privacy reasons, as for example valuable data such as genomics are used in digital farming, but also because it is part of the intelligence developed by ML application developers. Integrity of the training dataset is key to build reliable ML model and availability seems to only be a concern at the operational level. In addition, the confidentiality of the ML model is important in settings in which it is a monetizable resource such as ML as a Service (MLaaS), which is a form of pay-per-request service that could be compromised if the ML model was to be stolen. Furthermore, the ML model is a statistical representation of the training dataset as its confidentiality impact directly the confidentiality of the dataset. The integrity of the ML model is a concern for situations in which the ML predictions are used in a sensitive context such as farm management. Availability is a concern in time sensitive settings and for systems that cannot be substituted.

Wolfert *et al.* (2017) proposed a data chain in their framework for big data in smart farming. We adapted it to exclude network/infrastructure-based risk and focus on machine learning induced threats to assets described earlier. The principal node is data processing in which the ML model is developed. This node has two interfaces, the upstream data acquisition node in which the training dataset is being constituted and the downstream marketing node in which the end user is presented with a tool to query the model and obtain the associated predictions.

In Figure 1, we annotate the data chain to integrate actors found at each stage of the data lifecycle. The data provider is the main actor at the data acquisition stage, which is often the farmer but could also be laboratories in some cases (*e.g.* sample analysis on milk). We refer to the data collector to formalize the intermediary step consisting in

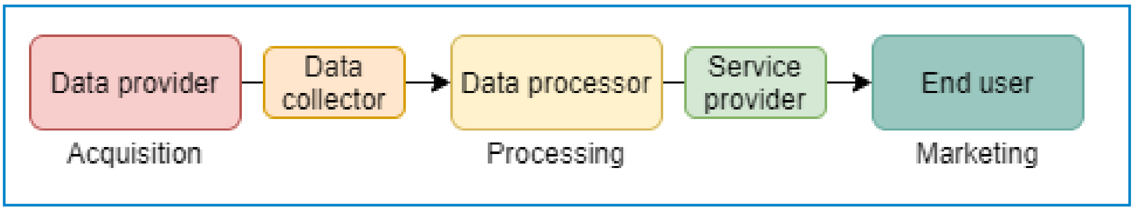

Figure 1. Actors involved in the data chain.

centralizing and storing the data. The data processor is the actor found at processing stage, who pre-process data to form the training dataset and train the model. Once the model is trained, the service provider refers to the actor in charge of wrapping the model in a product that can be distributed. Finally, the end user is the actor present at marketing stage that makes requests and applies predictions using the model through the marketed tool.

Adversarial model and threat vectors

We adopt the point of view of the data processor who forms the training dataset (preprocessing) and engineers the ML model (processing) because he is the actor having full and direct access to the resources that we aim to protect. We assume that the attacker could be or could impersonate a data provider (upstream) or an end user (downstream). For instance, in the case of Machine Learning as a Service (MLaaS), the attacker could target the model confidentiality for financial gain. In addition, if training dataset contains valuable information (e.g., genomics and/or production data), the attacker could target training dataset confidentiality. Finally, when the ML system is used for critical applications (food supply or seed production), the attacker could target integrity/availability of training dataset, ML model or prediction.

For the specific context of dairy digital farming, there can be a wide range of adversaries (insiders to the context of digital farming or not), thus leading to various levels of risks, ranging from a farmer seeking financial gain to eco-terrorists aiming to disrupt the food supply. As a result, the adversary is likely to have detailed knowledge about the digital farming and could have weak to strong technological skills. To study the attack surface and related threat vectors, we look at the interfaces of the ML system leaving aside all security concerns that are not inherently tied to ML (network, access control…).

Looking at the data chain, we have the upstream interface in which data is collected and preprocessed to form the training dataset and the downstream interface in which the trained model generates predictions upon user requests. At data collection stage, an attacker can craft and provide malicious data points to compromise the ML model and its predictions. At model interaction stage, malicious requests can lead to leak the ML model and the training dataset. More precisely, in controlled access settings, the attacker will have to compromise the model through a distant API whereas in model sharing settings the attacker is free to access both the program containing the model and the API locally, thus making the prediction access control harder and raising new concerns like reverse engineering.

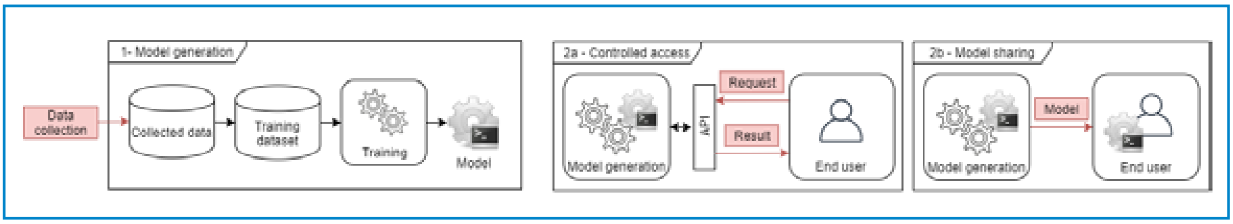

Figure 2. Attack vectors - (1) Upstream, (2) Downstream.

Risks to machine learning

Confidentiality of data and model

Membership inference attacks aim to deduce if a given data point is present in the training dataset or not. The first membership attack against a ML system was realized by Shokri *et al.* (2017). They targeted black box models in a context of MLaaS and were able to differentiate member data points only by sending requests to the model. Salem *et al.* (2018) have built upon this work to relax assumptions and extend the attack scenario. Others have studied membership on Generative Adversarial Network (Hayes *et al*., 2019) and even on robust Deep learning techniques (Song *et al*., 2019).

Model inversion attack aims to reconstruct information about a data point present in the training dataset. This type of vulnerability was introduced by Fredrikson *et al.* (2014) and demonstrated for a ML system used for personalized warfarin dosing. They were able to show that an attacker possessing the ML model and demographic information about a patient would be able to infer their genetic markers. Until recently, attacks failed to inverse higher complexity model such as neural networks but Y. Zhang *et al.* (2020) presented a technique that uses a small amount of auxiliary knowledge against neural network in white-box settings.

Finally, model theft attack aims to gain knowledge about a black box or grey box model such as type of algorithm, hyper-parameters or trained model parameters. Tramer *et al.* (2016) shows that model parameters at risk in a context of MLaaS, even when attacker does not have knowledge about the training data set or model algorithm. They use equation-solving attack to extract highly accurate model with a little number of requests. In their work presented earlier for membership inference Shokri *et al.* (2017) actually use a shadow model (*i.e.*, a model mimicking the target model behavior) as a step to mount their attack, essentially stealing a black-box version of the model.

Integrity of model and prediction

Data poisoning attack enables an adversary to influence the predictive power of a model by injecting malicious data points into the training data set. For example, Chen *et al.* (2017) present a scenario in which a back door is installed on a deep learning authentication system. Recently, the particular case of sequentially generated data for continuous learning system has been studied by X. Zhang *et al.* (2019). Finally, adversarial learning is a class of attacks in which an attacker exploits a predictive system by finding an input that induces an abnormal behavior of the system. For example, Al-Dujaili *et al.* (2018) successfully crafted adversarial example on malware binaries that allowed them to evade detection systems. Papernot *et al.* (2017) showed that they can instantiate such an attack in black-box settings targeting MLaaS.

Practical means of mitigation

Differential privacy is a designed privacy model to share information about a computation made on a dataset without compromising the privacy of each unique element. A possible implementation of differential privacy is through the addition of noise to the result to render unnoticeable the presence or absence of a particular profile in the data set. Differential privacy is a defense technique that is often used to counter membership inference and model inversion attacks. Since it can help generalizing the model, it can also be used to mitigate adversarial example and data poisoning attacks. Several attacks that we described take advantage of the accuracy of the predictions or use confidence levels shared along with the prediction. Controlling how the end user accesses the results of the predictive system (*e.g.*, by removing confidence levels) can help mitigating attacks such as membership inference and model inversion. Finally, robust models are designed to be more resilient to data perturbation both at training and prediction stage, helping mitigate adversarial crafting of data both at training stage (data poisoning) and at prediction stage (adversarial example). Several techniques can be employed to increase model robustness such as robust statistics, which are a class of estimation techniques that can be used to minimize impact of high diversity in statistical data distribution (*e.g.* outliers or small subgroups). Adversarial training is another technique in which adversarial examples are purposefully crafted and inserted in the training dataset to increase robustness against adversarial examples.

Discussion

In this paper, we have focused exclusively on threat vectors present at the data collection and the prediction interfaces. However, the security and privacy of ML systems security and privacy are also impacted by other concerns, which we briefly review in this section. In the data chain, in most cases the data collector (mostly the farmers) hand their data to data-processors. In addition, some of the data collected may be critical (*e.g.*, genomics) and considered as business secret (*e.g.*, production data). For this reason, the data collector might be reluctant to share its data which would break the first link of the data chain. In this scenario, homomorphic encryption is a cryptographic technique that could allow the data processor to train the ML system without the need for the data collector to divulge its valuable data, effectively maintaining our data chain functional. Data collectors have formulated concerns about the privacy of their data, and lack of cooperation between actors of the data chain have led to tensions within digital farming ecosystem. Doing our research, we have found multiple threat scenarios where the adversary is an insider to the digital farming context. Releasing tensions between actor would thus in itself help mitigate all ML induced risk to digital farming by lowering the likelihood of scenarios where the adversary is part of the data chain. A data trusts is a regulatory tool (a contract) designed to ensure that the management of a resource benefits each shareholder such as the resource provider (*i.e.* data-collector), resource processing agent (*i.e.* data-processor) and resource beneficiary (*i.e.* end-user).

Conclusion

As mentioned by organizational actors and looking at the context, it appears that security is a very concerning topic for digital farming that has been overlooked until now. We evaluated that ML was the left aside in terms of security and data privacy and dedicated our effort to help raise the attention as it is being used extensively. We have designed a Security model that helps framing the problem and investigated technical vulnerabilities and practical ways to mitigate them. During our study we also have found that related concern such as data ownership are directly impacting ML security and data privacy. Technical and regulatory tools such as homomorphic encryption and data trusts are available to help with these related concerns, effectively helping mitigating

ML induced risks. Agriculture has always been a technophile ecosystem and up to this day it has taken the most out of available technologies, leading to the digitalization of farming. We believe that digital farming sector should learn from other industries and take the opportunity to be ahead of the curve on security and data privacy concerns.

List of references

Abdullah Al-Dujail, Alex Huang, Erik Hemberg and Una-May OReilly, 2018. Adversarial deep learning for robust detection of binary encoded malware. 2018 IEEE Security and Privacy Workshops (SPW): 76-82.

Scott Champion, Linsky, Peter Mutschler, Brian Ulicny, Thomson Reuters, Larry Barrett, Glenn Bethel, Michael Matson, Thomas Strang, Kellyn Ramsdell and Susan Koehler, 2018. Threats to precision agriculture. U.S. Department of Homeland Security.

Xinyun Chen, Chang Liu, Bo Li, Kimberly Lu and Dawn Song, 2017. Targeted backdoor attacks on deep learning systems using data poisoning.

DLG Committee for Digitization, Work Management and Process Technology, DLG

Committee for Technology in Crop Production, DLG Committee for Technology in Animal Production, DLG Work Group Information Technology, Hans W. Griepentrog, Norbert Uppenkamp and Roland Hörner, 2018. Digital agriculture: A DLG position paper.

Gene L. Dodaro, 2020. Priority open recommendations: U.S. department of agriculture. U.S. Government Accountability Office.

Matthew Fredrikson, Eric Lantz, Somesh Jha, Simon Lin, David Page and Thomas Ristenpart, 2014. Privacy in pharmacogenetics: An end-to-end case study of personalized warfarin dosing. In 23rd USENIX Security Symposium (USENIX Security 14): 17-32.

Andrew Geil, Glen Sagers, Aslihan D. Spaulding and James R. Wolf, 2018. Cyber security on the farm: An assessment of cyber security practices in the United States agricultural industry. International Food and Agribusiness Management Review (3): 317-334.

Maanak Gupta, Mahmoud Abdelsalam, Sajad Khorsandroo and Sudip Mittal, 2020. Security and privacy in smart farming: Challenges and opportunities. IEEE Access (8):34564-34584.

Jamie Hayes, Luca Melis, George Danezis and Emiliano De Cristofaro, 2019. Logan: Membership inference attacks against generative models. Proceedings on Privacy Enhancing Technologies 2019(1): 133-152.

Nicolas Papernot, Patrick McDaniel, Arunesh Sinha and Michael Wellman, 2016. Towards the science of security and privacy in machine learning. Arxiv 1611.03814.

Nicolas Papernot, Patrick McDaniel, Ian Goodfellow, Somesh Jha, Z. Berkay Celik and Ananthram Swami, 2017. Practical black-box attacks against machine learning. Proceedings of the 2017 ACM on Asia Conference on Computer and Communications Security: 506-519.

Ahmed Salem, Yang Zhang, Mathias Humbert, Pascal Berrang, Mario Fritz and Michael Backes, 2018. Ml-leaks: Model and data independent membership inference attacks and defense son machine learning models. Network and Distributed Systems Security (NDSS) Symposium 2019.

Reza Shokri, Marco Stronati, Congzheng Song and Vitaly Shmatikov, 2017. Membership inference attacks against machine learning models. 2017 IEEE Symposium on Security and Privacy (SP).

Liwei Song, Reza Shokri and Prateek Mittal, 2019. Membership inference attacks against adversarially robust deep learning models. 2019 IEEE Security and Privacy Workshops (SPW): 50-56.

Michael Sykuta, 2016. Big data in agriculture: Property rights, privacy and competition in ag data services. The International Food and Agribusiness Management Review (19): 57-74.

Florian Tramèr, Fan Zhang, Ari Juels, Michael K. Reiter and Thomas Ristenpart, 2016. Stealing machine learning models via prediction apis. In Proceedings of the 25th USENIX Conference on Security Symposium (SEC'16): 601–618

Jason West, 2018. A prediction model framework for cyber-attacks to precision agriculture technologies. Journal of Agricultural and Food Information 19 (4):307-330.

Marc Window, 2019. Security in precision agriculture: Vulnerabilities and risks of agricultural systems. Luleå University of Technology - Department of Computer Science, Electrical and Space Engineering.

Sjaak Wolfert, Lan Ge, Cor Verdouw and Marc-Jeroen Bogaardt, 2017. Big data in smart farming - a review. Agricultural Systems (153):69-80.

Yuheng Zhang, Ruoxi Jia, Hengzhi Pei, Wenxiao Wang, Bo Li and Dawn Song, 2020. The secret revealer: Generative model-inversion attacks against deep neural networks. 2020 IEEE/CVF Conference on Computer Vision and Pattern Recognition (CVPR): 250-258.

Xuezhou Zhang, Xiaojin Zhu and Laurent Lessard, 2020. Online data poisoning attack. Proceedings of the 2nd Conference on Learning for Dynamics and Control, PMLR (120): 201-210.

Monitoring fattening pig's behaviour by RFID registrations

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Abstract

RFID identification can be used for health and welfare monitoring if tagged animals are recorded at drinking and feeding places. In an experiment, RFID readings from 144 fattening pigs (12 pens each with 12 pigs, one drinker and two feeders) were recorded with four readers (each with eight antennas) during almost four months. Tag readings were combined in visits and subsequently visits in meals. A model for the number of meals per pig per day was developed generating alerts when the number was less than expected. Most cases of culled pigs corresponded with alerts, but the sensitivity depended on the chosen setting. The related specificity was only high enough when a high threshold was chosen.

Keywords: RFID, pigs, eating behaviour, drinking behaviour, health and welfare monitoring.

Introduction

Pig identification with RFID ear tags can be used in pig production to certify antibiotics-free meat production, as is done by KDV (Sustainable Pork Value Chain, sustainable-pork.com). This RFID identification can also be used for health and welfare monitoring if tagged animals are recorded by readers at certain locations in the barn (Ruiz-Garcia and Lunadei, 2011, Maselyne *et al*., 2018). In a previous research a good correspondence between tag readings and animal behaviour was found in two cycles in a pen with 12 weaned pigs (de Mol *et al*., 2019). In the current research LF RFID readings in one cycle were available for 12 pens each with 12 fattening pigs. Recordings per animal can be used for individual monitoring of pigs. The goal of this experiment was the analysis of the individual patterns of the number of visits for monitoring by generating alerts when the number is deviating. These alerts are true in case of the culling or treatment of animals and false for healthy animals.

Materials and methods

Twelve pens with fattening pigs were involved in this experiment at the Dutch Swine Innovation Centre (VIC Sterksel) in the Netherlands.The experimental period started at March 11 2020 and ended at July 1 2020, this is from the start till the end of the fattening period. Each pig was equipped with an LF RFID tag in the right ear. Each pen was equipped with one drinker and two (combined) feeders (Figure 1). Four readers, each with eight antennas were available to register the tag readings at each drinker and feeder (equipment provided by Agrident, agrident.com). For four drinkers and all 24 feeders one specific antenna was available. Four antennas were applied for tag

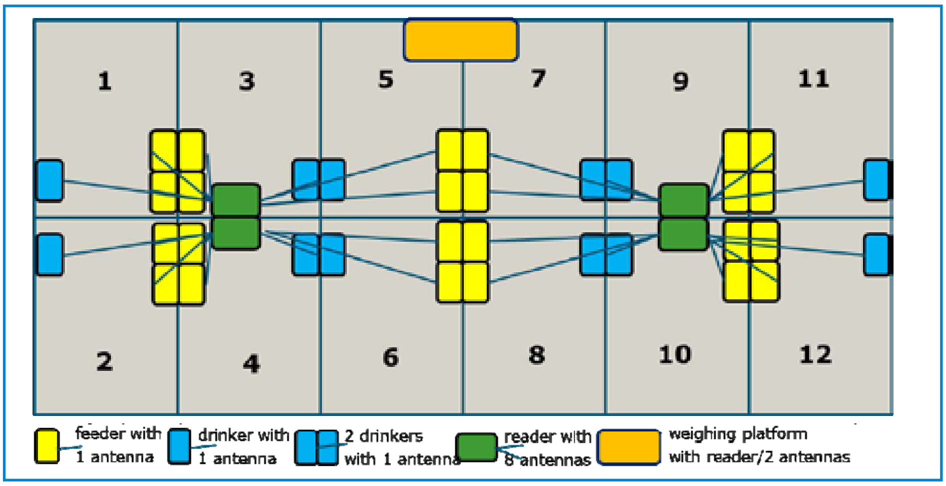

Figure 1. Layout of the barn with 12 pens each with one drinker and two feeders; 4 readers, each with 8 antennas; one weighing platform (with reader) connecting pens 5 and 7.

readings from two drinkers in neighbouring pens. In this way it was possible to register the visits of all drinkers and feeders (n=36) with 32 antennas (connected to four readers).

One weighing platform (Thomas® Animal Weighing system, www.hotraco-agri.com) was installed at a passage that was created at the back end of two neighbouring pens (Figure 1). Weight recordings were available anonymously and an additional reader with two antennas was installed on this weighing platform to register tag readings. Reference weight recordings by weighing by hand were available once a month.

Recordings of climate data (temperature, relative humidity, $CO_2$and NH_3) were also available (equipment provided by Hotraco, www.hotraco-agri.com).

Video recordings at selected spots were available for validation. Treatments, cullings and other management data could be used for the analysis of the monitoring results.

Readings of the RFID tags were recorded continuously. The tag readings became available as one csv file per reader per day with on each line:an identification, a time stamp and an antenna number. The combination of reader and antenna number identified a drinker, feeder or weigher. Tag readings were combined to visits by applying a bout criterion of 20 seconds (Maselyne *et al*., 2016). Visits were combined to meals by applying a meal criterion of 900 seconds(Tolkamp and Kyriazakis, 1999).Data were stored in an Access database. A procedure for reading the csv files was made where the tag readings of the same pig and same location were combined in visits with the time stamp of the first reading as starting time, the time stamp of the last time stamp as ending time and the number of readings included as an additional characteristic of the visit. In another procedure visits were combined in meals. This processing resulted in three types of readings, visits and meals: 1 = drinking, 2 = eating and 3 = weighing (only possible in the combined pen). The number of visits and meals per pig per day was used to develop a model for monitoring the pigs.

Results

Recorded visits were available per pig and per type. This is visualized in Figure 2 where the visits in one pen during a 6 hour period are shown. Tag readings were combined in visits. Visits can be to the drinker, to one of the feeders or to the weigher (not relevant for Pen 2 depicted in Figure 2). In general periods with activity from all pigs in the pen alternate with periods of rest where general activity is low. Evening and night periods appeared to be quieter (but not without activity).

The average weight by hand of the pigs was 35 kg at March 26 and 123 kg at June 17. Due to technical problems (mostly network problems) readings were missing for 28% of the days (uptime less than 20 hours per day).This missing percentage varied between readers from 11% to 49%. The data from the other days were used to get an indication of the usability of RFID readings for monitoring. Visits at one feeder were mostly combined with visits at the other feeder, therefore visits at the two feeders were combined in the further analysis. In total almost 17 million tag readings were recorded (Table 1), resulting in almost 1,3 million visits (131 readings per visit) and 266 thousand meals (5 visits per meal).The number of tags was at the same level for all antennas, the number of visits was higher for the antennas serving two drinkers and the number of meals was twice as high for these antennas. These results indicate that one antenna can be used to register the relevant readings of two drinkers.

Visits and meals are a characteristic of the behaviour of a pig. So, monitoring of the pig's behaviour can be based on monitoring the number of meals, the average interval between meals or the maximum interval between meals. Pigs with a decreased number of meals or with increased intervals should be alerted for inspection by the farmer. For these variables the daily level is predicted with a statistical model, e.g., for the number of meals given in Equation 1, where the number of meals today (d) is predicted by the number yesterday (d-1), the number on the day before yesterday (d-2) and the average number of the other animals in the same pen today.

$$\text{NrMeals(d)} = \alpha_1 + \alpha_2\ \text{NrMeals (d-1)} + \alpha_3\ \text{NrMeals (d-2)} + \alpha_4\ \text{AvgNrMeals (d)} \qquad (1)$$

Equation 1 was based on the analysis of the correlations of the number of meals with other variables that might be related. From this analysis it was concluded that it is not relevant to include climate variables in this equation. The values of the parameters

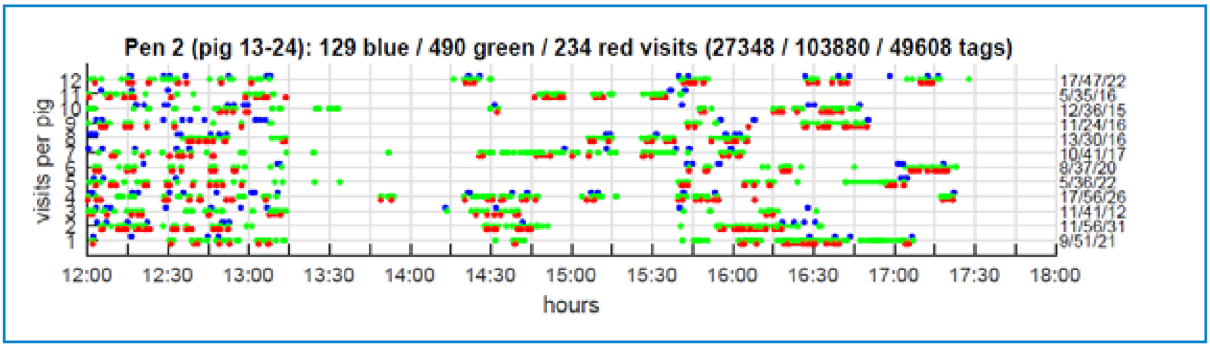

Figure 2. Example of all visits in Pen 2 between 12:00 and 18:00 hr om March 11, 2020; each line represents visits of one pig: blue visits correspond with readings at drinking nipple, green and red visits correspond with reading at one of the feeding throughs.

Table 1 Percentage of days with uptime less than 20 hours, number of readings, visits and meals per reader (reader 213 is on the weighing platform).

	Reader 209	Reader 210	Reader 211	Reader 212	Reader 213	Total
Days Uptime < 20 Hrs	49%	26%	24%	30%	11%	28%
Readings	31675394	46228483	43866491	44780502	1660406	168211276
Visits	227302	349709	327865	331887	43166	1279929
Meals	39688	71770	68736	63989	22262	266445

α_1, α_2, α_3 and α_4 might vary between pigs and might be time dependent. Therefore, a Kalman filter is used to fit these parameter values per pig on-line (de Mol *et al.*, 1999), resulting in updated parameter values per pig per day, together with a variance-covariance matrix for these values that can be used to calculate confidence intervals. Alerts are generated when the difference between predicted and real level for a pig on a day is too big, that is when the error is outside a confidence interval. Several options were considered: a 95%, 99% or 99.9% confidence interval.

The alerts were analysed in two ways:

- Alerts are true in case of a recorded case of culling or treatment: each case is either true positive (TP) or false negative (FN), resulting in the sensitivity: TP/(TP+FN), the percentage of detected cases.
- Alerts outside culling or treatment periods are false: each day in then either true negative (TN) or false positive (FP), resulting in the specificity: TN/(TN+FP),the percentage of healthy days without alert.

An example of the monitoring process for one pig is depicted in Figure 3. For each day the expected value was calculated based on Equation 1 using the parameter values from the Kalman model. The outcomes of the Kalman model made it also possible to calculate the confidence interval and to give alerts when the real value is outside the confidence interval. Sensitivity and specificity results for all pigs are included in Table 2 and Table 3.

Discussion

It was difficult to calculate the sensitivity as there were only a few cases of culled or treated pigs, furthermore some cases were in a period were most data were missing(so insufficient input for model) and there was one case only a few days after the start of the experiment (limited time for the model to adapt). However, in most cases of culling alerts at various levels were generated in the period before culling. Results for the treatment cases were not included, as these were only a limited number (n=11), for a great part(n=5) in the period before the culling of a pig.

For sensitivity there is no clear difference between the results of different variables. This is different for specificity, it is lower in case of the average or maximum interval between meals compared with the number of meals. The specificity is high for the

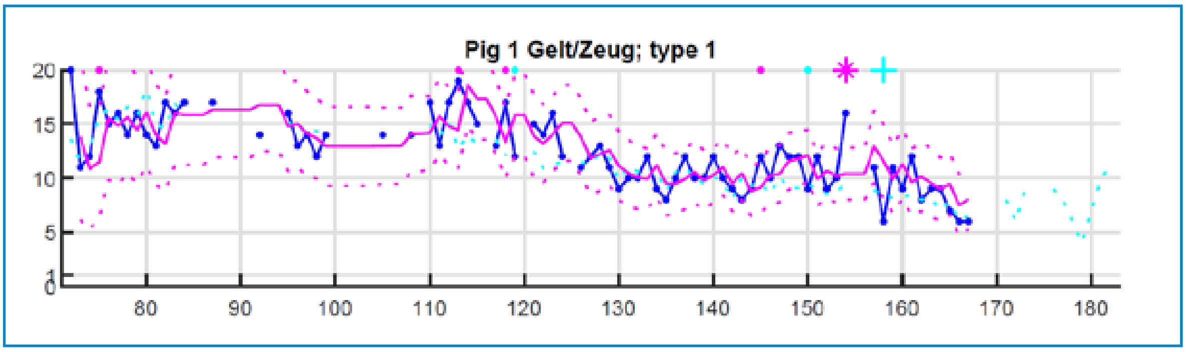

Figure 3. Example of monitoring per pig with results per day: blue solid line is number of meals, dotted cyan is average number for this pen, solid magenta line is fitted value (with dotted magenta line as 95% confidence interval) and alerts in top line when value is outside confidence interval (cyan for decreased values, magenta for increased; dot sign for 95%, plus sign for 99% and asterisk sign for 99.9% confidence interval).

Table 2. Sensitivity results based on number of meals per day, average interval between meals and maximum interval between meals for five cases of culled pigs (= outside 95%, ** = outside 99% and *** = outside 99.9% confidence interval).*

		Case 1[1]	Case 2	Case 3[2]	Case 4[2]	Case 5[2]
Decreased number of meals	Type 1 (drinking)	*	**	*	***	-
	Type 2 (eating)	**	***	*	*	**
	Type 3 (weighing)	n/a	**	**	n/a	n/a
Increased average interval between meals	Type 1 (drinking)	***	***	-	*	***
	Type 2 (eating)	-	-	-	-	***
	Type 3 (weighing)	n/a	***	**	n/a	n/a
Increased maximum interval between meals	Type 1 (drinking)	***	**	-	**	***
	Type 2 (eating)	***	-	**	-	***
	Type 3 (weighing)	n/a	***	***	n/a	n/a

[1] Case in the beginning of experiment

[2] Missing data in culling period

Table 3. Specificity results based on number of meals per day, average interval between meals and maximum interval between meals for all pig days outside treatment or culling periods.

			Confidence interval		
		Valid days	95%	99%	99.9%
Decreased number of meals	Type 1 (drinking)	9380	94.6%	98.4%	99.6%
	Type 2 (eating)	9115	95.4%	98.8%	99.7%
	Type 3 (weighing)	2245	94.7%	97.9%	99.5%
Increased average interval between meals	Type 1 (drinking)	9293	90.9%	93.9%	96.1%
	Type 2 (eating)	9040	90.2%	93.8%	96.2%
	Type 3 (weighing)	2051	91.6%	94.2%	95.8%
Increased maximum interval between meals	Type 1 (drinking)	9293	89.7%	94.3%	97.1%
	Type 2 (eating)	9040	90.6%	94.5%	97.0%
	Type 3 (weighing)	2051	91.5%	94.7%	97.5%

number of meals but might only be high enough for practical application in case of the 99.9% confidence interval. For practical application one should balance between wanted sensitivity and acceptable specificity.

Other variables should be studied as well, for example the visits per pig per day can be analysed in the same way. Other aspects that need further study are the influence of the group level on the individual pattern and the within-day pattern of the pig behaviour.

Two pens were different as they were connected by a weighing platform, the specific results also need further study: combining anonymous weights with RFID readings, differences in visiting patterns to the weighing platform compared with visits to the drinker and feeders.

Similar data are available from two other fattening round in the same pens, one in 2019 and one later in 2020. Comparing the results of these rounds with the present results might strengthen the conclusions.

Conclusions

We conclude that RFID readings can also be used for health and welfare monitoring in fattening pigs. Comparing the number of meals (or the average and maximum interval) per pig per day with the expected level results in alerts that can be useful is daily management. Sensitivity results were difficult to quantify, but alerts were given in most cases. Specificity was lower when results were based on average or maximum interval compared with average number of meals.

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List of references

de Mol, R.M., Keen, A., Kroeze, G.H., Achten, J.M.F.H., 1999. Description of a detection model for oestrus and diseases in dairy cattle based on time series analysis combined with a Kalman filter. Computers and Electronics in Agriculture, 22 (2-3), 171-185.

de Mol, R.M., P.H. Hogewerf, R.G.J.A. Verheijen, N.C.P.M.M. Dirx and J.B. van der Fels, 2019. Monitoring pig behaviour by RFID registrations. In: Precision Livestock Farming 2019 - Papers Presented at the 9th European Conference on Precision Livestock Farming, ECPLF 2019, p 315-321.

Maselyne, J., W. Saeys, P. Briene, K. Mertens, J. Vangeyte, B. De Ketelaere, E. F. Hessel, B. Sonck, and A. Van Nuffel, 2016. Methods to construct feeding visits from RFID registrations of growing-finishing pigs at the feed trough. Computers and Electronics in Agriculture 128: 9-19.

Maselyne, J., A. Van Nuffel, P. Briene, J. Vangeyte, B. De Ketelaere, S. Millet, J. Van den Hof, D. Maes, and W. Saeys, 2018. Online warning systems for individual fattening pigs based on their feeding pattern. Biosystems Engineering 173: 143-156.

Ruiz-Garcia, L. and L. Lunadei, 2011. The role of RFID in agriculture: Applications, limitations and challenges. Computers and Electronics in Agriculture 79(1): 42-50.

Tolkamp, B. J. and I. Kyriazakis, 1999. To split behaviour into bouts, log-transform the intervals. Animal Behaviour 57(4): 807-817.

Atypical spectra screening: applications for monitoring infrared instruments and model predictions

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Abstract

Fourier-Transformmid-InfraRed (FT-IR) spectrometry is a recognized and widely used method to determine the compositional quality of raw milk and other liquid milk products. In recent years, mathematical models were developed that are capable of detecting atypical milk samples. Here we show how atypical spectra screening can also be used to monitor variations in the spectra that are related to the instrument performance rather than to the characteristics of the milk. By studying the temporal dynamics of spectra anomaly scores, it is possible to detect measurement instabilities caused by changes in the measurement context (i.e., instabilities of FT-IR instruments). We also show how algorithms can be developed to detect episodes of measurement instabilities automatically and in real-time.Such information can provide insights in the maintenance status of infrared instruments and changes in the measurement context. This, in turn, is important to ensure a consistent quality of infrared measurements and chemical profiling of milk samples.

Key words: Milk, Infrared spectrometry, atypical spectra screening.

Introduction

Fourier-transform mid infrared spectrometry (FT-IR) is a recognized and widely used method to rapidly determine the compositional quality of raw milk and other liquid milk products. This is done by generating spectra based on the absorption of light at different frequencies that result from different chemical bonds present in the milk. On the basis of these spectra, mathematical prediction models can predict (i.e., calculate) the concentration of fat, protein, lactose, and many other parameters. For proper predictions in routine operation, the accuracy has to be continuously monitored. If needed, slope/intercept settings can be adjusted based on analysis of calibration sample sets with chemical reference values.

In the recent years, FT-IR spectrometry has gained increasing nterest for its potential to predic tindividual fatty acids or groups of fatty acids (Fleming *et al.*, 2017), minerals (Stocco, Cipolat-Gotet, Bonfatti, Schiavon, Bittante and Cecchinato, 2016), green house gas emissions (Vanlierde*et al.*, 2016), energy status of the cow (McParland *et al.*, 2012), pasture (Coppa *et al.*, 2020)dry matter intake (Dórea, Rosa, Weld and Armentano, 2018), adulteration of milk (Hansen and Holroyd, 2019), and others. These parameters are of interest not only to individual farmers for monitoring and improving herd management practices, but also to feed advisors, veterinarians, dairy processors and researchers. Due to the economic impact, quality control procedures need to secure that the predictions are valid. However, with more and more parameters that need to be monitored, the costs associated with chemical reference analyses of control milk samples become increasingly uneconomic. Moreover, for parameters such as pasture intake or gas emissions, chemical reference analyses that can be applied to the milk do not exist. As a consequence, a different and more general approach to monitor the validity of such predictions is required.

Since FT-IR predictions are eventually based on the information present in the spectra, the spectra need to be of good quality. Importantly, acquiring spectra from milk samples is, in fact, a measurement process that takes place under certain conditions (e.g., ambient temperature, physical sample characteristics) and by using a particular FT-IR instrument that is subject to wearing and measurement instabilities. Instabilities at the level of individual FT-IR instruments can manifest gradually (e.g., built up of a film inside the cuvette) or abrupt (e.g., suboptimal functioning of the homogenizer) and can occur over long (e.g., dissolving or wear of the cuvette) or short time scales (e.g., temporary issues with a moving mirror inside the cell). In principle, all these factors can lead to systematic changes in the obtained spectra. If this occurs at spectral bands containing information used by prediction models it can lead to erroneous or invalid predictions.

Monitoring routinely analyzed FT-IR spectra for systematic temporal deviations can therefore give valuable information about the validity of the predictions calculated from these spectra and, potentially, the functioning of the instrument. In the present paper we show how untargeted methods originally used to screen spectra for adulterated milk (e.g., Hansen and Holroyd, 2019) can be slightly modified to screen for systematic temporal deviations in routinely analyzed milk spectra. In short, this approach is based on establishing an FT-IR milk fingerprint that is unique to each FT-IR instrument and by tracking, over time, how individual milk spectra deviate from this fingerprint. Because the focus is on systematic deviations at the level of spectra rather than predictions calculated from them, the approach is more general compared to the use of pilot milk samples. Moreover, no cost and labor-expensive chemical reference analyses and pilot milk samples are required.

Materials and methods

Data

The data set contained 345473 spectra that correspond to Dutch bovine herd bulk milk samples randomly collected between January 2018 and November 2020. All milk samples were routinely analyzed for milk payment. For acquisition of the spectra, milk samples were randomly assigned to one of four FT-IR instruments (Milkoscan FT+, FOSS Analytical A/S, Hillerød, Denmark) where they all underwent the same pre-treatment before the scan took place. FT-IR spectra were obtained in the mid infrared region with wavelengths between 10.8 μm (926 cm^{-1}) and 1.995 μm (5012 cm^{-1}). AllFT-IR instruments were standardized monthly using the FOSS equalizer application in accordance with the manufacturer's instructions (Winning, 2015).

Untargeted spectra screening

For the development of a mathematical model to identify non-specific deviations in milk spectra, we followed a conceptually similar approach as described by (Hansen and Holroyd, 2019) and employed by manufacturers of FT-IR instruments (FOSS, 2014). For each of the four FT-IR instruments in the Qlip payment testing laboratory a separate model was developed on the basis of ca. 85000 spectra. Preprocessing of the spectra consisted of selecting wavenumbers between 925 cm^{-1} and 1550 cm^{-1}, 1710 cm^{-1} and 1900 cm^{-1}, and between 2700 cm^{-1} and 2971 cm^{-1}. Moreover, the spectra from each instrument were standardized to have, per wavelength, zero-mean absorption with unit-variance. We then used a principal component analysis (PCA) with ten components to project the spectra to the latent space. After transforming the spectra to the latent space, we computed the covariance matrix and calculated per spectrum the Mahalanobis distance. In the next step, we used the PCA to perform an inverse transformation on the spectra in the latent space in order to obtain the reconstructed spectra in the original space. By calculating, across all wavelengths, the root-mean-square error between the original and reconstructed spectra, we obtained the spectral residuals. In the last step, the Mahalanobis distances and the spectral residuals were each standardized to have zero mean with unit variance before they were summed to a single score per spectrum: the spectrum anomaly score. The higher the spectrum anomaly score, the more a spectrum deviates from all the spectra that were used to develop the screening model.

Identification and analysis of systematic deviations in IR spectra

The use of untargeted spectra screening for identifying instrument instabilities is grounded on two critical assumptions. First, instrument instabilities need to be events that have a duration such that a sufficient number of spectra is influenced. Instabilities affecting individual spectra entirely incidental and temporally unpredictable will not be detected. Second, the chemical composition of the milk samples has to be independent of the time at which the spectra are obtained. That is, milk samples from different groups (e.g. cow milk and goat milk) should not be analyzed in discrete groups. Only under these conditions it is possible to identify and relate systematic deviations in spectra to instabilities during their acquisition. The detection of systematic deviations in IR spectra therefore comes down to detecting deviations in the time course of anomaly scores at the level of individual instruments over a pre-defined time window. As can be seen in Figure 1, such systematic changes in the anomaly scores can present themselves as drifts (upper panel) or sudden jumps (lower panel) and can even impact routinely predicted milk compositional parameters such as urea and free fatty acids in the milk obtained from commercial prediction models installed on the respective instruments.

By visually inspecting for each instrument the anomaly scores on a daily basis over a period of three years, we identified 48 events with comparable instabilities. Of these, seven events were related to instrument A, sixteen to instrument B, eleven to instrument C, and fourteen to instrument D. For an actual implementation of such a tool in routine FT-IR analysis of milk samples, it is desirable to detect such incidences in real-time and as early as possible. To do so, we fed the anomaly scores of one day, one after another, to a Bayesian online change point detection algorithm (Adams and MacKay, 2017). The algorithm uses Bayesian inference to compute a distribution over the next unseen anomaly score in a time series, given only the anomaly scores it has seen before. It then computes for each individual anomaly score the probability that it reflects a change point on the basis of the past k anomaly scores. We found that $k = 24$ successive anomaly scores were sufficient to reveal most of the previously identified instrument instabilities. In other words, systematic changes in the anomaly scores could be detected with a latency of 24 measured spectra. The resolution at which a change point can be detected is at the level of an individual spectrum (see Figure 1 for examples).

So far, we focused on analyzing systematic deviations in anomaly scores on a spectrum-by-spectrum basis. However, instrument instabilities or drifts can also manifest over longer time scales. Figure 2 illustrates for each of the four instruments the change in anomaly scores over a period of three years. We also applied the Bayesian online change point algorithm to these time series. This time, however, we computed the average anomaly score over a time window of two consecutive days and computed the probability of each possible change point using the last three two-day-averages. This means that systematic changes in the anomaly scores could be detected with a latency of six days and a resolution of two days. As can be seen in Figure 2, most of the major peaks were readily identified by the algorithms.

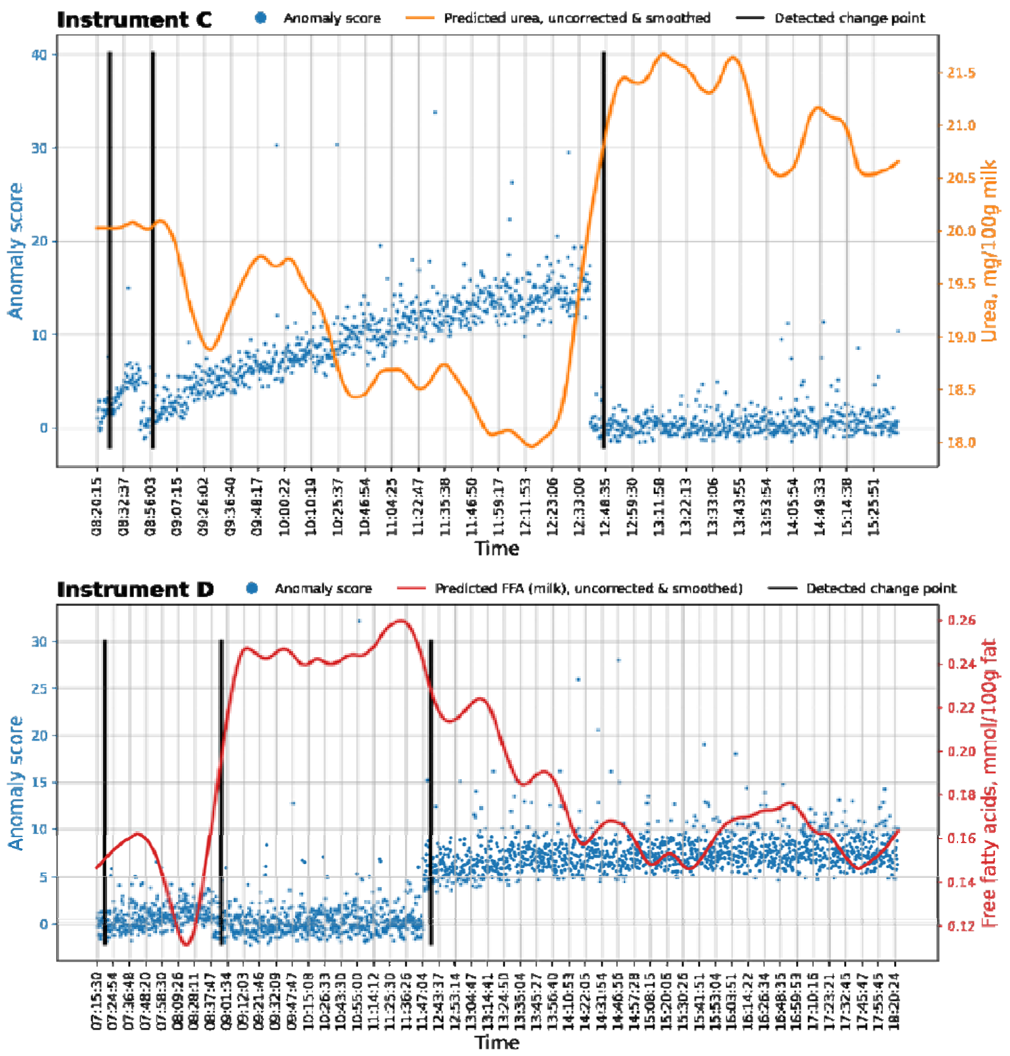

Figure 1. Examples of measurement instabilities that manifest as gradual (upper panel) or sudden (lower panel) temporal changes in the spectra anomaly scores over a day. Each blue dot corresponds to the spectrum anomaly score of an individual milk sample measured on that instrument. The orange and red lines depict LOWESS curves fitted to the uncorrected predictions for urea and free fatty acids in the milk, respectively. The vertical black bars reflect the moment in time where the Bayesian change point algorithm detected an unpredictable change in the anomaly scores. Note, how the change in the anomaly scores coincides with systematic biases in the predictions of urea and free fatty acids.

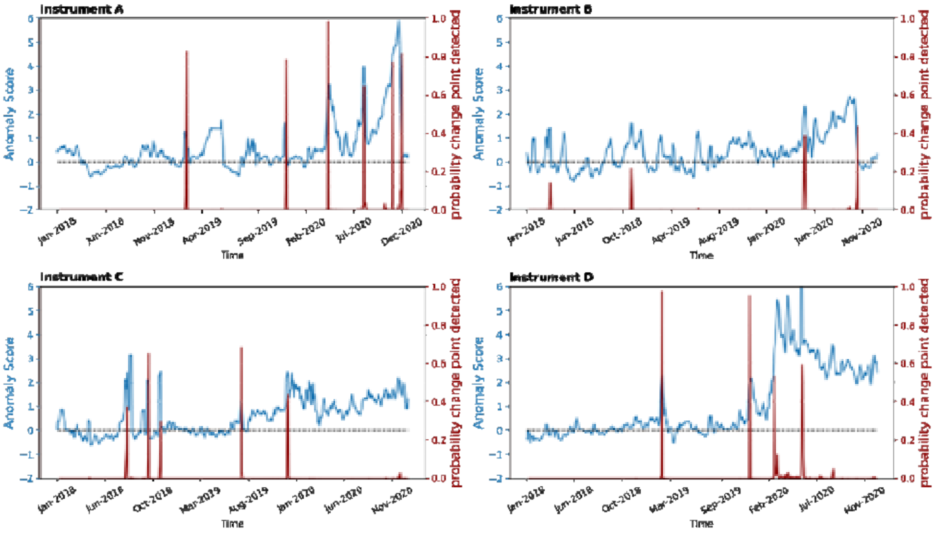

Figure 2. Long-term average of anomaly scores for each of four instruments from 2018 to 2020. The blue lines reflect the average anomaly score. The red spikes reflect the moment in time where the Bayesian change point algorithm identified an unpredicted change in the average anomaly score. The height of the spike corresponds to the probability or confidence that a change point has been detected.

Interestingly, the anomaly scores for instrument D appear to change drastically from early 2020 onwards. Around the same time, our colleagues began to notice increased fluctuations in the predictions of urea and free fatty acids on our pilot milk samples. These problems persisted until end of March 2020, when the cuvette was eventually replaced. Aside from abrupt and temporary fluctuations in the anomaly scores, all four instruments reveal a trend towards higher anomaly scores as time passes by. This can be a consequence of dissolving or wear of the cuvette or the built-up of a film inside of it.

For the majority of the instabilities that we discovered, it was not possible to relate it to documented maintenance work or other disturbances reported around that time. This is not surprising given that none of these events was detected or issued by the quality control sensors present in FT-IR instrument. Nonetheless, we can use the spectra to get a basic understanding about how systematic the effects of such instabilities are and whether certain types of instabilities reappear. To do so, we created a dataset with all milk spectra that were measured during the 48 episodes with instabilities. This amounts to ca. 20000 individual milk spectra. We then used a combination of PCA and *t*-distributed stochastic neighbor embedding to visualize possible patterns in the residuals. As can be seen in Figure 3, on a macro level, the four different instruments are well separated from each other. This emphasizes the need for the development of separate models per instrument. At the micro level, it can be seen that the number of distinct clusters (n = 23) is about half the number of identified incidences with instrument instabilities (n = 48). On the one hand, this can be an indication that we identified 23 distinct and unique categories of instrument instabilities. On the other hand, some of the clusters, particularly the small ones, only contain spectra from a single day. Because seasonal variations are not explicitly modelled in the untargeted screening model, they cannot be removed from the spectral residuals. This means that the same type of instrument instability that occurs during one day in the winter and one day in the summer can lead to distinct clusters. Nevertheless, some clusters contain spectra obtained during instrument instabilities that occurred on more than a single day. This suggests that certain instrument instabilities reappear.

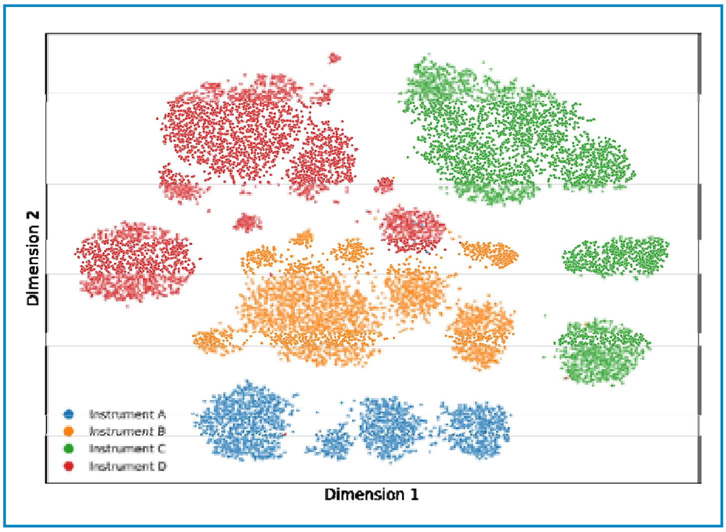

Figure 3. Clustering of milk spectra collected during episodes of instrument instabilities (n = 48). Each dot reflects an individual milk spectrum positioned on the two dimensions of a t-distributed stochastic neighbor embedding. The colors correspond to the different instruments.

Discussion

In the present paper, we demonstrated how untargeted models, originally developed to screen for adulterated liquid milk samples, can also be used to screen IRspectra for instrument instabilities. Our approach is based on the development of an FT-IR milk fingerprint that is unique to each instrument. When combined with change point algorithms, we have shown how instrument instabilities can be identified automatically and in real-time without requiring pilot milk samples. The spectral regions that our finger prints are based on contain chemically relevant information used by various prediction models. Instrument-related artifacts in these spectral regions can and occasionally did lead to biased predictions. Moreover, in the case of frequent short-term instabilities (in the range of minutes to hours) or strong drifts in the long-term (in range of months to years), additional maintenance measures could be considered.

In our admittedly small investigation, we have shown that instrument instabilities can be strong enough to bias predictions from commercial models of urea and free fatty acids in the milk. It should be noted, however, that instrument instabilities do not always have to affect model predictions and are certainly not the only factor affecting these predictions. Moreover, predictions of the chemical components such as fat, protein, and lactose are probably less susceptible to spectral deviations than models predicting parameters that are not directly present in the milk itself (e.g., fatty acids in the blood, pasture intake, or gas emissions). This is particularly problematic when these parameters are weakly correlated with the milk spectrum. In this case, prediction models are often more susceptible to spectral noise in general. It should also be noted that we could not relate the identified instrument instabilities to a particular cause. This is not surprising given that these instabilities were not detected or reported by the quality control sensors implemented in the instrument. Interestingly, the majority of temporary instabilities disappeared without human intervention. While individual episodes of instability may not always signal a need for maintenance, a change in the frequency of such episodes, however, deserves attention. Finally, it should be kept in mind that systematic changes in the spectra can be due to any change in the measurement conditions. These do not always have to be restricted to the FT-IR instrument but can also be due to changes in ambient temperature, humidity, sample pre-treatment, and even the physical properties of the milk sample itself.

When it comes to a practical application of our approach, the specific implementation may differ depending on the use case. When used as a quality control tool for monitoring model predictions, the change points in combination with the anomaly scores can be used to indicate whether an individual spectrum falls within an episode of instability or not. If it does fall in an episode of instability, one can compute the mean of the predictions before, during, and after the episode and compare them for statistical differences. When used as an additional quality control tool for instrument functioning, the detection of a change point in the anomaly scores can be used as a signal to have the respective instrument checked by the operator and, if needed, flagged for maintenance or repair.

List of references

Adams, R.P.and MacKay, D.J.C., 2007. Bayesian online change point detection. arXiv preprint arXiv: 0710.3742

Coppa, M., Martin, B., Hulin, S., Guillemin, J., Gauzentes, J.V., Pecou, A. and Andueza, D., 2020. Predictions of indicators of cow diet composition and authentication of feedings pecifications of protected designation of origin cheese using mid-infrared spectroscopy on milk. J. Dairy Sci., 104, 112-125. doi.: https://doi.org/10.3168/jds.2020-18468

Dórea, J.R.R., Rosa, G.J.M., Weld, K.A.and Armentano, L.E., 2018. Mining data from milkinfrared spectroscopy to improve feed intake predictions in lactating dairy cows. J. Dairy Sci.,101 (7),5878-5889. doi.: https://doi.org/10.3168/jds.2017-13997

Fleming, A., Schenkel, F.S., Chen, J., Malchiodi, F., Bonfatti, V., Ali., R.A., Mallard, B., Corredig, M. andMiglior, F., 2017. Prediction of milk fatty acid content with mid-infrared spectroscopy in Canadian dairy cattle using differently distributed model development sets. J. Dairy Sci., 100 (6), 5073-5081. doi.:https://doi.org/10.3168/jds.2016-12102

FOSS, 2014. Abnormal spectrum screening (ASM). Retrieved from:https://www.dairyfoods.com/ext/resources/White_Papers/WhitepaperDairyASM-US.pdf

Hansen, P.W. and Holroyd, S.E., 2019. Development and application of Fourier transform infrared spectroscopy for detection of milk adulteration in practice. International Journal of Dairy Technology, 72 (3), 321-331. doi.: 10.1111/1471-0307.12592

McParland. S., Banos, G., McCarthy, C., Lewis, E., Coffey, M.P., O'Neil, B., O'Donovan, M., Wall, E. and Berry, D.P., 2012. Validation of mid-infrared spectrometry in milk for predicting body energy status in Holstein-Friesian cows. J. Dairy Sci., 95 (12), 7225-7235. doi.: 10.3168/jds.2012-5406

Stocco, G., Cipolat-Gotet, C., Bonfattie, V., Schiavon, S., Bittante, G. and Cecchinato, A., 2016. Variations in major mineral contents of Mediterranean buffalo milk and application of Fourier-transform infrared spectroscopy for their prediction. J. Dairy Sci., 99, 8680-8686. doi.: https://doi.org/10.3168/jds.2016-11303

Vanlierde, A., Vanrobays, M.-L., Gengler, N., Dardenne, P., Froidmont, E., Soyeurt, H., McParland, S., Lewis, E., Deighton, M.H., Mathot, M. and Dehareng, F., 2016. Milk mid-infrared spectra enable prediction of lactation-state-dependent methane emissions of dairy cattle within routine population-scale milk recording schemes. Animal Production Science, 56, 258-264. doi.: http://dx.doi.org/10.1071/AN15590

Winning, H. 2015. Standardization of FT-IR instruments. Foss. Retrieved from: https://www.google.com/url?sa=tandrct=jandq=andesrc=sandsource=webandcd=andcad=rjaanduact=8andved=2ahUKEwji5IXX0L7wAhVJyaQKHXTeBI8QFjAAegQIAxADandurl=

https://www.fossanalytics.com/-/media/files/documents/papers/dairy-segment/standardization-of-ft-ir-instruments_gb.pdfandusg=AOvVaw2hu8dwViWhw6fAwVe47QO6

Beta-hydroxybutyrate and non-esterified fatty acids prediction using milk Fourier-transform infrared spectroscopy

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Abstract

Negative energy balance during the transition period predisposes dairy cattle to numerous metabolic conditions. Fourier-transform infrared spectroscopy (FTIR) presents one potential data source for estimating metabolic parameters used for the detection of cows suffering from these disorders. We created prediction models for blood BHB and blood NEFA using milk FTIR and production information from 622 milk samples subjected to FTIR measurements. The resulting R^2 for prediction of blood BHB was 0.5627 ± 0.2610(SD) and R^2 for blood NEFA prediction was 0.5093 ± 0.2473(SD). Balanced accuracies for detection of blood BHB greater than or equal to 1.2 mmol/L and for blood NEFA greater than or equal to 0.7 mmol/L were 83% and 73% respectively. Additionally, we predicted metabolic disorders in cows by applying the predicted blood BHB, predicted blood NEFA, and milk fat to protein quotient to an external dataset.

Keywords: Prediction modeling, metabolic disorders.

Introduction

Negative energy balance (NEB) following parturition predisposes dairy cattle to numerous metabolic disorders. While many detection methods for metabolic disorders exist, they suffer from poor accuracy, high costs, or intensive labor requirements. Fourier-Transform Infrared spectroscopy (FTIR) of milk may improve both understanding and detection of metabolic disorders, including those not detected by hyperketonemia alone. This prediction tool has the potential to allow for accurate prediction of biomarkers associated with negative energy balance, beta-hydroxybutyrate (BHB), and non-esterified fatty acids (NEFA), using a routine milk sample. The ability to predict multiple biomarkers from a single sample would allow better characterization of metabolic disorders in dairy cows.

Not all dairy cows experiencing adverse negative energy balance have elevated blood BHBA, the most common on-farm metric for evaluation. Ospina *et al*., 2013 reported that blood NEFA and blood BHB are not well correlated when measured on the same day. These affected cows are at risk of remaining undetected in an exclusively blood BHB focused testing strategy.

We used FTIR, milk production data, and ElasticNet regression to create prediction models for both blood BHB and blood NEFA. Using a combination of these we predicted the metabolic status of cows <60 DIM for an external dataset.

Materials and methods

Nine German dairy farms contributed data from December 2014 to December 2016 for this study. Farms visits occurred as often as once weekly and at least once monthly. Cows were dried off seven weeks before the expected calving date. Days in milk (DIM) targeted for sampling were between 0 and 60 days. Milk samples were taken between 8:00-10:00 AM using proportional milk samplers. A 10ml aliquot of milk was sampled and preserved using 1-2ml of bronopol (2-Bromo-2-nitropropane1,3-diol). Samples were transported at 4°C to regional milk testing facilities within one day of collection and analyzed using a MilkoScan FT-6000 (FossAnalytical A/S, Hillerød, Denmark). Milk FTIR absorbance variables were recorded for 1,060 wavenumbers.

A total of 622 observations from 478 cows had blood BHBA, blood NEFA, and milk FTIR samples collected. Blood samples were obtained following milking from randomly selected cows including milk samples from the same day.

Statistical analysis, variable selection, and editing

All statistical analyses were performed using R software version 3.6.3 (R Core Team (2020).). FTIR wavenumber variables were removed if greater than 15% of observations were missing values for a specific wavenumber. Wavenumber variables for values 900 –1,060 were removed as these 237 observations did not have these values recorded in the dataset. Following the removal of all observations with a missing value (n=134), the dataset contained 622 observations of 910 variables.

The IR spectra were transformed using the second derivative. The transformed spectra were reduced to 212 wavelength variables known to be informative (Grelet *et al.*, 2016). Model input was DIM, lactation group, milk production, and the 212 wavenumbers. Blood BHBA and blood NEFA were log-transformed after visualization using a histogram.

ElasticNet regression and classification for blood BHB and blood NEFA

The ElasticNet (ENET) is a regression method that combines ridge regression and lasso regression to apply regularization aimed at shrinking model coefficients and to reduce coefficient variance (Hoerl and Kennard, 1970; James *et al.*, 2013; Tibshirani, 1996). ENET is fit to the data using two parameters λ and α, and 7-fold cross-validation.

ENET in a logistic regression setting was also used to predict the binary outcome for above or below blood BHB and blood NEFA threshold values. The threshold value for elevated blood BHB was set at > 1.2 mmol/L and > 0.7 mmol/L for blood NEFA. In addition to the preprocessing described above, training data were balanced using Synthetic Minority Oversampling. The fitting of the ElasticNet classification model followed the same procedure as for the regression models.

External validation and metabolic disorder prediction

We evaluated our ENET regression models using an external dataset. The RMSE of predictions and Pearson's correlation coefficients between predicted and observed values were calculated. In addition to the prediction of metabolites independently, we predicted the overall metabolic status of the cow associated with the respective samples as either 'healthy' or risk for 'metabolic disorder'. Cows were identified as metabolically disordered if predicted blood BHB was greater than or equal to1.2 mmol/L, blood NEFA greater than or equal to 0.7 mmol/L, or fat-protein-quotient (FPQ) greater than or equal to 1.4. Predicted metabolic status was then compared to true metabolic status as determined by measured blood BHB and blood NEFA resulting in a confusion matrix from which diagnostic test parameters were derived.

Results and discussion

Descriptive statistics

Cows were sampled from 1 to 3 times. The median number of samples per farm was 55 (range: 2-180). A total of 152 cows were in their first lactation and 470 cows were in their second or greater lactation (median=3, range 1-10). A total of 563 Holstein and 59 Flekvieh samples were collected.

ENET for prediction of blood BHB and blood NEFA

Cross-validated R^2 values for blood BHB 0.5627 ± 0.2610(SD) and RMSE of 0.3873 $\underline{+}$ 0.1380(SD). The blood BHB classification had a balanced accuracy of 87% (80% – 91%), sensitivity of 90% (81% – 96%) and a specificity of 83% (80% – 86%). PPV and NPV were 43% (35% - 51%) and 98% (97% - 98%) respectively. Blood NEFA prediction RMSE was 0.4825 $\underline{+}$ 0.9260(SD) and R^2 was 0.5093 $\underline{+}$ 0.2473(SD). The balanced accuracy of blood NEFA predictions was 73% (68% – 79). These results are comparable to recent publications by Pralle *et al.*, 2018, who reported sensitivities of 76 to 81% and specificities of 71% to 81% for prediction of blood BHB levels greater than 1.2 mmol/L. The blood NEFA prediction results are similar to Luke *et al.*, 2019 and Tremblay *et al.*, 2019 who reported a sensitivity of 73%, and 77% respectively. These results show ENET is a good modeling algorithm for the prediction of blood BHB and blood NEFA.

External validation and metabolic disorder prediction

Validation of our ENET predictive model was performed using the Qcheck dataset with n=9660 observations to account for any overfitting bias. The resulting RMSE was 0.4018 (95% CI 0.3958 - 0.4082) for log-transformed blood BHB and 0.4043 (95% CI 0.3937 - 0.4159) for log-transformed blood NEFA prediction. Figures 1 and 2 display observed values compared to predicted values.

True observed blood BHB and true blood NEFA had a Pearson's correlation coefficient of 0.31 (P>0.001) while predicted blood BHB and predicted blood NEFA had a correlation coefficient of 0.69 (P>0.001). This increased correlation between predicted values of NEFA and BHB may be due to our dataset size.

Prediction accuracy for metabolic disorder classification using predicted blood BHB, predicted blood NEFA, and milk FPQ was 94% (9081 /9660). Sensitivity was 94% (3354 / 3542) and specificity was 94% (5727 / 6118). By using a combination of blood BHB, blood NEFA, and FPQ, this model enables the identification of cows suffering from hyperketonemia in addition to those with normal blood BHB levels suffering from the consequences of negative energy balance. By identifying these at-risk cows, we can direct necessary medical care to them before they lose massive amounts of body weight and develop more severe conditions resulting in death.

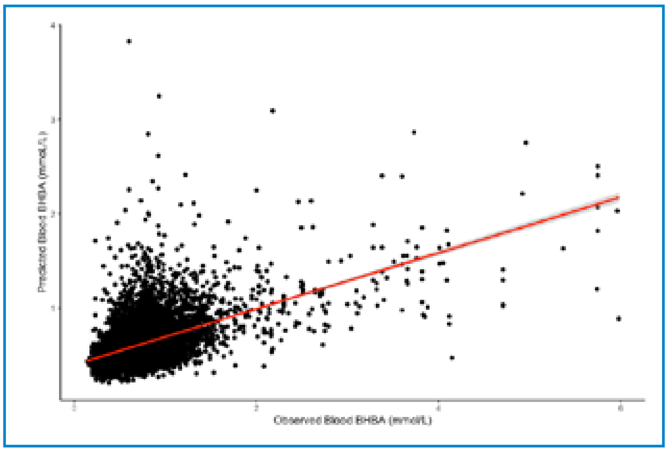

Figure 1. Observed versus predicted values of blood BHB for ElasticNet regression (n=9,660) using milk Fourier-transformed infrared spectroscopy and production variables (milk yield, DIM, lactation group).

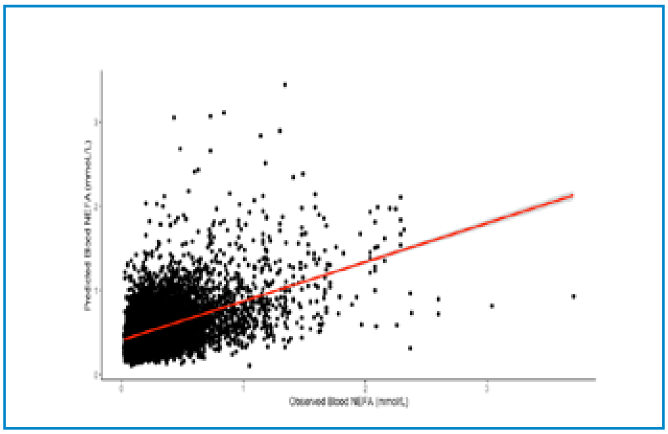

Figure 2. Observed versus predicted values of blood NEFA for ElasticNet regression (n=9,660) using milk Fourier-transformed infrared spectroscopy and production variables (milk yield, DIM, lactation group).

Conclusion

Metabolic disorders during the transition period remain a primary issue of concern for dairy cows. Milk FTIR measurement is one source of data for the detection of cows suffering from metabolic disorders with the benefits of obtaining multiple biomarkers from a single milk sample. Prediction of metabolic disorders using FTIR data can be accomplished by combining the predictions into a categorization into cows with and without metabolic disorders. This categorization results in rapid identification of these high-risk cows post-calving. Continued research into prediction modeling with regards to methods and input is warranted.

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List of references

Grelet, C., Bastin, C., Gelé, M., Davière, J.-B., Johan, M., Werner, A., Reding, R., Fernandez Pierna, J.A., Colinet, F.G., Dardenne, P., Gengler, N., Soyeurt, H., Dehareng, F., 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, ^{2}-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. J. Dairy Sci. 99, 4816–4825. *https://doi.org/10.3168/jds.2015-10477*

Hoerl, A.E., Kennard, R.W., 1970. Ridge Regression: Biased Estimation for Nonorthogonal Problems. null 12, 55–67. *https://doi.org/10.1080/00401706.1970.10488634*

James, G., Witten, D., Hastie, T., Tibshirani, R., 2013. An Introduction to Statistical Learning, Springer Texts in Statistics. Springer New York, New York, NY. *https://doi.org/10.1007/978-1-4614-7138-7*

Luke, T.D.W., Rochfort, S., Wales, W.J., Bonfatti, V., Marett, L., Pryce, J.E., 2019. Metabolic profiling of early-lactation dairy cows using milk mid-infrared spectra. Journal of Dairy Science 102, 1747–1760. *https://doi.org/10.3168/jds.2018-15103*

Ospina, P.A., McArt, J.A., Overton, T.R., Stokol, T., Nydam, D.V., 2013. Using Nonesterified Fatty Acids and ^{2}-Hydroxybutyrate Concentrations During the Transition Period for Herd-Level Monitoring of Increased Risk of Disease and Decreased Reproductive and Milking Performance. Veterinary Clinics of North America: Food Animal Practice, Metabolic Diseases of Dairy Cattle 29, 387–412. *https://doi.org/10.1016/j.cvfa.2013.04.003*

Pralle, R.S., Weigel, K.W., White, H.M., 2018. Predicting blood beta-hydroxybutyrate using milk Fourier transform infrared spectrum, milk composition, and producer-reported variables with multiple linear regression, partial least squares regression, and artificial neural network. Journal of Dairy Science 101, 4378–4387. *https://doi.org/10.3168/jds.2017-14076*

Tibshirani, R., 1996. Regression Shrinkage and Selection via the Lasso. Journal of the Royal Statistical Society. Series B (Methodological) 58, 267–288.

Tremblay, M., Kammer, M., Lange, H., Plattner, S., Baumgartner, C., Stegeman, J.A., Duda, J., Mansfeld, R., Döpfer, D., 2019. Prediction model optimization using full model selection with regression trees demonstrated with FTIR data from bovine milk. Preventive Veterinary Medicine 163, 14–23. *https://doi.org/10.1016/j.prevetmed.2018.12.012*

Implementation of the new certified reference material for somatic cell counting in milk

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Abstract

This study is sought to provide an overview on the implementation of the new certified reference material for somatic cell counting in milk, which was launched in 2020.

Milk somatic cell count (SCC) is a widely used indicator for monitoring the udder health of several mammalian species and is relevant in food quality regulations, milk payment testing, farm management and breeding programmes. Joint efforts of International Dairy Federation (IDF), the International Committee for Animal Recording (ICAR) and EC JRC resulted in the development and release of a new certified reference material. The availability of this new material allows better global equivalence in somatic cell counting in milk, which is a challenge today.

The newly available reference material has been tested in numerous countries around the globe. In some cases, an adjustment/re-anchoring of current SCC level would not be necessary, whereas it would be in others. Examples for both scenarios including evaluation of the impact of the transition on the results will be demonstrated. Suggestions for handling of possible transition issues in case of re-anchoring the current SCC level will be discussed. Further details and examples will be given on other possible applications of the certified reference material such as verification/adjustment of calibration settings of routine methods, assigning reference values to secondary reference materials, and usage in proficiency tests.

In conclusion, seeking global equivalence in somatic cell counting first countries already re-anchored their SCC level with more countries/laboratories to follow. These real-life examples are highly valuable to further promote the usage of the new reference material and help to establish procedures for its application.

Keywords: SCC, reference material, equivalence

Introduction

Somatic cell count (SCC) in milk is a widely used analysis and the number of tests done worldwide is estimated to be >500.000.000 per year. SCC test results from individual cow milk samples are used for udder health monitoring and management and breeding purposes. Beyond that, SCC result from bulk tank milk samples are relevant in food quality regulations and milk payment. A challenge in the industry is different SCC levels around the world as can be seen based on, for example, ICAR Proficiency Test results. These differences, in turn, lead to challenges in terms of trading dairy products.

New certified reference material for Somatic Cell Counting

A joint project team of experts from the International Dairy Federation (IDF) and ICAR together with the European Commission Joint Research Centre have developed a new certified reference material for somatic cell counting. The official name of the material is “EC JRC CRM® ERM-BD001“ and it can be ordered here: https://crm.jrc.ec.europa.eu/p/ERM-BD001

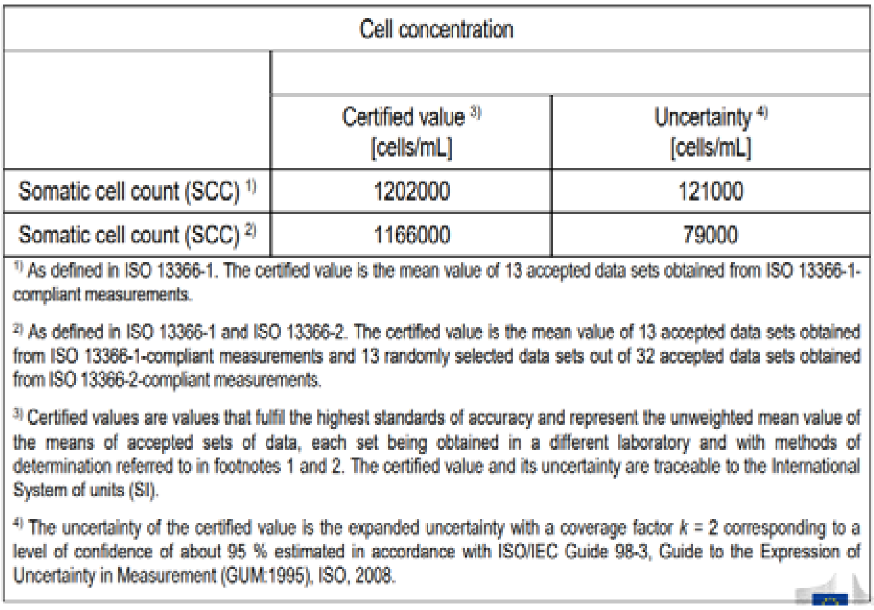
The material consists of two samples. One sample has a low SCC of about 50,000 cells/mL and the second one has a high SCC of about 1,000,000 cells/mL (Figure 1). The samples are produced based on bulk tank cow milk. Milk cells are preserved through spray drying and samples are afterwards homogenized, bottled, and labelled.

The two samples are produced from raw bulk cow’s milk which has been powdered by spray drying at NIZO in the Netherlands. The homogenization of the samples, bottling and labelling was performed by the Joint Research Centre in Belgium.

Application of the new primary reference material

The primary reference material for somatic cell counting can be applied in different ways in laboratories. When reference and/or routine methods are operational at your laboratory the primary reference material can be used to check the performance of the methods. This way the correctness of the two methods can be verified and when necessary adjusted.

Another way to apply the primary reference material could be to check on the calibration settings of the routine methods. When the routine method is calibrated with the primary reference material, the obtained results will be traceable to the reference results.



	Cell concentration	
	Certified value [3] [cells/mL]	Uncertainty [4] [cells/mL]
Somatic cell count (SCC) [1]	1202000	121000
Somatic cell count (SCC) [2]	1166000	79000

[1] As defined in ISO 13366-1. The certified value is the mean value of 13 accepted data sets obtained from ISO 13366-1-compliant measurements.

[2] As defined in ISO 13366-1 and ISO 13366-2. The certified value is the mean value of 13 accepted data sets obtained from ISO 13366-1-compliant measurements and 13 randomly selected data sets out of 32 accepted data sets obtained from ISO 13366-2-compliant measurements.

[3] Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and with methods of determination referred to in footnotes 1 and 2. The certified value and its uncertainty are traceable to the International System of units (SI).

[4] The uncertainty of the certified value is the expanded uncertainty with a coverage factor k = 2 corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

Figure 1. Somatic cell count levels of the two samples included in the EC JRC CRM ERM-BD001 as illustrated on the Certificate of Analysis.

Another application of the primary reference material is to assign values to secondary reference materials. In practice, the routine instruments for somatic cell counting are often calibrated by using secondary reference materials produces by difference providers. When the values assigned to these secondary reference materials are based on primary reference material, the alignment of the results, consecutively obtained with the routine methods in different laboratories will be ensured.

Last, the primary reference can be used in a proficiency test, where the results obtained at the same time from different laboratories and with difference methods are compared. Including the primary reference materials in proficiency testing schemes allows for a proper comparison of the methods and the laboratories performance.

A webinar entitled “Development and application of a certified reference material for somatic cell counting in milk” to introduce the reference material and elaborate on its application was conducted in December 2020. A recorded version of the full webinar and pdf-copies of the presentations are available here: https://www.icar.org/index.php/technical-bodies/sub-committees/milk-analysis-sub-committee-landing-page/webinar-3-december-2020-on-development-and-application-of-a-certified-reference-material-for-somatic-cell-counting-in-milk/

The new reference material including examples on its application are also described in detail in the recently published IDF Bulletin 508/2021, which is available for free download here: https://store.fil-idf.org/product/bulletin-of-the-idf-n-508-2021-guidance-on-application-of-ec-jrc-certified-reference-material-for-somatic-cell-counting-in-milk/

Status on implementation of the new certified reference material around the world

We conducted a small survey to learn more about the actual status on the implementation of the new SCC certified reference material and observed that different countries are mainly in four different phases of the implementation:

1. Material tested and adopted - in Lithuania and Switzerland.
2. Material not tested so far – In countries such as China and Chile the new material has not yet been tested so far. Among other reasons, the shipment of the SCC reference material was not possible due to COVID 19 restrictions.
3. Material tested, no need for adjustment of SCC level – The new primary SCC reference material has been tested and it was observed that the current SCC level and the SCC level of the primary reference material are well in alignment. Thus, it was concluded that no adjustment of SCC counting levels is needed. Nevertheless, the new primary SCC material is considered a valuable product because it opens up the possibility to monitor SCC counting levels on a regular basis (e.g. once every quarter) and verify correctness. This situation applies to many countries around the world, e.g. Denmark, Germany, Italy, Japan, New Zealand, UK, USA. A dialogue on using the new primary SCC reference material as an official and mandatory material has been initiated with the respective authorities in Japan and USA. Below a case report for this implementation phase coming from Germany.
4. Material tested, need for adjustment of SCC level – The test of the new primary SCC reference material revealed that the current SCC level requires some adjustment. Following this finding, the respective laboratories started to initiate the dialogue with their stakeholders and agreed upon a strategy for transition of SCC levels. Regular application of the new SCC reference material is seen as highly valuable in the transition period and thereafter. This situation applies to, e.g. Canada, France, Israel, and the Netherlands and is further described in below case report from the Netherlands.

It is common practise in most milk testing laboratories around the globe to work with secondary SCC reference material. In this context, we would like to recommend that secondary SCC reference materials are to be checked for alignment with the new primary reference material and that customers of secondary reference materials ask their providers for such alignment checks.

Conclusions

Equivalence in SCC levels around the world has been a challenge for many years. A joint IDF/ICAR project team developed and launched a new primary reference material for somatic cell counting. The application of this material can help to obtain global equivalence in somatic cell counting.

How can cow-individual sensor data, national data and drone images improve our understanding of resilience

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Abstract

Resilient dairy cows can be characterized by completing multiple lactations, with good (re)productive performances, facing no or few health problems that they overcome easily, and that are efficient and consistent in milk production. Improving resilience has clear advantages, but phenotypic information on this trait is lacking. We explored whether we can fill this gap with information from sensor technologies as these offer high-frequency, continuous, and longitudinal data of individual cows. We did this in three studies: the first study developed cow-specific resilience proxies using milk yield sensors, and correlating these proxies to national data to study herd factors impacting resilience. The natural logarithm transformed variance (LnVAR**)** in daily milk yield appeared an interesting cow-individual resilience proxy: a low LnVAR was genetically correlated to better udder and hoof health, better longevity and fertility, a higher body condition score, and lower ketosis and milk yield. A low LnVar, thus, represents a cow with a good resilience. Subsequently, herd-year effects for LnVar were estimated and correlated with herd performance parameters derived from the national milk recording system. This revealed large differences in resilience between herd-years: the LnVar in the herd-year with the largest effect was >6 times larger than the LnVar in the herd-year with the smallest effect. The positive correlation with the proportion of cows with a rumen acidosis indication (r = 0.31) suggested that feed management may have an important effect on resilience. The second study used sensor data collected during the first lactation to predict a lifetime resilience score using logistic regression or random forest. Both methods had a similar classification performance (accuracy 45-50%). However, random forest required much less data pre-processing to get to this performance. This makes random forest an attractive method to derive information from sensor data, particularly when input becomes even more complex with new sensor technologies entering the market. One of these new technologies could be camera-mounted drones, which were explored in the third study. These drones were used during several field trials, and artificial intelligence was used to detect, locate and identify cows, and obtain specific cow characteristics (height, volume, weight) from the images. Accuracies of >95% for detecting cows, ~91% for identifying cows, and ~88% for obtaining cow characteristics (lying, standing or grazing) were achieved. This makes camera-mounted drones a promising new technology in monitoring traits that can be used for resilience assessment.

Keywords: Resilience, proxies, prediction, sensor technologies.

Introduction

Resilience is the ability of animals to be minimally affected by environmental disturbances, or to quickly recover from them (Colditz *et al.*, 2016). In that perspective, resilient dairy cows can be characterized by completing multiple lactations, with good (re)productive performances, facing no or few health problems that they overcome easily, and that are efficient and consistent in milk production (Adriaens *et al.*, 2020). Improving resilience has clear advantages, e.g., improved animal health and welfare (Mulder and Rashidi, 2017) and reduced antibiotic usage (König and May, 2019). Despite the importance of resilience is clear in itself, breeding or managing for resilience is hampered due to the complexity of the trait and the lack of phenotypic information.

During the past decade, there are fast developments in sensor technologies that are increasingly being adopted on farms. These technologies primarily aim at monitoring specific traits (e.g., milk production), or at facilitating management-by-exception by detecting specific events that require farmer attention, e.g., heat or clinical mastitis. These sensors generate high-frequency, longitudinal and continuous time-series of data of individual cows, and it is this specific characteristic that makes sensors also interesting for phenotyping complex traits such as resilience. More recently, vision and image technologies are increasingly used to retrieve information on, e.g., cow posture for lameness detection (Van Hertem*et al.*, 2018), or for identification of Holstein cattle (Bhole*et al.*, 2019). Both technologies require specific approaches to retrieve the relevant information. For example, regression analyses can be used to analyse the sensor technologies that produce structured data, but this methodology also requires pre-processing to transform raw sensor data into information that can be used by the method itself. Machine learning approaches are increasingly being used (Lokhorst*et al.*, 2019) in the domain of precision dairy farming, but not yet to study resilience. The vision or image technologies require artificial intelligence (deep learning) approaches to retrieve relevant information from the unstructured data. But the feasibility of vision technologies to study resilience has not been explored yet. The usefulness of sensor data to develop cow-specific proxies for resilience has been explored in the current study. We also explored the differences between regression analysis and machine learning (random forest) in predicting lifetime resilience score (as described in Adriaens *et al.*, 2020) using sensor data collected during the first lactation. Finally, we explored the feasibility of camera-mounted drones, in combination with deep learning, to retrieve information of interest to assess resilience in an outdoor situation.

This paper summarizes the main results and lessons we have learned in our attempt to utilize new and commercially available sensors, in combination with national data, in our understanding of resilience.

Developing cow-specific resilience proxies

Daily milk yield recordings were explored for the development of a cow-individual sensor-based proxy for resilience. We used data provided by Cooperation CRV and CRV BV (Arnhem, the Netherlands)from198,745 first-parity Dutch Holstein Frisian cows milked by automatic milking systems. Three steps were subsequently taken: first, individual lactation curves were fitted, using daily milk yield and 4th order 0.7 quantile regression curves, that reflected production potential in absence of disturbances. Second, the natural transformed variance (**LnVar**) of the deviations between measured milk yield and the fitted curve were computed, with the expectation that resilient cows have a smaller range of deviations from their fitted curve, resulting in a lower value for LnVar. Third, LnVar was genetically correlated with health, longevity, fertility, metabolic and production traits. This demonstrated that low LnVar was indeed genetically correlated to better udder and hoof health, better longevity and fertility, a higher body condition score, and lower ketosis at the same level of milk yield. This made LnVar a good cow-individual sensor-based proxy for resilience, which could be used for breeding purposes (Poppe *et al.*, 2020).However, we expected herd management to have an effect on this cow-individual resilience. Therefore, herd-year estimates of the LnVar were assessed for 9,917 herd-year classes based on records of 227,655 primiparous cows from 2,644 herds from the years 2011-2017, while corrected for genetics and year-season effects. These herd-year estimates were considered a herd resilience indicator, expecting that herd-year classes with a low estimate for LnVar contain cows that have, on average, a consistent daily milk yield and are, thus, resilient. Large differences in herd-year estimates were observed (mean 1.34, range 0.38-2.56). Moreover, herd-year estimates of the same herds between years were positively correlated: if a herd had a high LnVar in a certain year, this same herd tended to have high LnVars in other years too. This indicated an effect of management on this trait, and thus, herd-year estimates were related to herd performance parameters derived from the national milk recording system. Herd-year classes with a high LnVar estimate tended to have a high proportion of cows with a rumen acidosis indication (r = 0.31), high somatic cell score (r = 0.19), low fat content (r = -0.18), long calving interval (r = 0.14), low survival to second lactation (r = -0.13), large herd size (r = 0.12), low lactose content (r = -0.12), and high milk production (r = 0.10). These correlations supported the hypothesis that herds with a high LnVar estimate are not resilient. The high correlation with rumen acidosis indication suggests that feed management may play an important role in resilience (Poppe *et al.*, 2021).

Predicting lifetime resilience using sensor data and machine learning

The fluctuations in daily milk production were useful as a proxy for breeding for resilience (Poppe *et al.*, 2020). However, there are more sensor technologies available on farm. This provides the opportunity to combine data from different sensors that are collected early in life to predict lifetime resilience. Adriaens *et al.* (2020) reported on an approach to compute a lifetime resilience score, and they used sensor information to predict this score. They reported that adding features based on activity sensor data improved prediction accuracies significantly ($P < 0.01$), compared to predictions based on daily milk features alone. Poppe *et al.* (2020) and Adriaens *et al.* (2020) have in common that they pre-process the sensor data to retrieve biologically meaningful features to be included in their regression models. However, machine learning approaches, as random forest, are reported to find (non)linear relationships between variables, while requiring little effort for pre-processing (Touw*et al.*, 2013). We studied whether random forests can predict lifetime resilience scores, and whether these algorithms require less pre-processing efforts than a more traditional method like logistic regression. Data for this study originated from Dairy Campus (Lelystad, the Netherlands), and included data of 370 dairy cows that had daily sensor data available for activity, milk yield, rumination, and weight, for at least 100 days during 1-300 DIM of their first lactation. We followed the same approach as Adriaens *et al.* (2020) to compute lifetime resilience scores, and divided the cows into three evenly distributed groups (High, Medium, Low lifetime resilience). Also following Adriaens *et al.* (2020), we aggregated sensor data to daily measurements. We derived 14 features from these daily measurements for each of the four sensor time series, totalling to 56 sensor features. These 56 features were used to develop a stepwise ordinal logistic regression model (considered as reference). We also developed three random forest models: one that used the same features that were deemed significant in the ordinal regression analysis, and one that used all 56 sensor features. The third random forest used the aggregated daily sensor measurements and lactation averages as predictive features.

Table 1. Accuracy of classifying cows for lifetime resilience score.

Model	Accuracy	Range
Logistic Regression	45.1	8.1
Random Forest		
6 features derived from sensor measurements	45.7	8.4
56 features derived from sensor measurements	51.2	10.9
Daily measurements and lactation averages	50.5	6.3

Table 2. Number of cows used in field studies, and performance (in terms of precision) of deep learning models to detect, identify, and characterize poses, height and weight.

	Carus			Juchowo Farm
	2018	2019	2020	2019
Number of cows	4	6	16	100
Performance				
Detecting cows (%)	95.0	96.2		97.3 shaded 99.9 unshaded
Identifying cows (%)	87.6	91.3		--
Characterization of poses (%)	88.7			
Height and Weight (Mean error)		6cm[1]	31kg[2]	

[1]Mean error in height with LIDAR; [2]Mean error with RBG, after removing two extreme outliers.

All models were validated using 10fold cross validation, and the accuracy was used as performance evaluation metric. The accuracy was 45.1% for the logistic regression. The random forest that used the same six features resulted in an accuracy of 45.7%. Using all 56 pre-processed features in a random forest had an accuracy of 51.2%. Lastly, the random forest that used aggregated daily values and lactation averages had an accuracy of 50.5% (Table 1). We concluded that a random forest can reach similar performance as logistic regression, even with less pre-processing efforts (Ouweltjes *et al.*, 2021).

Camera-mounted drones to obtain cow characteristics for resilience

Unmanned Airborne Systems, better known as drones, are an example of a new technology that might be helpful in monitoring cows that are outdoors and further away from the farm, e.g., in rangeland beef production systems. This study aimed specifically at assessing the feasibility of fixed wing and multirotor camera-mounted drones to identify, locate, and retrieve characteristics of cattle that can be of relevance for resilience. Four field studies were conducted on two research farms (Carus, the Netherlands; Juchowo biological farm, Poland) to collect images and video footage by flying the mounted drone over herds on pasture. Material was annotated using a graphical annotation tool called Label Img which uses label object bounding boxes in images. Annotation was done such that analyses could determine whether (1) cows can be detected in the field, (2) individual cows can be identified, (3) cow characteristics can be classified, (4) height and weight can be derived. Analyses were done through deep learning API Nanonets and AgisoftMetshape for imagery, and Yolo for video footages. Results are promising for detection, identification and characterization (Table 2). Detecting cows in the field reached accuracies >95%, where detecting cows in fields without shade reached higher accuracies (99.9%) than in shaded fields (97.3%). Identifying cows reached an accuracy of 91%. This was reached for a small group of animals, each having a distinct coat pattern. Identification of cows that will have the same uniform colour will be challenging with the techniques explored. Characterization of cows into standing, grazing and lying the analyses revealed no difficulties in separating grazing from lying, but separating grazing and standing is challenging. The mean error in estimating height was 6cm with LiDAR, and in estimation of weight 31kg with RGB 3D after removing two extreme outliers. These results suggest that camera-mounted drones could be a promising new technology in monitoring traits that can be used for resilience assessment.

Conclusions

The natural logarithm transformed variance of daily milk is a good proxy for resilience, as a low value of this proxy is genetically correlated to better udder and hoof health, better longevity and fertility, a higher body condition score, and lower ketosis and milk yield. There were large differences in resilience between herds. Correlating with herd-level data from the national milk recording systems revealed a positive correlation with the proportion of cows having a ketosis indication. This indicates that feed management may have an important effect on resilience. Using on-farm sensor data to predict lifetime resilience resulted in similar performance accuracies between regression and random forest. However, the random forest reached this performance with hardly any data pre-processing, in contrast to regression analysis. Finally, camera-mounted drones are a promising approach to locate cows and retrieve cow characteristics that can be related to resilience.

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List of references

Adriaens, I., N.C. Friggens, W. Ouweltjes, H. Scott, B. Aernouts, and J. Statham. 2020. Productive life span and resilience rank can be predicted from on-farm first-parity sensor time series but not using a common equation across farms. J. Dairy Sci. 103: 7155-7171.

Bohle, A., O. Falzon, and G. Azzopardi. 2019. A computer vision pipeline that uses thermal and RGB images for the recognition of Holstein cattle. In: Proceedings of the International Conference on Computer Analysis of images and patterns. Vento M., and G. Percanella (eds). CAIP 2019, LNCS 11679, p 108-119.

Colditz, I.G., and B.C. Hine. 2016. Resilience in farm animals: Biology, management, breeding and implications for animal welfare. Anim. Prod. Sci. 56: 1961-1983.

Lokhorst, C., R. de Mol, and C. Kamphuis. 2019. Invited review: Big Data in precision dairy farming. Animal. 13: 1519-1528.

König, S., and K. May. 2019. Invited review: Phenotyping strategies and quantitative-genetic background of resistance, tolerance and resilience associated traits in dairy cattle. Animal 13:897-908.

Mulder, H., and H. Rashidi. 2017. Selection on resilience improves disease resistance and tolerance to infections. J. Anim. Sci. 95: 3346-3358.

Ouweltjes, W., M. Spoelstra, D. Ducro, Y. de Haas, and C. Kamphuis. 2021. A data-driven prediction of lifetime resilience of dairy cows using commercial sensor data collected during first lactation. *Under review.*

Poppe, M., R.F. Veerkamp, M.L. van Pelt, H.A. Mulder. 2020. Exploration of variance, autocorrelation, and skewness of deviations from lactation curves as resilience indicators for breeding. J. Dairy Sci. 103: 1667-1684

Poppe, M., H.A. Mulder, C. Kamphuis, and R.F. Veerkamp. 2021. Between-herd variation in and relations to herd performance. J. Dairy Sci. 104: 616-617

Touw, W. G., J.R. Bayjanov, L. Overmars, L. Backus, J. Boekhorst, M. Wels, and S.A. van Hijum. 2013. Data mining in the Life Sciences with Random Forest: a walk in the park or lost in the jungle? Briefings in bioinformatics. 14: 315-326.

Van Hertem, T., A. Schlageter Tello, S. Viazzi, M. Steensels, C. Bahr, C.E. Bites Romanini, K. Lokhorst, E. Maltz, I. Halachmi, and D. Berckmans. 2018. Implementation of an automatic 3D vision monitor for dairy cow locomotion in a commercial farm. Biosystems Engineering. 173: 166-175

Dairy cows enabling circular production systems

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Abstract

The transition towards circular agriculture is about careful management of soil, feed, animals, biodiversity and money, to achieve a near-to-closed-loop system of resources. A tailored breeding approach is an essential building block, to ensure cows' health, welfare and production in new farming systems related to resource availability. In this study, we defined circular production systems and related cow traits and compared the performance of two types of cows on a dairy farm in the Netherlands that strives to be circular. To define characteristics of circular dairy production systems and breeding goal traits required to transition towards circular production, a workshop was organized with multidisciplinary experts. In this workshop, nine characteristics of circular dairy production systems were defined: flexible, cooperative, efficient without losses, healthy cows, low input without concentrates, extensive nature and landscape, multipurpose, pasture based, and closed. Connecting to these characteristics, cow traits were prioritized which fitted to one or more types of circular dairy production systems, for example, roughage efficiency, grazing behaviour, coping with dietary fluctuations, and environmental footprint. A quick scan was performed at research farm "Knowledge Transfer Centre De Marke", where innovative measures are designed and tested to minimize nutrient losses to work towards circular farming. The performance and variation in breeding goal traits of two types of cattle was investigated. Half of the in total ninety dairy cows at "De Marke" is of the Holstein Friesian breed, the other half is a three-breed rotational cross (Holstein Friesian, Montbéliarde, and Scandinavian Red cattle), which are managed together. Preliminary analysis on production and reproduction traits indicate that both types of cattle perform well within this extensive farming system. In recent years, the variation within breeding goal traits for Holstein Friesian AI bulls greatly increased and in combination with the implication of genomic selection, this helped to improve longevity and fertility traits. The quick scan highlights the importance of a large pool of genetic variation within or between cattle breeds. This variation will be essential for breeding programs when cows have to perform in a different environment, as will be the case when transitioning towards circular dairy farming.

Keywords: Dairy cows, circular farming, genetic diversity, animal breeding.

Introduction

To reduce the impact of agriculture on climate change, the ministry in the Netherlands calls for a transition to circular farming by 2030 (Rijksoverheid, 2018). In this transition there is an aim to achieve a near-to-closed loop system of resources involving the nutrient cycle between livestock, manure, land and crops (Figure 1). This transition may impact the resources available to dairy cows, which can have an impact on health, welfare and production. Animal breeding can support the transition towards circular farming, where we make use of the natural variation between cows, to select cows that perform well and are easy to manage in new farming systems. Breeding is a process of which the impact is observed after multiple generations, therefore it is important to think ahead when defining breeding goals.

The current selection index for the Dutch national breeding goal for dairy cattle consists of fifteen characteristics for the following categories: milk production, milk components, feed efficiency, health, reproduction, longevity and conformation (CRV, 2018). The selection index is the sum of weighted breeding values, whereby weighting is currently mainly determined on the basis of economic value and desired gains. To enable the circularity of dairy farming, the environmental impact can be considered as changing the desired gain and reweighing the index traits. Also, new traits can be added to if they are clearly described, measurable on a large enough scale for an affordable price, have phenotypic variation, are heritable, and have limited adverse genetic correlations with other breeding goal traits. These factors have to be investigated before a trait can be applied to breeding practices. Furthermore, common traits for which breeding values exist, may have to be re-evaluated in an environment that differs from conventional Dutch dairy farms.

The aim of our study was to investigate which cow traits can be used in breeding programs to ensure the health, welfare and production of dairy cows in new farming systems related to resource availability. First, we defined characteristics of a circular dairy farm and corresponding cow traits. Second, we performed a case study on research farm “De Marke”, a dairy farm where innovative measures are designed and tested to minimize nutrient losses. Here we investigated the effect of resource availability on two types of dairy cows: Holsteins and three-way-crosses.

Figure 1. The nutrient cycle between livestock, manure, land, and crops.

Material and methods

A workshop with fourteen multidisciplinary experts (with expertise on dairy farming, animal breeding and genetics, animal production systems, the effect of livestock on the environment, nature inclusive dairy farming, and plant systems) was organised to define characteristics of circular dairy production systems. The system definitions give a framework of the environment under which a dairy cow should perform, and is the first step before a new breeding goal can be defined (Oldenbroek & Van Der Waaij, 2015). Based on the system definitions, cow traits were defined relating to one or more of definitions of circular dairy production systems. Traits can be either new or existing traits that may have to be re-evaluated in a new farming system.

A case study was performed on data from research farm “De Marke”, a dairy farm where innovative measures are designed and tested to minimize nutrient losses, where we investigated the effect of resource availability on the production and reproduction of two types of dairy cows: Holsteins and three-way-crosses. The crosses were a three-breed rotational cross, between Holstein, Viking Red and Montbéliarde cattle, for which crossbreeding started in 2010. The analysis included 446 lactations between 2014 and 2019 from 187 cows. A correction was applied for parity number and calving season.

Results and discussion

In the workshop with multidisciplinary experts nine characteristics for circular dairy farms were defined. We came to the conclusion that there will likely not be one circular system that fits all, but tailor-made solutions for individual farms. The characteristics of circular dairy farms were: flexible, cooperative, efficient with minimal losses, healthy cows, low input, extensive with the focus on nature and landscape, extensive which is pasture based, multipurpose, and closed housing.

Flexibility is important when the input of nutrients becomes variable in circular systems where, for example, a farm strives to use regionally produced feed. Similarly, when the feeding of substrates is reduced the cow becomes more dependent on the quality or quantity of roughage and grass. In such a system cows will need to be resilient and produce milk efficiently from a roughage based diet. Circular farms can also aim to be cooperative, where cows are used to process losses of regional arable farmers (Van Zanten, 2016) which can cause variability in the feed available. To be able to process a variable diet, cows need a healthy rumen. An important characteristic applicable to all circular farms is to minimize the losses of nutrients, both on the farm, crop and animal level. On cow level there is interest in monitoring the losses of nitrogen, phosphate, and potassium, because of their negative environmental impact when oversupplied. Similarly, the reduction of greenhouse gasses emitted directly into the air, such as methane, offers potential to reduce the environmental impact of both circular and conventional dairy farming (van Bruggen et al., 2019). It is important to monitor the wellbeing of cows when the farming systems changes, as healthy cows make a farm easier to manage for a farmer. Furthermore, good health contributes to the longevity of a cow, which reduces the number of replacements to be reared and helps to increase both sustainability as well as profitability of the sector (Van Pelt, 2017). A characteristic that was highlighted before is low input. Currently, many soy-based substrates are fed to livestock which can be used as food for humans (Dei, 2011). A grass-based diet reduces the feed-food competition between humans and livestock. To improve a cows’ production from a grass-based diet, it can be of interest to study the grazing behaviour in the future. This grass-based diet fits into the view of extensive farming systems. Which can either be pasture based, with pastures with highly nutritional grass types, or adapted to maintain nature and landscapes, where the diversity of plants and animals (e.g., insects and meadow birds) is a priority. Another characteristic of a circular farm can be to keep cows for multiple purposes. A high quality and quantity of meat, after the milk-productive lifetime of a cow, can reduce the environmental impact per kg of product. Furthermore, it may be of interest to investigate the manure composition of cows, to be used as a qualitatively good fertilizer in arable farming. A final system definition can be closed housing, which to many does not correspond with circular farming. However, closed housing offers potential to minimize to spilling of nutrients into the environment by using, for example, air washers.

Many cow traits can be applied in various types of systems, and all contribute to the reduction of the environmental impact of dairy farming in circular and conventional farming systems. Some traits already exist but may have to be re-evaluated in a new farming system. Some traits are new and should be investigated before they can be applied in breeding programs. The cow traits that are important for a dairy cow to perform well in circular farming systems, and traits that help to reduce the environmental impact of dairy farming, that we defined are: longevity, health, claw health, udder health, rumen health, robustness, conformation, fertility, resilience, milk components, milk yield, roughage intake and efficiency, water use, nitrogen, phosphate, and potassium efficiency, greenhouse gas emissions, grazing behaviour, and multipurpose (meat and manure).

The case study on dairy farm “De Marke” showed that both types of cattle performed well (Ducro et al., 2021). The Holstein cows produced more kilograms of milk, whereas the crosses had a higher fat and protein percentage in the milk. Furthermore, the crosses had a shorter time between calving partly due to less time between the first and last insemination. Information on health was not available. Differences in production and reproduction between the two types were reflected by different breeding goals, and also by the estimated breeding values for these traits. Which shows that the breeding goal largely influences the desired characteristics of different types cattle. The variation within breeding goal traits for Holstein AI bulls greatly increased over the recent years, which helped to improve longevity and fertility of Holstein cows. Together with genomic selection, genetic variation helps to breed for circularity and improve traits for Holstein cows and all other breeds.

Conclusion

Breeding is a process that may take multiple generations, and therefore it is important to think ahead when defining a breeding goal. In the transition towards circular agriculture, we need to re-think breeding goals. In this study we defined cow traits which can enable the transition towards circular farming, for example: longevity, rumen health, resilience, roughage efficiency, water use, grazing behaviour, and greenhouse gas emissions. For some traits breeding values exists, however re-evaluation may be required in environments that differ from conventional Dutch dairy farms. Some traits are new and need further investigation before they can be added to the selection indexes.

List of references

van Bruggen, C., Bannink, A., Goenestein, J. F. M., Huijsmans, L. A., Lagerwerf, H. H., van der Sluijs, S. M., Velthof, G. L., & Vonk, J. (2019). Emissies naar lucht uit de landbouw in 2017. Retrieved from Wageningen:

CRV. (2018). NVI. Retrieved from

Dei, H. K. (2011). Soybean as a feed ingredient for livestock and poultry. In D. Krezhova (Ed.), InTech.

Ducro, B., Hoving, H., Stilting, L., & Mentink, H. (2021). Vergelijking tussen Holsteins en kruisingen op melkveeproefbedrijf De Marke in de periode 2014-2019. Retrieved from Wageningen:

Oldenbroek, K., & Van Der Waaij, L. (2015). Textbook Animal Breeding and Genetics for BSc students. Wageningen, The Netherlands: Centre for Genetic Resources and Animal Breeding and Genomics Centre.

Van Pelt, M. L. (2017). Genetic imporvement of longevity in dairy cows. Wageningen University, Wageningen, The Netherlands.

Rijksoverheid. (2018). Visie landbouw natuur en voedsel: waardevol en verbonden. Den Haag, The Netherlands

Van Zanten, H. (2016). Feed sources for livestock: recycling towards a green planet. Wageningen University, Wageningen, The Netherlands.

RZ€ - The new German total merit index expressing breeding impact in Euro

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Abstract

Breeding values for Holsteins are expressed in Germany on a relative scale with average 100 and genetic spreading 12. This includes the established total merit index RZG. EBV on relative scale have advantages but an important disadvantage is that they do not show the economic impact of selection decisions directly. The new, additional total merit index RZ€ (spell RZ Euro) is therefore expressed in Euro margin. The margin refers to the total margin realized by a cow during her lifetime of about three years in comparison to the basis that are 4 – 6 years old cows. Included in RZ€ are the evaluated traits with their economic impact based on current margin calculations. Economic weights purely follow transparent margin calculations without any additional aspects from a breeding policy point of view. In this aspect the RZ€ is unique compared to other internationally used total merit indices.

Keywords: Total merit, breeding index, economic based, Holstein .

Introduction

In Germany estimated breeding values (**EBV**) for dairy breeds are expressed as relative breeding values on a scale with average 100 and a genetic standard deviation of 12. Only exceptions are the EBV for milk production traits that are expressed on the phenotypic scale (kg, %), respectively. The relative scale is used for single traits but also for all indices including the total merit index (**TMI**) RZG, established in 1996. The advantage of a relative scale is that EBV can be compared directly across traits and indices in the sense that it is known where the animal ranks within the trait/index (e.g., +2 standard deviation >= rank 97,5%). The disadvantage of a relative scale is that differences on the phenotypic scale between animals are not directly visible. For the total merit index this means that the economic impact of higher or lower RZG is not clear. Therefore, there was an increasing demand of farmers and industry to have an additional TMI on an economic scale i.e., in Euro (**€**) to clearer indicate the often-underestimated economic impact of selection for animals with higher or lower TMI, the RZ€. For Holstein, the RZ€ was introduced in August 2020. For the other dairy breeds RZ€ is not available because the direct health traits as important part of RZ€ are so far only available for Holstein.

Method

To calculate the economic impact per relative point of published EBV (resp. per genetic standard deviation) two basic figures are needed:

- phenotypic units that equal ±1 relative point of published EBV.
- margin per trait unit on the phenotypic scale (e.g. per case).

Phenotypic units per relative point EBV

Due to technical reasons phenotypes used in genetic evaluation are often different compared to traits/scales used for economic calculations in dairy farms. Examples are longevity or calving ease traits. In genetic evaluation for longevity not the total productive lifespan in time units is used, but survival as 0/1 for certain time periods in a multi-lactation-model. Calving ease uses scores for ease of calving on a 1to 4 scale that is different to percentage of difficult calvings. Therefore, the genetic standard deviations from the evaluation models cannot be used for economic calculations. According to that the genetic standard deviations on the phenotypic scale were derived from the observed daughter differences between top and bottom 25% of daughter proven Holstein AI bulls from the latest three full proven bull birth years. These were AI bulls born 2008 to 2010 (for the trait longevity the bulls were born 2003 to 2005). Only daughters that had the chance to show performance over three lactations were included (for longevity to survive eight years). For most traits the difference between the top and bottom 25% of bulls was in the range of 20 to27 relative points of EBV i.e. about two genetic standard deviations. The daughter difference between the two bull groups were standardized to 24 points so that the daughter difference represents one genetic standard deviation (50% from sire). Table 1 shows the results i.e. what difference on the phenotypic scale equals 12 points or 1 genetic standard deviation of relative breeding value.

In the German genetic evaluation system 13 individual health traits or disorders are included, but breeding values are published for four traits/indices only. The EBV for mastitis resistance (RZudderfit) is the only one health trait comparable to the single

Table 1. Description of phenotypic units corresponding to a genetic standard deviation S_g (12 points) for each trait/EBV.

Relative EBV	Daughter trait	Daughter phenotype (Ø all lact.)	Realized S_g
RZS	Cell count (k/ml)	218	83[1]
RZN	Longevity (days)	1115	259
1st-to-last heifer	1st-to-last heifer (days)	31.3	6.2
NR heifer	NR heifer (%)	72.0	5.0
calv.-1st	Calv.-1st (days)	84.2	9.0
1st-to-last cows	1st-to-last cows (days)	51.5	10.1
NR cows	NR cows (%)	55.7	6.3
CE direct	Difficult calvings (%)	3.5	2.0[1]
SB direct	Still born calves (%)	5.8	2.4[1]
CE daughter	Difficult calvings (%)	3.2	1.7[1]
SB daughter	Still born calves (%)	5.8	3.1[1]
Dairy type	Dairy type (scores)	81.9	0.9
Body	Body (scores)	82.1	1.1
Feet & legs	Feet & legs (scores)	80.6	1.0
Udder	Udder (scores)	81.2	1.0
RZD	Milking speed (kg/min.)	2.42	0.40
RZcalffit	Young stock survival (15 mo.) %	93.0	4.4[1]

[1] Spreading on phenotypic scale is skewed.

Table 2. Description of phenotypic units of each health trait corresponding to a genetic standard deviation (12 points) of its corresponding health index EBV.

Relative EBV	Daughter trait	Daughter phenotype (Ø all lact.)	Realized S_g
RZudderfit	Mastitis %	25.6	12.0[1]
RZhoof	Mortellaro %	24.1[1]	12.0[1]
	Sole ulcer %	15.1[1]	13.2[1]
	Digital phlegmon %	8.4[1]	10.8[1]
	White line defect %	7.6[1]	6.4[1]
	Laminitis %	6.8[1]	3.5[1]
	Tylom %	5.1[1]	4.4[1]
RZrepro	Ovarian cycle disorders %	19.7[1]	11.5[1]
	Metritis %	13.1[1]	7.4[1]
	Retained placenta %	7.5[1]	4.9[1]
RZmetabol	Displaced abomasum %	1.4[1]	3.1[1]
	Milk fever %	1.9[1]	1.7[1]
	Ketosis %	3.1[1]	2.4[1]

[1] Spreading on phenotypic scale is skewed.

traits in table 1. Because the economic calculations should be based on published EBV, the impact of one genetic standard deviation of published health trait indices for hoof health (RZhoof), metabolic stability (RZmetabol) and resistance to reproduction disorders (RZrepro) were derived as shown in table 2. Differences in these published health indices have impact on three to six underlying traits economic weight can be calculated for. Within each health trait index the included single traits are positively correlated so that improving genetic level for the health index by one genetic standard deviation improves the level for all included traits at the same time as given in table 2.

Margin per trait unit

The margin per trait unit is calculated as direct marginal cost minus direct marginal return under average German production conditions keeping all other traits constant (ceteris paribus condition). It is important to consider only direct economic impact but not indirect impact via other traits included in RZ€. For the example of mastitis resistance, the direct economic impact of one unit more mastitis (= one additional case of mastitis) are the extra costs for treatment (veterinarian, drugs, labor) and produced milk that is not tradable due to antibiotic treatment. Costs for extra culling of cows with severe mastitis is not considered because this is included in economic impact of longevity. EBV longevity is based on all involuntary culling including culling due to mastitis. By exclusively considering the direct costs and returns of each trait double counting of economic effects is avoided. The first column in table 3 gives the calculated margins per trait unit.

Genetic differences for somatic cell count and conformation traits have only very limited economic impact and therefore are not included in RZ€. Somatic cell count is mainly an indicator trait for mastitis resistance that is included in RZ€. Under ceteris paribus condition (same incidence rate of mastitis) differences in cell count have no economic impact on farm level. Greater 99% of all produced bulk milk meats the threshold of 400,000 cells/ml that would lead to restrictions in milk sale. The importance of conformation traits in dairy breeding practice is based on expected indirect effects via other functional traits (longevity, fertility, calving, health) that are included already with their direct economic impact in RZ€. Conformation traits have a direct economic impact when selling breeding animals because the breeding cattle market values animals with better conformation. Even though German Holstein farmers sell relative to many other countries more breeding heifers (e.g. per year 80,000 German Holstein heifers exported) the absolute number of sold heifers per cow and lifetime is limited to <0.2. This leads to a direct economic impact of conformation traits for an average farm of only about 0.5% relative weight in RZ€. More details on the economic calculations for all traits can be found on *www.vit.de* (publications – 2020 – The new RZ€).

Economic impact per cow lifetime

The focus of dairy farmers making selection decisions on the base of EBV is the (economic) superiority of the selected female resp. the daughters of the selected bull. Therefore, the RZ€ is the sum of the economic impact for all traits based on the entire productive lifespan of a cow. The average productive life of a German Holstein cow was assumed with a productive life of about 1100 days (3,01 years) giving birth to three calves and completing 2,75 lactations with average 360 milking days. Based on these assumptions and the genetic standard deviations from table 1 and 2 the economic impact of one relative point EBV per cow lifetime was calculated (see table 3).

For every animal and trait € margin is calculated as (relative) points EBV (deviation from 100) multiplied with economic impact per (relative) point EBV and lifetime. The sum of all trait margins is the total merit in Euro = RZ€. Because the base for all EBV are 4 to 6 year old cows (= 100, representing active cow population) the RZ€ of a female expresses the difference in margin she will realize in average during her lifetime compared to an average cow. The standard deviation of RZ€ is about 535 € and top young genomic females and bulls reach up to almost +3,000 RZ€.

From the ratio of the margins per genetic standard deviation and lifetime the relative weights of the traits and trait complexes can be calculated. In RZ€ the milk production traits have 41% weight, followed by longevity with 27% and direct health traits with 16%. Fertility traits sum up to 7%, young stock survival has 6% and calving traits 3% weight (see table 4).

Selection response

The correlation of the new TMI RZ€ with the established TMI RZG is high with 0.97. This even though the RZG includes 15% conformation traits and the ratio of weights included

Table 3. Margin per trait unit and margin per point relative EBV and lifetime.

EBV trait	€ per unit resp. per case	S_g	€/S_g per lifetime	€/EBV unit+life
Fat (kg)	2.56	25.1	197.72	7.88
Protein (kg)	4.09	19.7	248.76	12.56
Lactose in F/P free milk (kg)1)	-0.024	690	-51.13	-0.07
RZN/Herdlife (day)	1.00	259	258.69	21.56
Calving-to-first (day) 2)	0.33	9.0	6.05	0.50
First-to-last-insemination heifers (day)	1.67	6.2	10.35	0.86
First-to-last-insemination cows (day)	3.67	10.1	52.06	4.34
Stillbirth rate maternal	137.5	3.1	12.81	1.07
Stillbirth rate direct	137.5	2.4	9.87	0.82
Calving ease maternal	59.4	1.7	4.03	0.34
Calving ease direct	59.4	2.0	5.03	0.42
RZudderfit	186.0	12.0	61.39	5.12
RZhoof	32-74	4-13	30.13	2.51
RZrepro	28-100	5-12	17.10	1.43
RZmetabol	131-289	2-3	39.86	3.32
RZcalffit (young stock survival)	449.7	4.4	54.61	4.55

Table 4. Relative weight of traits and trait complexes in RZ€.

EBV trait	€/S_g per lifetime	Resulting weights (%)		
Fat (kg)	197.72	20.7	41	Milk production traits
Protein (kg)	248.76	26,0		
Lactose in F/P free milk (kg)1)	-51.13	-5.3		
RZN/Herdlife (day)	258.69	27.0	27	Productive life
Calving-to-first (day) 2)	6.05	1.1	7	Daughter fertility
First-to-last-ins. heifers (day)	10.35	0.6		
First-to-last-ins.cows (day)	52.06	5.4		
Stillbirth rate maternal	12.81	1.3	3	Calving traits
Stillbirth rate direct	9.87	1.0		
Calving ease maternal	4.03	0.4		
Calving ease direct	5.03	0.5		
RZudderfit	61.39	6.4	16	Health traits
RZhoof	30.13	3.1		
RZrepro	17.10	1.8		
RZmetabol	39.86	4.2		
RZcalffit	54.61	5.7	6	Young stock survival

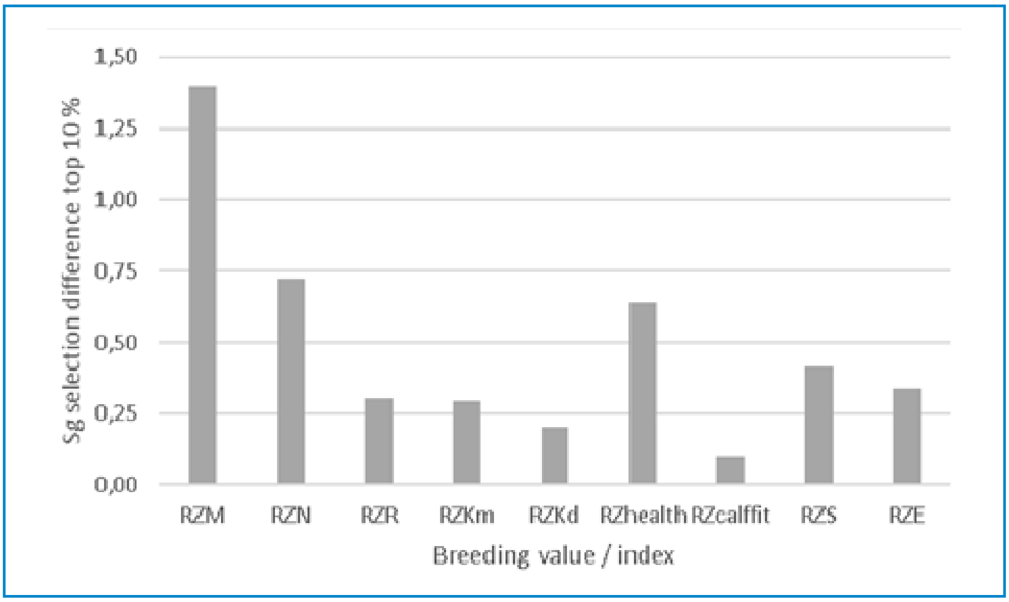

Figure 1: Selection response selecting for RZ€ (selection difference top 10% of 256,352 females from herd genotyping born 2019+2020, gEBV 04-2021). RZM=index milk production traits, RZN= relative EBV longevity, RZR= daughter fertility index, RZKm/d=calving trait index maternal/direct, RZhealth= total health index, RZcalffit= relative EBV young stock survival, RZS= relative EBV somatic call score, RZE= overall conformation index

in both TMI is partly different (for composition of RZG see *www.vit.de* - description of breeding values). The expected selection response for selection by RZ€ overall follows the weights for the trait complexes. The expected progress for traits longevity, direct health, fertility, and calving traits is slightly higher compared to the relative weight in RZ€ because these traits have significant positive genetic correlations among each other creating additional indirect selection response. Expected progress in young stock survival is small compared to e.g. fertility traits with comparable weight in RZ€. Young stock survival has no correlation to longevity and health traits of cows.

The importance of conformation traits in farmers selection decisions was already mentioned. Therefore, a concern of farmers regarding selection by RZ€ is the selection response for conformation and for somatic cell count. Even though not included directly in RZ€ there can be expected significant positive indirect selection response for overall conformation by selecting for RZ€ (see figure 1). Overall feed and legs and udder are positively correlated to longevity and direct health traits. Dairy type is correlated to milk production. The indirect selection response for overall conformation by selection for RZ€ equals almost two-thirds of the response selecting for RZG including 15% conformation traits. For somatic cell count the same good selection response is expected as in former times for RZG with 7% direct weight for somatic cell count but no mastitis resistance. This could be expected because now mastitis resistance has 6.4% weight and genetic correlation to somatic cell count is high.

Summary and outlook

The new and additional TMI for Holsteins RZ€ shows economic impact of selection decisions directly because of the scale € margin per lifetime. The composition follows strictly economic calculations without additional aspects from breeding politics. This is different to most other TMI that e.g. mostly include conformation traits without a true calculation on (direct) economic impact of conformation traits.

Correlation to established TMI RZG including conformation and weighting of trait complexes partially based on breeding policy aspects is high. Nevertheless, impact of RZ€ should not be underestimated. The scale € margin compared to an average cow can make Holstein dairy farmers more aware of the big impact on economy of genetics and selection decisions. Especially the growing number of Holstein dairy farmers that use herd genotyping for selecting their females experience how big the differences in EBV among their animals are and how good prediction of performance differences by genomic EBV is. With this information they can comprehend those differences in RZ€ give a realistic picture of the economic differences based on genetics between animals and how much extra margin is to gain following a strict economic breeding goal.

Finally, the RZ€ shows that an economically based breeding goal must not be in contradiction with animal health and the expectation of the society. Health traits including fertility, calving and longevity get 59% weight in the breeding goal because of economic reasons and breeding goal is no longer dominated by milk production traits and conformation.

Squaring the bovine circle – An Irish perspective

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Summary

The concept of circular breeding goals necessitates looking at the bovine industry in its entirety and becomes broader than the agri-industry circle when issues such as climate, environment, animal welfare and consumer preferences are being considered. Many of the potential solutions discussed have impact across the bovine sectors or have downstream impacts on the other sectors. Economically balanced breeding goals delivering genetic gain across a wide range of measurable traits are a core building block on the journey to breed more sustainable cattle.

Background

The bovine sector comprised of dairy and beef cattle is worth up to 6.5 bn in yearly export revenue to the Irish economy. For the context of this paper the sector can be broadly divided into four distinct but intrinsically linked sub-sectors namely the dairy sector, the commercial suckler sector, the bull breeder sector, and the dairy beef sector. Currently the national bovine herd stands at circa 1.5 million dairy cows, 950,000 suckler beef cows and their progeny giving a total inventory of circa 6 million animals. In recent years since the abolition of milk quotas in 2015 the dairy herd has expanded by 400,000 additional cows with suckler cow numbers declining in the same period by circa 85,000 cows. Recent national farm survey results (Donnellan *et al*, 2020) indicate dairy herd income averaged €1,118 per ha compared to €285 and €381 per ha for suckler cowherds and other cattle rearing herds respectively. From the perspective of circular breeding goals this paper will outline a number of initiatives aimed to increase productivity, profitability and reduce the carbon footprint across all the sectors simultaneously. ICBF have responsibility to harness genetic gain to increase profitability in a sustainable way for all Irish cattle farmers and the wider agri-industry. This is achieved through an integrated database which continues to evolve to meet the demands of the industry. Figure 1 shows the current level of integration or the within agri-sector circle which currently allows genetic evaluations, profit index and genetic gain generation to occur across the dairy and beef sectors. In addition to genetic gain the database is also accessible to farm advisors to access phenotypic data to help improve profitability at the individual herd level but also at discussion group and milk co-operative level. Figure 1 also envisages the potential future circle as the challenges facing the sector such as the environment and greenhouse gas emissions are tackled and new opportunities are identified such as consumer experiences and perceptions and potential animal product related human health benefits.

Climate and the environment

In 2020 the Irish Government launched a national “Climate and Air Roadmap” for the agriculture sector which aims to reduce the current agriculture carbon footprint from biogenic methane by 10% by 2030 and to become carbon neutral by 2050.Many of the assumptions in that report were based on the Teagasc Marginal Abatement Cost Curve (MACC) regarding greenhouse gas (GHG) and ammonia emissions (Lanigan *et al* 2018). Figure 2 outlines some the actions presented in the MACC report ranging from adopting new management practises to the improved efficiency from increased genetic merit.

Role of current Dairy Breeding in Greenhouse gas mitigation

Improving carbon efficiency through adoption of the EBI for dairy cattle breeding was identified as high value in the MACC report from both a low cost and a high impact viewpoint in terms of emissions abatement. Work by Ramsbottom *et al.* (2012) using individual cow-based and herd-based data reported an increase in net margin per cow of €1.94 per one euro improvement in EBI (expectation of €2). Figure 3 is a diagrammatic representation of the current emphasis within the dairy EBI. More recent work by Shalloo *et al.*, (2021) has elucidated 6 primary traits included in the EBI which have contributed significantly to trends in dairy GHG; calving interval, cow survival, liveweight, milk, fat and protein. Cumulatively, genetic progress (€12.6 EBI/yr) for these key traits is improving gross GHG mitigation by 24.9 kg CO2e/lactation (or /cow/year). Table 1 shows the genetic trend of these 6 traits and the impact on GHG emissions. Results indicate increased genetic merit for milk production traits and liveweight and the associated response in phenotypic performance actually resulting an increase in GHG emissions per unit change in those traits while a reduction in calving interval (related to earlier turnout to avail of less costly feed) and better cow survival (due to less replacements needed as cows live longer)both contribute to a reduction in GHG.

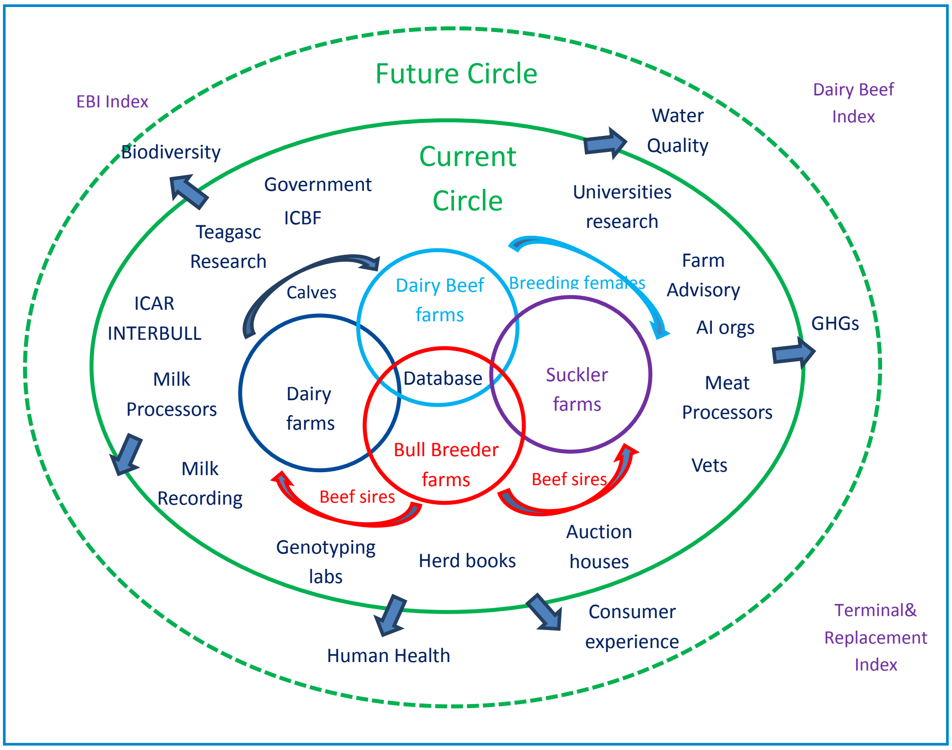

Figure 1. Current and future level of industry integration or circle of engagement for bovine genetic improvement in Ireland

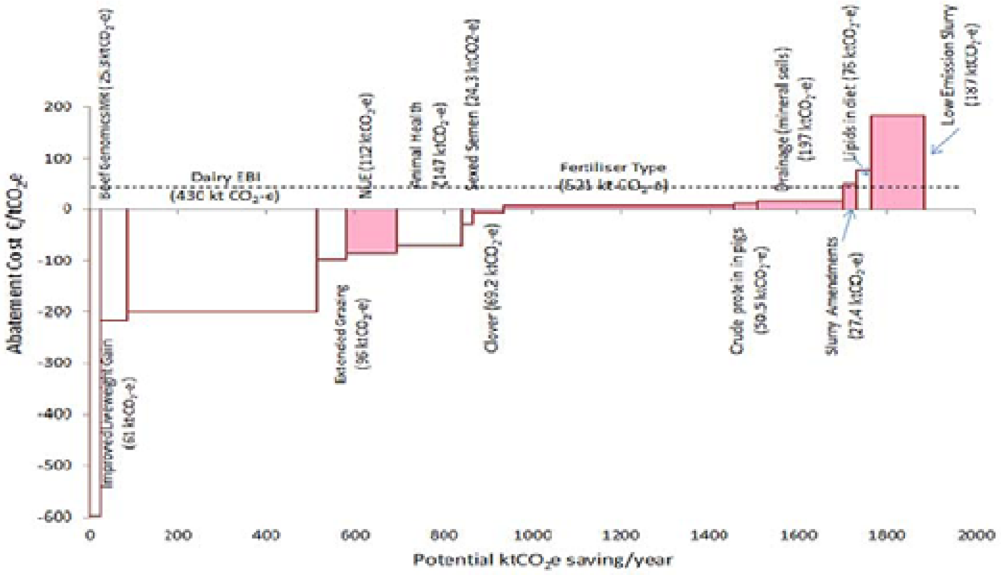

Figure 2. The Teagasc Marginal Abatement Cost Curve for GHG and ammonia (Lanigan et al *2018).*

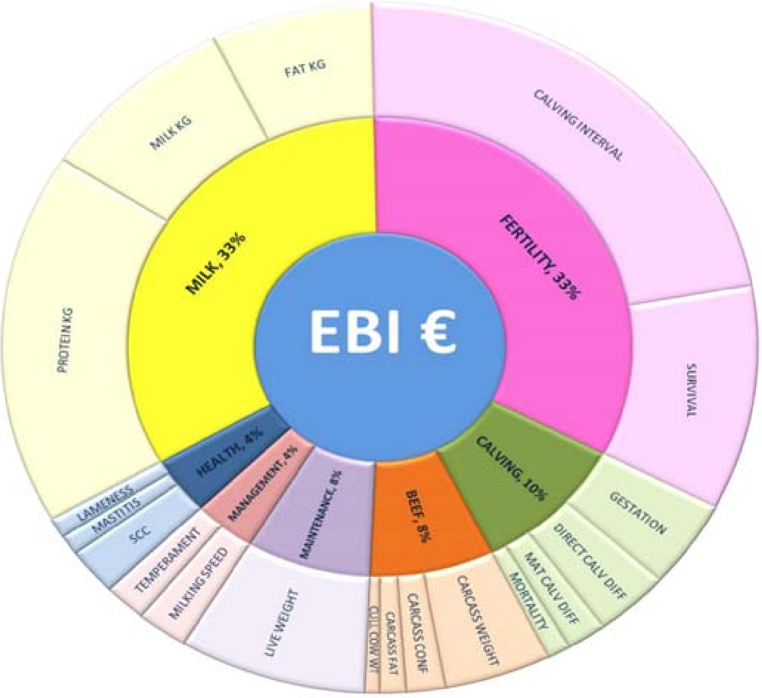

Figure 3. Current trait emphasis in the Economic Breeding Index for Irish dairy cattle

Role of current Beef Breeding in Greenhouse gas mitigation

Genetic progress in the Suckler herd is driven by the Terminal and Replacement economic indexes. The indexes were launched in 2011 but in 2016 the Replacement index was revamped to place more emphasis on maternal traits within the index. Using measures of genetic merit and economic based profit indexes is a more recent concept for suckler beef farmers compared to their dairy comrades. While many dairy herds have been engaging with the ICBF database since 2001 the majority of beef farmers commenced engagement in 2008 with the launch of the Suckler Cow Welfare Scheme (SCWS) where farmers received a monetary payment for recording ancestry, calving, weanling and docility measures. This data provided a significant boost to genetic evaluations and as can be seen in Figure 4 the genetic trend of the suckler herd for Terminal traits increased noticeably from 2010 to 2014 even though there was no stipulation for genetic improvement in that scheme. In 2016 the Beef Data and Genomics Programme was launched which in addition to many of the measures in the SCWS also contained a genotyping requirement along with a genetic improvement stipulation based on the Replacement index. The scheme has resulted in the generation of circa 2 million genotypes from 25,000 herds where the focus was on the breeding males and females in those herds. Those genotypes combined with the phenotypes from these herds have become the corner stone for the ICBF beef genetic evaluations.

Figure 5 is a graphical representation of the traits in the Replacement and Terminal indexes. All Terminal index traits are also included in the Replacement index. The Replacement index places heavy emphasis on Milkability, carcass weight, fertility, longevity and the maintenance cost of the suckler cow as measured through the cow liveweight trait. Work by Quinton *et al*. (2018) estimated that a €1 increase in Replacement index for beef suckler cows reduced enteric methane emissions intensity

Table 1. Key traits in the Dairy EBI which are influencing Enteric methane emissions intensity.

Trait	PTA Trend (units/year)	Change in kg CO2e/trait unit
Calving interval (days)	-0.21	59.8
Survival (% per lactation)	0.12	-34.4
Milk fat (kg)	0.60	5.41
Milk protein (kg)	0.55	5.65
Milk kg	3.12	0.22
Liveweight (kg)	0.22	2.05

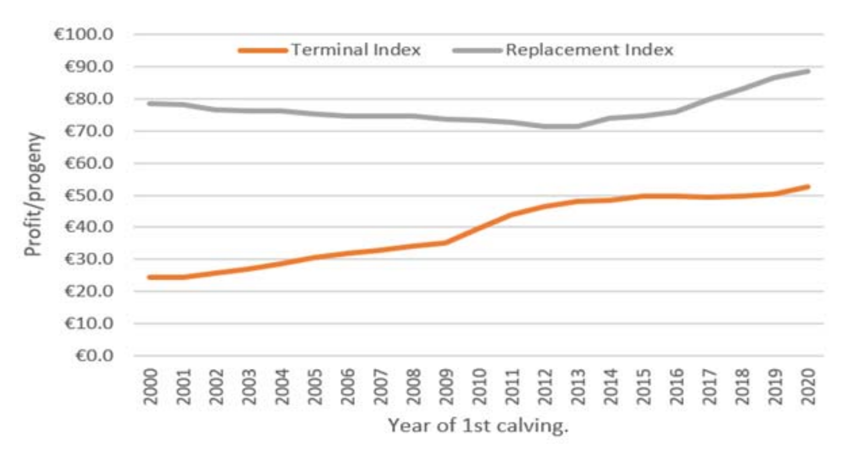

Figure 4. Trend in Terminal and Replacement Index for Suckler herd females.

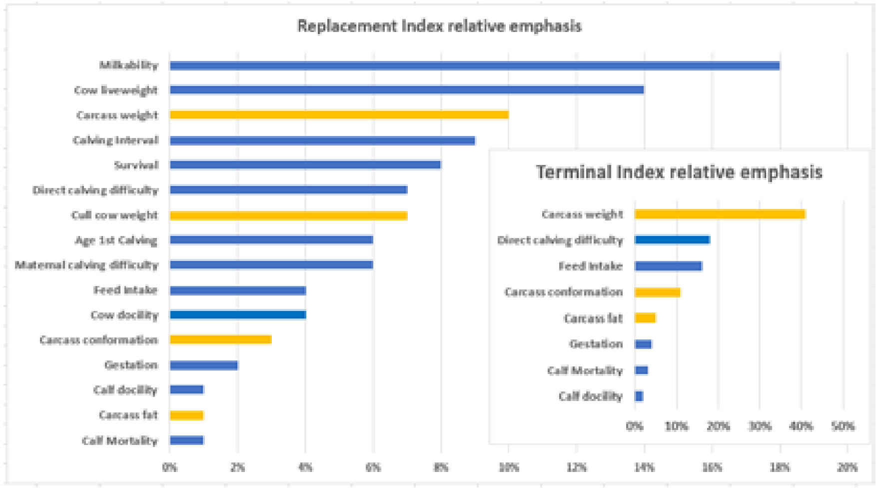

Figure 5. Relative emphasis in the current Replacement and Terminal indexes. Revenue traits are in orange with costs of production traits in blue.

by 0.009 kg CO2e/kg meat/cow/year, and that a €1 increase in Terminal index reduced emissions intensity by 0.021 kg CO2e/kg meat/cow/year.

Can we accelerate genetic progress?

There are a range of avenues available to improve the genetic progress currently occurring but for the purposes of this paper they have been classified into two broad categories namely a) increasing the gain in the current traits and b) incorporating new traits which have either an economic or environmental benefit not captured in the existing trait suite.

Increasing genetic gain in the current traits

Below are 6 areas which can be improved to facilitate increased genetic gain.

Increased AI sire usage and sire recording

Currently the level of AI usage in the dairy herd to generate replacements is high with 73% of dairy sired calves in 2020 sired by an AI sire with the remainder either sired by a natural service sire (10%) or no recorded sire (17%). For those 2020 dairy sired calves the average superiority of AI sires versus natural service sires in EBI was €73. In those same dairy herds the level of AI usage of beef sires on dairy cows is low at 26% with the remainder either sired by natural service sires (34%) or no sire recorded (40%). The average superiority of AI sires versus natural service sires in Dairy Beef Index (DBI) was €22. Similarly in the 2020 suckler herd calves only 19% were sired by AI with 57% sired by natural service sires and 24% with no sire recorded. The average superiority of AI sires versus natural service sires was €7 for Terminal and €36 for Replacement index.

Increased engagement in milk recording

Milk recording drives the Milk sub-index of the dairy EBI but also has a large effect on the Health sub-index which includes the somatic cell count trait. Currently circa 55% of cows are milk recorded. Various initiatives are underway such as engagement with milk recording organisations to provide more value added at the individual cow level (detailed SCC reports, harnessing of spectral for new traits like energy balance and potential GHG emissions) but also with milk co-operatives to build on the existing within co-op benchmarking reports currently available.

Increased cow liveweight recording

Cow live weight is a key component of both the dairy and beef profit indexes where there is a penalty on increased liveweight due to the increased maintenance feed costs associated with larger cows. To date most of the cow liveweight prediction has come from cows which are weighed in auction houses before slaughter or from a numerically smaller dataset from Teagasc research herds which provide comprehensive data throughout lactation. Genetic correlations are also utilised in the genetic evaluation with traits with larger volumes of data such as weaning weight, carcass weight and cull cow weight. The launch of the Beef Environmental Efficiency Pilot scheme in Suckler herds (2019 and 2020) has resulted in over 700,000 new cow and calf liveweight records potentially available for inclusion in genetic evaluations. A nationwide weighing service infrastructure has been put in place to facilitate the low- cost rental of scales by farmer which will be available to both beef and dairy farmers to avail of.

Genotyping all animals through DNA based calf registration

Three pilot projects have been initiated since 2018 to assess the feasibility of DNA based calf registration. The most recent in Spring 2021 included 400 dairy herds. Participating herds must have their full list of breeding females and males genotyped which then allows the ICBF database to parentage verify the calf based on the returned genotype and this information is then relayed to the Department of Agriculture (DAFM) for calf registration. The vision is to migrate the full national herd to DNA based registration by 2030 which will have many benefits in terms of removing parentage errors from evaluations and enhancing genomic training populations.

Harmonisation of bio-economic models

Currently the economic values used in the dairy and beef indexes are derived from the Teagasc Moorepark Dairy Systems Bioeconomic model (Shalloo *et al*., 2004) and the Teagasc Grange Beef Systems Model Bioeconomic model (Crossan *et al*., 2006). The two models are independent of each other and designed to accurately assess the economic impact of the traits within that particular sector. However, with issues such as environment and Greenhouse gases and the obvious links between the sectors described in Figure 1, initial discussions have commenced on the potential to combine both models into a single entity to allow synergies such as the ability to model national inventory for aspects such as beef production and GHG emissions.

In 2020 ICBF launched a new profit index called the Dairy-Beef index (Berry *et al* 2019). This index is for dairy farmers who want to use beef sires on their dairy cows. In 2020 690,000 calves were born to a dairy dam and a beef sire which represented 46% of the total 2020 dairy calves. In recent years with dairy expansion, herd size has increased from an average of 44 cows calving in 2010 to 77 cows in 2020. Farmers have placed more priority on calving ease and short gestation for both the dairy and beef sires used on their cows. As a result the carcass merit of the dairy x beef male

Adoption of the Dairy Beef Index

calf available to the dairy-beef rearing herds has been declining. The Dairy-Beef index aims to maintain calving traits for the dairy farmer while improving feed efficiency and carcass traits for the beef rearer and finisher.

Incorporating new traits

Resistance to Tuberculosis and Liver Fluke

Tuberculosis (TB) and Liver Fluke infection are two common diseases which afflict Irish dairy and beef cattle. Levels of TB in the population over the last 5 years has averaged 16,800 cases with 4.3% of all herds experiencing a reactor in the annual herd test. This is despite decades attempting to eradicate the disease and annual costs almost reaching €100m in 2020. Liver Fluke is a common parasite in Irish cattle afflicting 7-8% of steers and heifers and up 40% of cows in wet years. Animal Health Ireland have estimated an average cost of €70 per condemned liver in slaughtered steers factoring in condemned livers and lack of thrive due to infection. The launch in 2019 of genetic evaluations for Tuberculosis (Ring *et al*., 2019) and Liver Fluke resistance (Twomey *et al*., 2016) were the culmination of 10 years of collaborative work involving Teagasc, ICBF, DAFM and Animal Health Ireland (AHI). Heritability of TB and Fluke is estimated at 9% and 1% respectively. Work is underway to derive economic values for these new traits to facilitate inclusion is the EBI and beef profit indexes.

Meat Eating Quality

Recent UK retailer research has indicated that if a consumer has a negative experience eating beef, it will be six weeks before they purchase beef again. These results are against a backdrop of declining beef consumption within the EU, compared to pork and chicken alternatives. To address these trends, it is necessary to develop systems to help improve the eating quality attributes of beef for the traits that are of value to consumers, most notably tenderness, juiciness, and flavour. Genetic evaluations for meat eating quality were launched in 2020 as part of ICBF's participation in an industry backed programme called the Meat Technology Centre (MTI) which is a consortium involving Teagasc, Irish Universities and the Irish meat processors. The evaluations are based on circa 5,300 trained sensory panel phenotypes for tenderness, juiciness and flavour of prime cattle steaks following a standard operating procedure around rearing, slaughter and sensory assessment. Resulting heritability estimates (Berry *et al*., 2021) ranged from 13% for flavour to 15% for tenderness. Genetic evaluation results are transformed to an *expected satisfaction rating* of the progeny from a given sire. The average expected rating for each trait is 80% (i.e., 80% of progeny are expected to have satisfactory tender, flavour or juicy score), with sires higher than this expected to produce progeny with superior meat-eating quality for the relevant trait. The range in average satisfaction values across AI sires with published evaluations (September 2020) is 70% to 90%. While clear breed differences exist (Figure 1), of similar relevance is the variability in meat eating quality that exist within breeds.

Age at slaughter

Reducing age at slaughter is viewed as a potential low hanging fruit in terms of reducing GHG emissions. Initial modelling suggests that if the current prime cattle kill (equivalent to 1.32m cattle in 2020) was slaughtered 1 month earlier than the current 802-day average this has the potential to remove 247 Kilotonnes of GHG from the system or the equivalent of not having to cull 97k cows from the National herd. Initial parameter estimation work suggests the trait has a heritability broadly equivalent to the current carcass traits but that the data needs more stringent editing to remove management effects related to the age of the current kill which are driven by penalties once an animal is killed over a certain age.

Methane yield

The collection of direct methane yield in a meaningful volume is now feasible with the availability of the Greenfeed System (C-Lock, Rapid City, South Dakota).The ICBF progeny performance test centre has evaluated 670 animals to date over the last 2 years using the Greenfeed system alongside the Insentec Feed system (Hokofarm, The Netherlands). Evaluation is being carried out on a range of animal's types (steer, heifer, bulls) and diet types (high concentrate and mixed roughage and concentrate). In addition, the Moorepark Research station is actively collecting methane yield on lactating dairy cows at pasture using the Greenfeed systems. It is hoped that data collected at both centres will help to initially inform researchers of the phenotypic relationships between GHG emissions with diet, animal type, feed intake but as volumes grow it will become feasible to estimate genetic parameters and generate genetic evaluations for methane yield or residual methane yield.

List of references

Berry, D.P., Amer, P.R., Evans, R.D., Byrne, T., Cromie, A.R. and Hely, F. 2019. A breeding index to rank beef bulls for use on dairy females to maximise profit. JDS, 102, 11. 1—56-10072

Berry, D.P., Conroy, S., Hegarty, P.J., Evans., R.D., Pabiou., T., Judge., M., 2021. Inter-animal genetic variability exist in organoleptic properties of prime beef meat. Meat Science 173 (91): 108401

Crosson, P., O'Kiely, P., O'Mara, F.P. and Wallace M. 2006).The development of a mathematical model to investigate Irish beef production systems. Agricultural Systems, 89, 349-370

Donnellan, T., Moran, B., Lennon, J. and Dillon, E., 2020. Teagasc National Farm Survey 2019 Results. *https://www.teagasc.ie/media/website/publications/2020/Teagasc-National-Farm-Survey-2019.pdf*

Lanigan, G., al. 2019. An Analysis of Abatement Potential of Greenhouse Gas Emissions in Irish Agriculture 2021-2030. Teagasc Greenhouse Gas Working Group. *https://www.teagasc.ie/media/website/publications/2018/An-Analysis-of-Abatement-Potential-of-Greenhouse-Gas-Emissions-in-Irish-Agriculture-2021-2030.pdf*. *https://assets.gov.ie/10206/d042e174c1654c6ca14f39242fb07d22.pdf*

Quinton CD, Hely FS, Amer PR, Byrne TJ, Cromie AR. Prediction of effects of beef selection indexes on greenhouse gas emissions. Animal. 2018 May;12(5):889-897. *https://doi: 10.1017/S1751731117002373.*

Ramsbottom., G., Cromie, A.R., Horan, B., Berry, D.P. 2011. Animal, 6:7 pp 1031-1039 Relationship between dairy cow genetic merit and profit on commercial spring calving dairy farms.

Shalloo, L., Dillon, P., Rath, M. and Wallace, M. 2004 Description and validation of the Moorepark Dairy Systems model Journal of Dairy Science 87, 1945–1958

Shalloo *et al.*, In preparation (preliminarily presented at IGC 2020)

Ring, S.C., D.C. Purfield, M. Good, P. Breslin, E. Ryan, A. Blom, R.D. Evans, M.L. Doherty, D.G. Bradley, and D.P. Berry. 2019. Variance components for Bovine Tuberculosis Infection and Multi-Breed Genome-Wide Association Analysis Using Imputed Whole Genome Sequence Data. PLoS ONE. 14 (2): e0212067. *https://doi.org/10.1371/journal.pone.0212067*

Twomey, A.J., R.G., Sayers, R.I. Carroll, N. Byrne, E. O'Brien, M.L. Doherty, J.C. McClure, D.A. Graham, and D.P. Berry. 2016. Genetic parameters for both a Fasciola hepatica-liver damage phenotype caused by and Fasciola hepatica antibody response to phenotype in dairy and beef cattle. J. Anim Sci. 94(10):4109-4119. *https://doi.org/10.2527/jas.2016-0621*

Improving dairy feed efficiency, sustainability, and profitability by impacting farmer's breeding and culling decisions

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Abstract

Enhancing feed efficiency should improve the profitability and sustainability of dairy farming due to reduced use of feed and land resources while potentially reducing emissions of greenhouse gas (GG) per liter of milk. The selection of animals that are genetically superior for feed efficiency requires precise measurements of feed energy intake and milk energy output from enough cows to predict genetic merit for feed efficiency with reasonable reliability. Previously, a consortium of dairy science experts from North America and Europe created a pool of data including 5,000 cows genotyped and phenotyped for feed intake and related traits. Using this database, the researchers showed that dry matter intake (DMI) and residual feed intake (RFI) had sufficient heritability to enhance genetic progress for feed efficiency. Data from that study projected that the US dairy sector could save $540 million/year with maintained milk production by breeding for more efficient cows. The project presented herein was launched in 2019 to build on previous results and is the next logical step for implementing the selection for feed efficiency in the US and to address concerns about greenhouse gas emissions. Specific objectives are to:

1. Increase the reliability of genomic predictions for feed efficiency.
2. Develop a feed intake index that uses sensors to predict feed intake on individual cows,
3. Initiate a long-term program for updating genomic predictions of feed efficiency, and
4. Determine if genomic predictions of feed efficiency can decrease methane emissions.

The project protocol calls for the acquisition of data related to feed intake, milk yield and composition, and body weight for 42 days in 3600 mid-lactation cows (50-200 DIM) over a 5-year period. Additionally, a subset of cows will be fitted with sensors to monitor body temperature, feeding behavior, and locomotion. Mid-infrared spectral profiles will be collected from all milk samples. Methane emission will be measured in 300 cows. Data collection is in progress at all research stations. These data will be used to develop a genomic evaluation for feed efficiency in U.S. Holsteins and support the development of management tools.

Keywords: Efficiency, residual, feed, intake, dairy, methane, sensor, genomic, Holstein.

Introduction

Increases in population and consumption of dairy products will translate into a need for approximately 600 billion kg more milk in 2067 than is produced today (Britt *et al*., 2018). This need might be constrained by environmental challenges. Scientists need to provide tools to farmers and their advisers to achieve their environmental sustainability goals in an economically viable manner. Genetic selection has yielded remarkable gains in the yield and efficiency in livestock production, bringing milk production of US Holstein cows from 5,904 to 13,015 kg/lactation in the period 1957-2019 (CDCB, 2021), 56% of this improvement was due to genetic selection. Over this time period, increased focus on "functional traits" has led to a breeding goal, the Lifetime Net Merit index, including non-production traits, such as female fertility, calving ability, udder health, and longevity. Genetic selection has been revolutionized by genomic selection (Meuwissen *et al*., 2001), coupling low cost animal genotyping stored in large repositories housing thousands of DNA samples from dairy bulls, and milk-recording databases with millions of performance records from their progeny. As a result, US dairy farmers now carry out genomic testing on more than 90,000 calves per month, increasing genetic progress dramatically over the past decade (García-Ruiz *et al*., 2016). Currently, the database of the US Council on Dairy Cattle Breeding (CDCB) contains more than 5 million dairy genotypes.

Genetic selection for higher milk production has increased efficiency of energy utilization in dairy cattle however, variation among cows in their ability to digest and metabolize nutrients and perform maintenance functions has not been exploited in genetic improvement programs. Residual Feed Intake (Fig 1) is a measure of the amount of feed energy a cow consumes each day relative to her expected energy requirement, computed from Dry Matter Intake (DMI), secreted milk energy, body weight (BW) and BW change measured over a period of time. Davis *et al*. (2014) and Yao (2016) showed that selection for RFI is feasible, and that low RFI values selection might impact feed costs. The key to improving feed efficiency through breeding programs is to establish a reference population of animals with performance data and genomic testing data. Reference genotypes and phenotypes can be matched with the genotypes from the

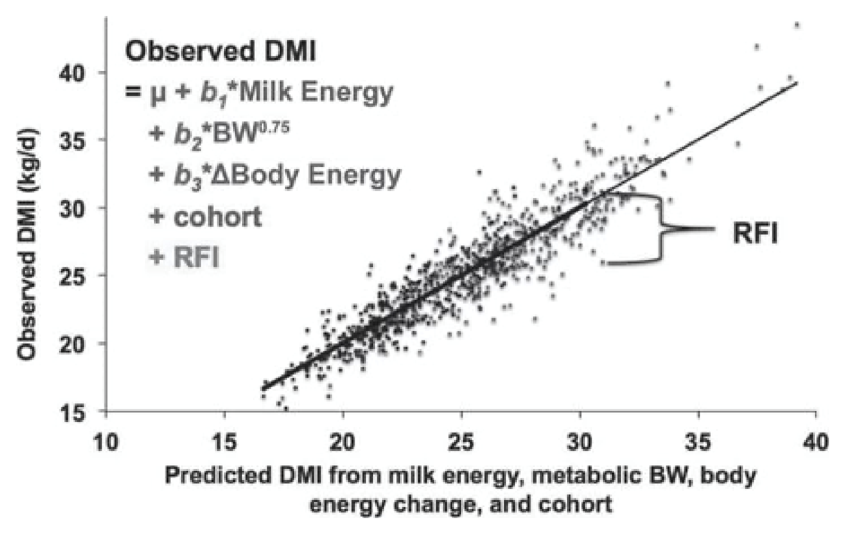

Figure 1. Residual feed intake (Van de Haar et al*., 2016)*

national population that have economic test results but lack performance data for feed efficiency, to compute genomic estimated breeding values (GEBV) (Meuwissen *et al.*, 2001), and hence make informed selection decisions. GEBV of new animals can then be calculated at birth (or on embryos), allowing greater genetic gains over a shorter time interval (Boichard *et al.*, 2015). Previous studies with a 5000-cow database with feed efficiency phenotypes and SNP genotypes demonstrated that genomic selection for feed efficiency is possible and could improve dairy farm profitability (Van de Haar *et al.*, 2016; Yao, 2016). The heritability for RFI has been estimated at 0.16 (Tempelman *et al.*, 2015, 2020; Hardie *et al.*, 2017; Lu *et al.*, 2018; Li *et al.*, 2020), showing that genetic selection for RFI can improve feed efficiency. The reliability for EBV has been estimated at 34% and 13% for phenotyped and genotyped cows, respectively. These low reliability values are attributed to the limited data size (Li *et al.*, 2020). Increasing prediction reliability for RFI requires more feed intake data (van Raden *et al.*, 2018). Preliminary analysis of genomic evaluation of feed efficiency for US Holsteins indicated that, calculating the range in GEBV for 16,000 sires, the top 20 % most efficient cows require 635 kg of feed less per lactation than the least efficient cows (bottom 20%) (VanRaden *et al.*, 201; Yao, 2016) hence, RFI has economic value. The use of genomic selection for feed efficiency in US dairy will depend upon maintenance of a reference population to re-calculate marker effects and maintain GEBV accuracy. Currently, measures of feed efficiency, like RFI, are limited to research facilities that can determine individual cow feed intake, BW, body condition score, and milk energy output. Today, automated sensor technology is being used on many commercial dairies in the US, providing an opportunity to use this data for genetic improvement (Neethirajan *et al.*, 2017). Sensor data, combined with biological traits like milk spectra, may allow development of predictive models for feed efficiency traits like RFI (Pahl *et al.*, 2015).

Although economic benefits of selecting for improved feed efficiency are clear, the environmental impact is not. Enteric methane represents ~30% of US methane emissions from agriculture (US EPA, 2018). Predictions of cattle methane emissions are based primarily on DMI and are not very accurate (Ellis *et al.*, 2010). The relationships between feed efficiency and enteric methane emissions in dairy cows, have not been sufficiently studied. Some attempts have been made to use mathematical models to estimate a cow's enteric methane emissions from milk spectral data (Vanlierde *et al.*, 2018). Such models could allow enteric methane emissions to be estimated without direct measurement.

This project will allow direct selection for improved feed efficiency based on DMI data recorded on thousands of cows. In addition, it will allow indirect selection for improved feed efficiency based on predicted intakes of hundreds of thousands of cows, where these predictions are derived from sensors that record location, physical activity, and rumination data in real-time, as well as routine laboratory analyses that provide mid-infrared (MIR) spectral data of milk samples. The impact of this project is to increase the efficiency and sustainability of dairy production addressing the following identified gaps: 1) The reliability of the GEBV for RFI is too low, 2) The cost of measuring DMI of individual cows is too high, and measuring individual intakes on commercial farms for genetic or management purposes is infeasible at present, 3) The dairy industry needs leadership to translate research to practice in the form of a sustainable reference population for feed efficiency, and 4) The relationship of RFI to overall sustainability, and particularly enteric methane emissions is not known. The project presented herein has 4 specific aims:

1. to increase the reliability of genomic predictions for feed efficiency,
2. to develop a feed intake index that uses sensors to predict feed intake on individual cows,
3. to initiate a long-term program for updating genomic predictions of feed efficiency, and

4. to determine if genomic predictions of feed efficiency can decrease methane emissions.

Ultimately, feed efficiency would be improved by including genomic breeding values for feed intake in the US Net Merit Index.

Methods

A total of 3600 lactating Holstein cows will be assigned to the project for a period of 5 years (2019-2024) and will be studied for 42 days between 50 and 200 days of lactation in cohort groups (Fig 2). Only data between 50 and 200 DIM are collected in this project, BW is more stable within this window than outside it, minimizing errors in RFI determinations due to BW change. A cohort group of cows is fed the same diet at the same time at the same location. Diets are total mixed rations fed *ad libitum.* Briefly, Milk yields (**MY**), DMI, BW, Body condition score (BCS) fat (**FAT%**), protein (**PROT%**), and lactose (**LACT%**) components, and other sensor-based data are collected from Holstein cows on 5 research stations: Iowa State University (**ISU**; Ames), Michigan State University (**MSU**; East Lansing), the University of Florida (**UF**; Gainesville), the University of Wisconsin-Madison (**UW**), and the USDA Animal Genomics and Improvement Laboratory (**AGIL**; Beltsville, MD) as in Tempelman *et al.* (2015). Body temperature is recorded by sensors in the vagina (iButton thermosensor) and MIR milk spectra are collected during the experimental period. Methane emission will be measured on 300 cows using a GreenFeed system (C-Lock, Inc., Rapid City, SD). Cows visit the system throughout the day. Only cows with more than 36 methane measurements taken within a 24-hour will be included in the result analysis.

Results

Since the beginning of the project, feed intake phenotypes corresponding to a total of 1824 cows have been collected and added to the current CDCB feed intake database which contains a total 6577 records. From these records, 6,221 phenotypes of residual

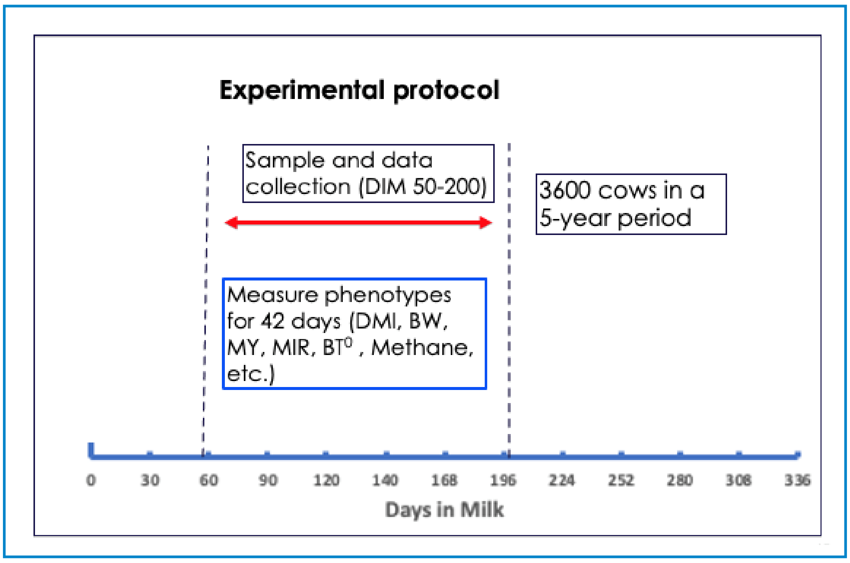

Figure 2. Experimental protocol.

feed intake (RFI), from 5,023 Holsteins born from 1999 to 2017, were included in the official predicted transmitting abilities (PTA) for Feed Saved in Holsteins, released by the CDCB in December 2020. Additionally, middle infrared (MIR) milk spectral data have been collected from 1149 cows, and methane emission were measured in 81 cows at the AGIL-USDA research station. During the year 2020 the CDCB-FFAR project has involved 16 undergraduate students, 8 graduate students, and 3 postdocs. In terms of visibility and technology transfer the project has generated 2 scholarly publications, 2 abstracts/conference presentations, 7 presentations, 3 extension trade articles, and various popular publications. This study is supported by the Foundation for Food and agricultural Research (FFAR) and the CDCB and will continue to add phenotypes to the US feed efficiency database until 2024.

List of references

Boichard, D., V. Ducrocq, and S. Fritz. 2015. Sustainable dairy cattle selection in the genomic era. J. Anim. Breed. Genet. 132:135–143.

Britt, J H., R.A. Cushman, C.D. Dechow, H. Dobson, P. Humblot, M.F. Hutjens, G.A. Jones, P.L. Ruegg, I.M. Sheldon, and J.S. Stevenson. 2018. Invited review: Learning from the future - A vision for dairy farms and cows in 2067. Journal of Dairy Science 101:3722-3741.

CDCB 2021, *https://queries.uscdcb.com/eval/summary/trend.cfm*

Davis S.R., K.A. Macdonald , G.C. Waghorn, R.J. Spelman. 2012.feed intake of lactating Holstein-Friesian cows predicted from high-density genotypes and phenotyping of growing heifers. J Dairy Sci. 2014 Mar;97(3):1436-45. doi:10.3168/jds.2013-7205.

Ellis, J.L., A. Bannink, J. France, E. Kebreab, and J. Dijkstra. 2010. Evaluation of enteric methane prediction equations for dairy cows used in whole farm models. Global Change Biology 16:3246– 3256.

García-Ruiz, A., J.B. Cole, P. M. VanRaden, G.R. Wiggans, F.J. Ruiz-López, and C.P. Van Tassell. 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Proceedings of the National Academy of Sciences of the United States of America 113: E3995-4004.

Hardie, LC., M.J. Van de Haar, R.J. Tempelman, K.A. Weigel, L.E. Armentano, G.R. Wiggans, R.F. Veerkamp, Y. de Haas, M.P. Coffey, E.E. Connor, M.D. Hanigan, C. Staples, Z. Wang, J.C.M. Dekkers, and D.M. Spurlock. 2017. The genetic and biological basis of feed efficiency in mid-lactation Holstein dairy cows. J. Dairy Sci. 100:9061–9075.

Li, B., P M. van Raden, E. Gunal, D. J. Null, E.E. Connor, M.J. Van de Haar, R.J. Tempelman, K.A. Weigel, and J.B. Cole. 2020. Genomic prediction of residual feed intake in U.S. Holstein dairy cattle. Journal of Dairy Science 103:2477-2486.

Lu, Y., M.J. Van de Haar, D.M. Spurlock, K.A. Weigel, L.E. Armentano, E.E. Connor, M. Coffey, R.F. Veerkamp, Y. de Haas, C.R. Staples, Z. Wang, M.D. Hanigan, and R.J. Tempelman. 2018. Genome-wide association analyses based on a multiple-trait approach for modeling feed efficiency. J. Dairy Sci. 101:3140–3154.

Meuwissen, T. H. E., B. J. Hayes, and M. E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819-1829.

Neethirajan, S., S. K. Tuteja, S.-T. Huang, and D. Kelton. 2017. Recent advancement in biosensors technology for animal and livestock health management. Biosensors and Bioelectronics 98:398-407.

Pahl, C., E. Hartung, A. Grothmann, K. Mahlkow-Nerge, and A. Haeussermann. 2015. Suitability of feeding and chewing time for estimation of feed intake in dairy cows. Animal 10(9):1507-1512.

Tempelman, R.J., D.M. Spurlock, M. Coffey, R.F. Veerkamp, L.E. Armentano, K.A. Weigel, Y. de Haas, C.R. Staples, E.E. Connor, Y. Lu, and M.J. Van de Haar. 2015. Heterogeneity in genetic and nongenetic variation and energy sink relationships for residual feed intake across research stations and countries. J. Dairy Sci. 98:2013–2026.

Tempelman, R.J., and Y. Lu, Y. 2020. Symposium review: Genetic relationships between different measures of feed efficiency and the implications for dairy cattle selection indexes. J Dairy Sci 103:5327-5345. *https://doi.org/10.3168/jds.2019-17781*.

US EPA. 2018. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2016. EPA 430-R-16- Washington, D.C. *https://www.epa.gov/sites/production/files/201801/documents/2018_complete_report.pdf*.

Van de Haar, M.J., L.E. Armentano, K. Weigel, D. M. Spurlock, R.J. Tempelman, and R. Veerkamp. 2016. Harnessing the genetics of the modern dairy cow to continue improvements in feed efficiency. J. Dairy Sci. 9:4941–4954.

Vanlierde, A., H. Soyeurt, N. Gengler, F.G. Colinet, E. Froidmont, M. Kreuzer, F. Grandl, M. Bell, P. Lund, D. W. Olijhoek, M. Eugène, C. Martin, B. Kuhla, and F. Dehareng. 2018. Short communication: Development of an equation for estimating methane emissions of dairy cows from milk Fourier transform mid-infrared spectra by using reference data obtained exclusively from respiration chambers. J. Dairy Sci. 101:7618–7624.

Van Raden, P.M., O'Connell, E.E. Connor, M. Van de Haar, R.J. Tempelman, and K. Weigel. 2018. Including feed intake from U.S. Holsteins in genomic prediction. Page 125 in Proc. World Congress on Genetics Applied to Livestock Production, Auckland, NZ.

Yao, C. 2016. Genomic and genetic evaluation of feed efficiency and stillbirth in dairy cattle. Ph.D. dissertation, Department of Dairy Science, University of Wisconsin-Madison.

Differences in milk composition associated with enteric methane emissions

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Abstract

Milk samples from sheep were analysed for fat, protein, lactose, somatic cell counts and detailed fatty acid composition. The sheep were from two lines each of 100 adult ewes, grazed together but differing by an average of 10% in enteric methane yield (g CH_4 /kg dry matter intake). Milk samples were taken at 2, 4 and 6 weeks post lambing. Rumen fluid was also sampled for volatile fatty acid profiles and sequencing of the rumen microflora. There were significant differences in the rumen microbiome, rumen volatile fatty acids and milk fatty acid composition between the two lines. This suggests that the ruminant hosts have been co-selected for divergent fermentation profiles affecting milk composition. The next step is to explore whether milk composition profile in ruminants is a potential predictor of enteric methane status. These results have important implications for the selection of low methane emitting ruminants and subsequent effects on product composition.

Keywords: methane, milk, rumen, microbiome.

Introduction

Around 1/3 of New Zealand's greenhouse gases are emitted as enteric methane, a by-product of ruminant digestion (Mfe, 2020). Heritable individual variation in enteric emissions has been shown in ruminant livestock populations (Pinares-Patino *et al*. 2013, Jonker *et al*., 2018), and selection for lowered methane emissions has been shown to be an effective mitigation tool in sheep (Pinares-Patino *et al*., 2013). As enteric methane is produced during the fermentation of ingested feed to energy sources for the animal, this selection against methane production has been associated with different microbial populations in the gut and differing amounts of volatile fatty acids in rumen outflow (Jonker *et al*., 2019, 2020). Sheep selection lines exist that have been bred divergently for 3 generations creating two divergent lines that differ in methane emissions by approximately 11% (Rowe *et al*., 2019). Because these sheep selection lines that are divergent for methane emitted per kg DMI also differ in fermentation energy sources to the animal, and in rumen microbial composition, we hypothesised that synthesis of milk fatty acids and therefore milk composition may also vary.

Methods

Ewes To create the selection lines, the 100 lowest emitting and the 100 highest emitting ewes and the 10 lowest and 10 highest emitting sires were selected from an initial population of 1000. Lines were closed and selected over 3 generations for high and low enteric methane emissions (CH_4) per kg of dry matter eaten (DMI) to attain an average of 11% difference. Ewes from both lines were grazed together on a mixed ryegrass-clover pasture.

Milk At 3 time-points post lambing, at 2-weekly intervals starting 2 weeks after the first ewe lambed, all ewes were taken off pasture at 8am. Lambs were removed and after one hour a milk sample was collected from each ewe. The foremilk was discarded and a mixed sample of ~25ml was collected from both teats. A 5-ml sample of milk was processed, and the fatty acids measured as methyl esters, using gas chromatography as described by Agnew *et al*. (2019). The remaining 20ml was sent to the LIC testing laboratory (Christchurch, NZ) for standard herd test profiling of fat, protein, lactose and somatic cell count.

Rumen Fluid. Within 30 min of milking, a 30-ml rumen fluid sample was collected via stomach intubation. Short chain fatty acid analysis was carried out using a 2-ml subsample, as described by Attwood *et al*. (1998). The remainder was snap-frozen and freeze dried prior to DNA extraction and microbial sequencing as described by Hess *et al*. 2020.

Methane All selection line ewes were measured for methane emissions through portable accumulation chambers (Jonker *et al*., 2018) at 4 and 6 weeks post lambing. Breeding values for each ewe were estimated to ensure that differences were retained in early lactation.

Analysis Data were analysed using univariate linear mixed models. Models (1) and (2) were fitted for each trait using ASREML v4.1 (Gilmour *et al*. 2015).

$$y = \mu + cdat*bg + age + nll + lwt + line + pe \qquad (1)$$

$$y = \mu + cdat*bg + age + nll + lwt + M \qquad (2)$$

where

y is the trait of interest, cdat is the collection date of the sample, bg indicates if the ewe lambed late or early, age is the ewe's age (years) at sampling, nll is the number of live lambs, lwt is the ewe's live weight (kg) at sampling, line is the methane line (low or high), pe is the permanent environment random effect, and M is the reference-based microbial relationship matrix computed as described by Hess *et al*. (2020). Model (1) was fitted to investigate the effect of selection line on each trait while Model (2) was fitted to estimate the microbiability (proportion of variance explained by the rumen microbial profile).

Results and discussion

Average milk constituents for the two selection lines from a standard milk test are given in table 1. No significant differences were found between the high and low methane selection line sheep for total fat, total protein or lactose. Significant differences were reported for somatic cell count and for methane breeding value. There were no obvious reasons why the low line sheep should have higher somatic cell counts. Udders were in good condition with no signs of infection. Sheep had been grazed together since immediately post mating. The low line sheep have been previously shown to have greater parasite resistance (Rowe *et al*., 2019). This may suggest potential differences in immune status.

In contrast to the non-significant differences in total fat percentage, there were clear differences in individual milk fatty acids analysis between the selection lines (Table 2).

In particular, medium chain fatty acids were lower in the low methane emitting sheep, and polyunsaturated fatty acids were higher. There was also a significant difference between the lines in iso C14 and anteiso C15 and C14:1 (Table 2). The relationship between rumen fermentation, bio-hydrogenation and the presence of odd and branched chain fatty acids in milk was reviewed by Vlaeminck (2006). These iso and anteiso acids in the milk might be related to the ruminal iso volatile fatty acids (Table 3). Regardless of whether this is the case and they are from de novo synthesis in the rumen or derived from propionate in the mammary gland, they warrant further investigation as potentially important predictors in milk.

Table 3 reports differences in the volatile fatty acids measured in rumen fluid of the lines. In particular, there were differences in acetic acid and in the ratio of acetic to propionic acids. Propionate dominated fermentations are often associated with lower methane emissions due to lower hydrogen production. Similar results were reported by Jonker *et al*. (2020). Table 4 shows the variance explained by the rumen microbes present, which was estimated using the microbial relationship matrix M in model 2. There is a clear link between the microbial community composition obtained by sequencing and described by Hess *et al.*(2020), and the fatty acids found in the milk and the volatile fatty acids in the rumen. Next steps are to measure methane emissions from animals identified with divergent profiles and to explore the impact that changes in fatty acid composition of milk may have on neonatal nutrition and product processing.

Table 1. Average milk constituents of high and low selection line sheep.

	Low	High	P-value
Fat (%)	5.07	5.00	0.910
Protein (%)	5.04	5.14	0.169
Lactose (%)	5.79	5.78	0.451
Total solids (%)	16.28	16.28	0.797
Somatic cell count ,000	260	182	<0.001*
Methane yield g/kg DMIbreeding value	–1.22	+1.14	<0.001*

*Significant at 5% threshold.

Table 2. Fatty acid composition of milk from high and low selection line sheep.

Fatty Acid (%)	Low – High	% Diff from high	P-value
iso C14	0.009	8.2	<0.001*
C14:0	–0.199	–2.6	0.218
iso C15	0.008	3.2	0.077
anteiso C15 +C14:1[1]	0.022	4.0	0.021*
C16:0	–0.455	–2.6	0.024*
C18:1 t9	0.012	4.8	0.036*
C18:1 t11	0.613	8.7	0.001*
C18:1 c11	0.032	9.7	0.018*
C18:2 n6	0.082	13.0	<0.001*
C18:3 n3	0.176	17.3	<0.001*
C20:0	–0.006	–5.0	0.008*
CLA	0.255	10.9	0.001*
SFA[2]	–1.220	–2.8	<0.001*
PUFA[3]	0.512	12.9	<0.001*

[1] Antesiso C15 and C14:1 could not be separated in the spectral analysis and are reported together.
[2] SFA = saturated fatty acids = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0.
[3] PUFA = polyunsaturated fatty acids = CLA + C18:2 n6 + C18:3 n3.
*Significant at 5% threshold

Table 3. Volatile fatty acid (VFA) profile of rumen fluid.

Rumen VFAs	Low – High	% Diff from high	P-value
Concentrations			
Acetic (mM)	–4.423	–7.9	0.009*
Butyric (mM)	–0.555	–7.0	0.060
Caproic (mM)	0.013	6.1	0.243
Isobutyric (mM)	–0.106	–9.4	0.012*
Isovaleric (mM)	–0.124	–10.2	0.030*
Propionic (mM)	–0.837	–5.2	0.122
Valeric (mM)	–0.062	–6.5	0.113
Total(mM)	–4.423	–7.9	0.009*
Proportions[1]			
Acetic		–0.4	0.346
Butyric		–0.8	0.073
Caproic		4.6	0.013*
Isobutyric		–1.5	0.282
Isovaleric		–2.1	0.261
Propionic		1.1	0.003*
Valeric		–0.3	0.733
Ratios			
Acetic/Propionic	–0.102	–2.9	0.012*
(A + B)/(P + V)[2]	–0.104	–2.7	0.009*

[1] Proportions were log transformed to satisfy assumptions of homogeneous variance when fitting the models.

[2] (A + B)/(P + V) = (Acetic + Butyric)/(Propionic + Valeric).

*Significant at 5% threshold

Table 4. Proportion of variance of milk fatty acids (FA) and volatile fatty acids (VFA) explained by the rumen microbial profile.

Milk FAs (%)	Variation explained	Rumen VFAs (%)	Variation explained
iso C14	0.13 ± 0.05 *	Proportions	
C14:0	0.07 ± 0.04	Acetic	0.30 ± 0.07 *
iso C15	0.10 ± 0.04 *	Butyric	0.34 ± 0.06 *
anteiso C15 + C14:1	0.10 ± 0.05 *	Caproic	0.23 ± 0.06 *
C16:0	0.11 ± 0.04 *	Propionic	0.28 ± 0.07 *
C18:1 t9	0.09 ± 0.05 *	Valeric	0.34 ± 0.07 *
C18:1 t11	0.28 ± 0.06 *	Ratios	
C18:1 c9	0.16 ± 0.05 *	Acetic/Propionic	0.26 ± 0.06 *
C18:1 c11	0.13 ± 0.05 *	(A + B)/(P + V)	0.26 ± 0.06 *
C18:2 n6	0.17 ± 0.06 *		
C18:3 n3	0.26 ± 0.07 *		
C20:0	0.14 ± 0.05 *		
CLA	0.25 ± 0.06 *		
SFA	0.16 ± 0.05 *		
PUFA	0.31 ± 0.07 *		

*Greater than 2 standard deviations above zero.

List of references

Agnew M.P., Craigie C.R., Weralupitiya G., Reis M.M., Johnson P., Reis M.G. (2019). Metabolites 9: 189.

Attwood G.T., Klieve A.V., Ouwerkerk D., Patel B.K.C. (1998). Ammonia-hyperproducing bacteria from New Zealand ruminants. Applied and Environmental Microbiology 64:1796-1804

Gilmour A.R., Gogel B.J., Cullis B.R., Welham S.J. and Thompson R. (2015). ASReml User Guide Release 4.1, VSN International Ltd, Hemel Hempstead, HP1 1ES, UK.

Hess, M.K., Rowe, S.J., Van Stijn, T.C., Henry, H.M., Hickey, S.M., Brauning, R., McCulloch, A.F., Hess, A.S., Kirk, M.R., Kumar, S., Pinares-Patiño, C., Kittelmann, S., Wood, G.R,, Janssen, P.H., McEwan, J.C.. (2020). A restriction enzyme reduced representation sequencing approach for low-cost, high-throughput metagenome profiling. PLoS One 15: e0219882.

Jonker, A., Hickey, S.M., McEwan, J.C., Rowe, S.J., Janssen, P.H., MacLean, S., Sandoval, E., Lewis, S., Kjestrup, H., Molano, G., Agnew, M., Young, E.A., Dodds, K.G., Knowler, K., Pinares-Patiño, C.S. (2019). Genetic parameters of plasma and ruminal volatile fatty acids in sheep fed alfalfa pellets and genetic correlations with enteric methane emissions. Journal of Animal Science 97: 2711-2724.

Jonker A., Hickey S., Boma P., WoyimoWoju C., Sandoval E., MacLean S., García RendónCalzada, M., Yu W., Lewis S., Janssen, P.H., McEwan J.C. and Rowe S. (2020). Individual-level correlations of rumen volatile fatty acidswith enteric methane emissions for ranking methane yieldin sheep fed fresh pasture.Animal Production Science 61: 300-305.

Jonker, A., Hickey, S.M., Rowe, S.J., Janssen, P.H., Shackell, G.H., Elmes, S., Bain, W.E., Wing, J., Greer, G.J., Bryson, B., MacLean, S., Dodds, K.G., Pinares-Patiño, C.S., Young, E.A., Knowler, K., Pickering, N.K., McEwan, J.C. (2018). Genetic parameters of methane emissions determined using portable accumulation chambers in lambs and ewes grazing pasture and genetic correlations with emissions determined in respiration chambers. Journal of Animal Science 96:3031-3042.

MfE (2020). New Zealand's Greenhouse Gas Inventory 1990-2018. In Ref. MFE 1496. Ministry for the Environment, Wellington, New Zealand.

Pinares-Patiño C.S., Hickey S.M., Young E.A., Dodds K.G., MacLean S., Molano G., Sandoval E., Kjestrup H., Harland R., Hunt C., Pickering N.K., McEwan J.C. (2013) Heritability estimates of methane emissions from sheep. Animal 7:316-321.

Rowe S.J., Hickey S.M., Jonker A., Hess M.K, Janssen P., Johnson T., Bryson B., Knowler K., Pinares-Patiño C., Bain W., Elmes S., Young E., Wing J., Waller E., Pickering N., McEwan J.C. (2019). Selection for divergent methane yield in New Zealand sheep - a ten-year perspective. Proceedings of the 23rd Conference of the Association for the Advancement of Animal Breeding and Genetics (AAABG), Armidale, New South Wales, Australia, pp.306-309.

Vlaeminck, B., Fievez, V., Demeyer, D., Dewhurst, R. J.(2006). Effect of forage:concentrate ratio on fatty acid composition of rumen bacteria isolated from ruminal and duodenal digesta. J Dairy Sci 89(7): 2668-2678.

Minimization of fertility economical loss by MIR based energy balance prediction

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Abstract

The impact of the pregnancy stage of dairy cows on milk yield, milk fat and milk protein content is well known. During the last decade researchers underlined the possibility of spectral predictions of pregnancy stage due to the detailed milk composition which can be approached thanks to the Mid-infrared (MIR) spectroscopy. MIR is using the infrared light from the electromagnetic spectrum which shows specific absorption patterns, when sent through a milk sample caused by frequency dependent interactions with the chemical bonds of the chemical milk components. MIR is a non-expensive and routinely used method for major milk components and also for fine milk composition analysis. One of the outcomes from those researchs was the proposed use of MIR pregnancy tools for choosing the better insemination period. The results were indicating that at the beginning of the pregnancy some areas of the MIR spectra were more specifically affected, than the absorption patterns of the fat or protein content. The aim of this paper is to study the impact of the energy balance (EB-NEL) at the insemination day and to underline the economic loss based on milk MIR spectra predictions. The objective was to show, that it is possible to help milk recording organization advisors to use EB-NEL-based MIR milk spectra predictions not just in health issues, but also in choosing the right insemination period. It was observed that this approach could be used for further studies aiming to develop MIR strategies based on EB-NEL for management strategies for improved insemination success and the reduction of fertility economical loss.

Keywords: Fertility, insemination management, energy balance, mir milk spectra, dairy cow, dairy farming, controlling.

Introduction

Mid Infrared (MIR) spectroscopy is using the infrared light from the electromagnetic spectrum which shows specific absorption patterns when sent through a milk sample caused by frequency dependent interactions with the chemical bonds of the chemical milk components. With the help of milk MIR spectra, a wealth of information can be obtained by establishing relationships with reference methods. MIR is a non-expensive and routinely-used method for major milk components and also for fine milk composition analysis. During the last 15 years different researchers were developing new calibration models with the help of milk MIR spectral data linked to milk main components such as fatty acids (Grelet C. *et al*. 2014) or minerals (Soyeurt H. *et al*. 2009), milk biomarkers such as ketone bodies (Grelet C. *et al*. 2016) in milk, inflammation indicators or complex components such as blood components (BHB, NEFA, Glucose, IBF1, Insulin, Calcium) (Dale *et al*. 2020) and traits like energy balance (NEL and ME) (Dale L. *et al*. 2019). At Regional Association for Performance Testing in Livestock Breeding of Baden-Württemberg (LKV B.W.), due to the collaborations with German Association for Quality and Performance Testing e.V. (DLQ) and optiKuh Consortium, data from feeding and breeding experiments from the 12 optiKuh research farms, together with spectral data was used to perform MIR calibrations based on energy balances NEL (GfE, 2001) and ME (Susenbeth, 2018). The models were robust and statistical accurate and can be used for rough screening (Dale *et al*., 2019). Moreover, in the last decade the research focus was on the proposal of using milk MIR spectral data for predictions of pregnancy information into tools that could give the information to the farmer if a cow is open or pregnant. In the early 2013 a Belgian team has developed a first model based on MIR standardized spectral data and pregnancy information. Laine *et al*., (2015) was the pioneer study, trying to build a model based on open and pregnant cows with the help of MIR spectral data. The results showed that there is a strong link between the pregnancy and the lactation stage. Also, the Bavarian team (Kammer *et al*., 2015) tried to build a model in late lactation stage but was unsuccessful. Laine *et al*., (2017) pointed out, that for some specific wave numbers of the milk MIR spectrum the relative effect of pregnancy is higher than on fat and protein content at the beginning of the pregnancy stage. In 2020, an Australian team (Dehlez *et al*., 2020) tried to develop a similar model as the Belgium team with the result, that in the first 100 lactation days it is not possible to see a difference between open and pregnant cows. Meanwhile, a Scottish team developed a deep learning model, where the cow pregnancy status was predicted using MIR spectral data (Brand *et al*., 2021). Unfortunately, until now no researcher found a proper model to be useful for the Milk Recording Organization as a working tool of the monthly milk recording. For dairy farmers a large economic loss is caused by fertility issues. An early identification of pregnant cows could be a key element for the improvement of reproductive performances and reduction of economic losses. It is well known that the veterinary services cost is varying from 30€ to 90€ per day and depending on the issues in the farm the costs are increasing. Also, the insemination costs by veterinary are ranging from 15€ to 35€ per cow depending on the quality of insemination material, while the insemination costs by farmer are between 12€ and 20€ per cow for work plus 6€ to 20€ for insemination material. Seen all this information it was decided to explore what was wrong at the insemination day and to try to have a better insemination rate. The objective of LKV B.W. is to help dairy farmers with the close monitoring of their dairy cows, including the detection of fertility problems using EB-NEL predictions. The aim of this study was to see the influence of pregnancy stage in the spectral data and also the impact of the EB-NEL at insemination day and to underline the economic loss due to multiple inseminations. The objective was to show, that it is possible to help milk recording organization consultants and advisors to use EB-NEL-based MIR milk spectra predictions not just in choosing the proper feeding ration or healthy issues but also in choosing the right insemination period.

Material and methods

From the LKV BW cattle database about 288 dairy farms were selected. The selected farms are taking part in the health monitoring program “GMON Rind BW”. The data covered the complete years of calving period and the variability of days in milk for the population. The selection milk samples covered a complete period of 6 years and considered the relevant breeds Holstein, Simmental and Brown Swiss as well as the season variability. For this study indicator data and insemination data was combined together, followed by adding the veterinary diagnosis information to the selected data. Once the data was prepared the spectral data for the selected milk recording samples was added. All milk recording data from LKV B.W. is analyzed on Bentley Spectrometers. The spectral data set was first standardized by applying the OptiMIR/EMR method (Grelet *et al.*, 2015) and pre-processed by Savitzky-Golay first derivative to remove the offset differences between samples for baseline correction, before performing Legendre polynomial modeling. To identify the main variables that were positively or negatively associated with pregnancy information a Pearson correlation analysis with all MIR predictions available at EMR level but also at LKV B.W. was performed using the “corrplot” R package (Figure 1.).In order to identify the pregnant and open cows it was applied first the definition code for the pregnancy status such as: code 0 = non-pregnant, code 1 = pregnant because of calving date registration and official insemination date within the 9 months period and code 2 = open because of multiple inseminations. The 212 OptiMIR wave numbers of spectral data were used for the comparison between the pregnancy statuses (Figure 2.). Then pregnancy status data were combined with EB-NEL data.

Results and discussions

The first results were indicating that at the beginning of the pregnancy some areas of the MIR spectra were more specifically affected than the absorption patterns of the fat or protein content. Due to the Pearson correlations, itcan be observed in the Figure 1, that the EB-NEL is affected the most comparing open versus pregnant cows, showing positive correlations 0.53. Similar results in Pearson correlation were obtained for EB-NEL during the lactation stage varying between 0.50 and 0.85 for Holstein and Simmental cows respectively (Becker *et al*. 2021).Inversely, the long chain fatty acids (LCFA) were negatively correlated with -0.53. As described in literature positive correlations with pregnancy status could be found for protein, blood insulin, glucose, calcium, IGF1, fatty acids such as C10, C12 and De-Novo, EB-ME as well as all minerals such as calcium, magnesium, phosphor and potassium. Negative correlations could be found for energy corrected milk (ECM), fat to protein ratio (FEQ), Natrium, fatty acids such as C17, C18 and Preformed, as well as Ketosis indicators like BHB and NEFA in blood and milk.

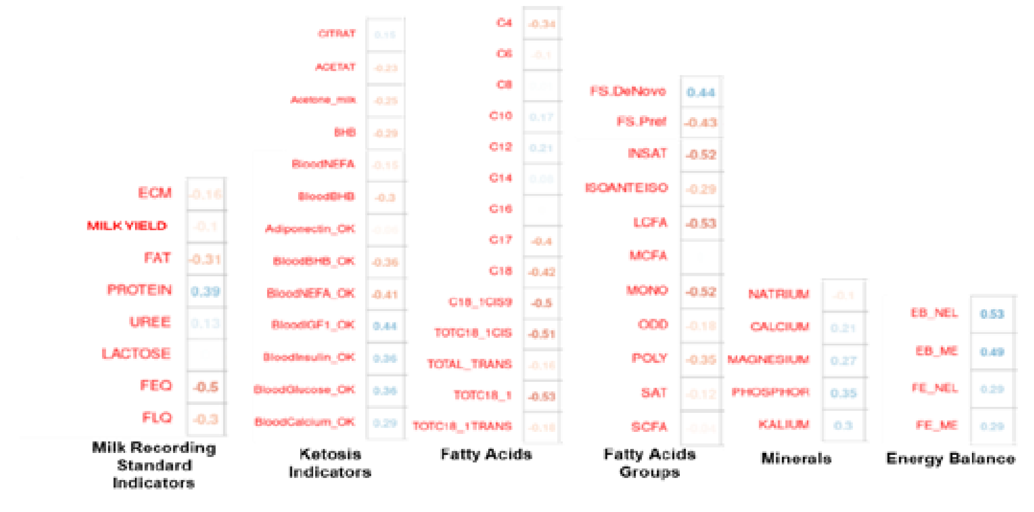

Figure 1. Pearson Correlations comparing open versus pregnant cows.

After seeing these positive and negative correlations the question came up whether there are differences in the spectral data during early lactation between open and pregnant data before and after insemination as well. Since, as described before, there is no possibility to detect with the spectral data differences between the status pregnant or not pregnant during the first 100 days in milk (DIM) it was decided to perform more statistical analysis in order to check if the spectral data are different. Especially differences in the first 4 lactation weeks, in the first 28 DIM and also in the weeks 4 to 8, from 29 to 56 DIM were of interest (Figure 2.). As the Belgian team in the 2017 explained, that the lactation stage can be seen in milk MIR spectral data, from our study it can be confirmed, that there are visible differences between pregnant and open cows before and after insemination. The green color is showing not pregnant data before being inseminated and the red one are the cows after insemination. Figure 2shows cows with 2nd insemination comparing data from 4th and 8th lactation week, between 22 and 56 DIM. Green spectra are from cows before insemination, red spectra are from cows with successful inseminations and there is blue color, that appears for spectra of open cows after an unsuccessful insemination. It was also confirmed that cows with 3rdinsemination compared with cows from the 5thand 9thlactation week, between 36 and 63 DIM, could be distinguished as open and pregnant cows after insemination.

After analyzing the spectral data and after observing the influence of the EB-NEL in the Pearson correlation, the EB-NEL influence at insemination day was analyzed. It can be underline that there were differences between spectral data after insemination, and it can be distinguished between pregnant and open cows during the first 90 DIM. The EB-NEL MJ/day was first predicted for all farms and afterwards differentiated by primiparous and multiparous cows. In Figure. 3 can be seen the mean value of the ECM and the EB-NEL in MJ/day. This differences between open and pregnant cows after inseminations were also studied per breeds and insemination numbers (Table 1). This response may be explained because high ECM dairy cows use a great deal of energy to cope with the marked increase in ECM at the beginning of lactation. This increased energy requirement is partially met by increased feed intake but at a slower rate than milk production; and by retrieval of body energy reserves which results in cows with a negative EB-NEL (Gomez *et al.*, 2018). It can be seen in the Table 1 that at the 1st insemination for all the farms the EB-NEL was positive for the pregnant cows while for the open cows it was negative.

It can also be underlined that there are differences between the breeds by regarding all farms or individual Holstein (HOL), Brown-Swiss (BSW), Simmental (SIM) farms. For the reason of better comparison, individual farms of each breed were selected having a similar feeding and husbandry system. In the case of HOL farms the 1st insemination was always after 7 till up to 10 lactation weeks (WIM), showing that the

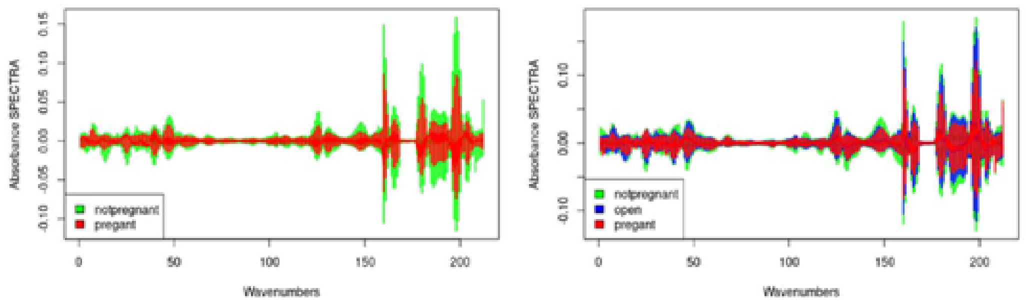

Figure 2. Spectral differences of cows after 2nd insemination between not-pregnant, open (not successful insemination) and pregnant data at 22-56 days in milk (DIM)

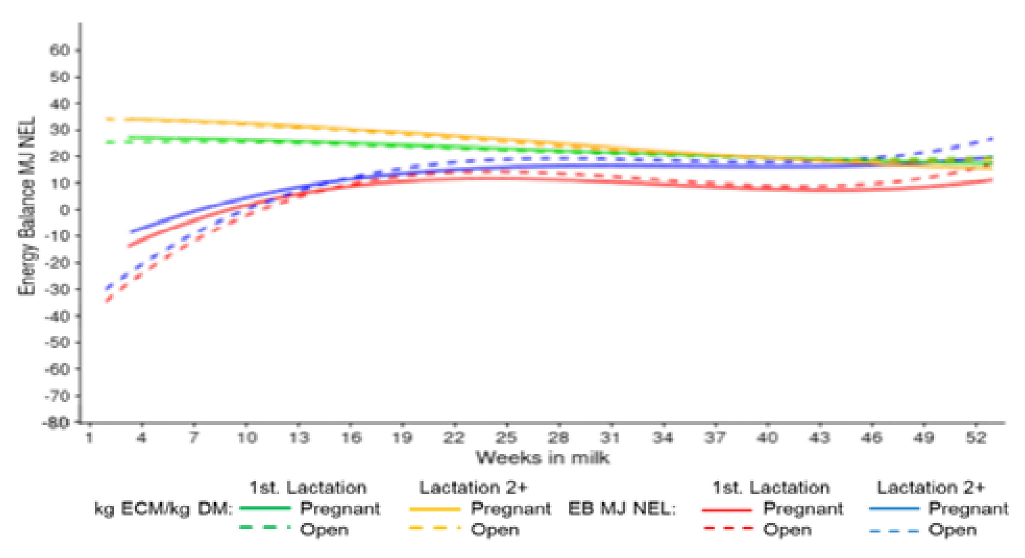

Figure 3. Mean ECM (kg/day) and EB-NEL MJ/day by primiparous and multiparous and open and pregnant cows in different lactations

herd manager has chosen a late insemination period. Therefore, the EB-NEL MJ/day has been analyzed and by comparing open versus pregnant cows after insemination in the first 10 weeks of lactation, it can be seen, that the EB-NEL is lower in the later ones. Pregnant cows had a higher net energy level (EB-NEL) as expected. In general, low pregnancy rates in dairy cows of actual breeding lines are expected due to excessive milk production. For the open cows after the 1st insemination, it can be seen, that the EB-NEL was less than -10 MJ/day and is consistent with published studies in this field (Gomez *et al*., 2018). In the case of BSW farms it can be seen, that at the 1st insemination in comparison with HOL farms the EB-NEL is higher and still open cows after the 1st insemination have EB-NEL -2 MJ/day, while the pregnant cows have more than 15 MJ/day EB-NEL. In the case of SIM farms in comparison with all other farms the EB-NEL for the open cows after the 1st insemination was almost -17 MJ/day, while for the pregnant cows was more than 9 MJ/day. The negative EB-NEL leads normally to a decrease in body condition score at calving and an increased level of beta-hydroxybutyrate, which could lead more likely to a delay of the first ovulation (Torres *et al*., 2019). Torres *et al*., 2019 found out, that cows with energy deficit are slow to ovulate and show a reduced conception rate to first service or have an increased probability of abortions and an increased calving to conception interval. In the case of 2ndinseminations, the ECM of open cows was higher as for cows that were successful inseminated and the EB-NEL for the HOL cows was lower for open cows after insemination as for the cows successful inseminated, the EB-NEL was lower than -11MJ/day for open cows and for the successfully inseminated cows was bigger as 9MJ/day, while the SIM cows was less than -20 MJ/day for open cows and for the successful inseminated cows was bigger than 10 MJ/day. In the case of BSW cows the EB-NEL for open cows was less than 2 MJ/day while for the successful inseminated was bigger than 17 MJ/day. In the case of cows with 3rd inseminations, the EB-NEL was lower for open cows than for cows that are successful inseminated, HOL: -11 vs. 9, BSW:-2 vs 18and SIM: -18 vs.12 MJ/day. Regarding the differences between the breeds, it can be notified, that open cows of the SIM are showing the lowest EB-NEL values at 1st insemination. It can be stated, that the SIM breed has therefore a better tolerance for a deficient energy level and can compensate better, than the other two

Table 1. Predicted energy-balance(EB-NEL) for cows with different pregnant status and insemination numbers according breed and farm

Insemination number	Pregnant status	All farms		All Holstein farms		One Holstein farm		All Brown-Swiss farms		One Brown-Swiss farms		All Simmental farms		One Simmental farms	
		N°	EB NEL	N°	EB NEL	N°	EB NEL	N°	EB NEL	N°	EB NEL	N°	EB NEL	N°	EB NEL
1	Pregnant	243262	13.1	93466	11.4	1368	6.6	74544	16.9	1216	15.8	66517	11.3	994	8.9
	Open	89468	-4.7	37055	-6.21	602	-10.9	28405	0.2	405	-1.8	20993	-8.7	280	-17.3
2	Pregnant	130043	14.3	53320	12.7	582	9.1	35984	12.7	591	17.8	36074	12.7	683	10.6
	Open	44530	-6.7	19216	-8.3	273	-11.5	13049	-8.3	183	2.4	10917	-10.3	158	-20.8
3	Pregnant	28367	15.2	66511	13.9	364	9.0	6744	19.0	381	17.9	18304	13.7	496	11.9
	Open	10522	-7.6	23754	-9.1	196	-10.9	17699	-1.9	125	-2.1	5829	-11.4	117	-18.3

Table 2. Percentage of open cows per different classes of predicted energy-balance (EB-NEL) MJ/day and per breed

Breeds	EB MJ NEL < - 25	EB MJ NEL -25 <--->0	EB MJ NEL = 0	EB MJ NEL 0 >---<25	EB MJ NEL > 25	Total
All Breeds	9.5%	24.2%	3.8%	55.8%	6.7%	100%
HOL	9.9%	25.5%	4.2%	56.0%	4.4%	100%
BSW	7.2%	18.5%	2.8%	59.5%	12.0%	100%
SIM	11.3%	28.2%	4.1%	51.5%	4.9%	100%

breeds with respect to fertility rates. This high tolerance is also visible at the 2nd and 3rd insemination. After checking this difference, it has been observed that the EB-NEL MJ/day was different for animals that were open after the inseminations (1st, 2nd or 3rd). The EB-NEL was different also between breeds and it can be seen that there were 2.8 % of the open BSW cows were around 0 EB-NEL in MJ/day while SIM and HOL cow had more than 4%. Also 12% of this BSW cows were open and had an EB-NEL in MJ/day more than 25 while SIM and HOL just 4.4% and 4.9%. The EB-NEL was also different in between breeds at the insemination period (Table 2). Table 2 indicates that open cows were in more than 50% in the 4th energy level (between 0 >—<25), thus in most cases they have been at a good energy level: But is is also showing, that for SIM this level was lower than for the other breeds, while in case of a negative EB-NEL the percentage in SIM was in total higher, than compared to the other breeds. This underlines again the finding of the SIM breed being more tolerant to a high variability in the energy level and showing a more constant fertility, than the other breeds in case of a decreased EB-NEL.

Conclusions

To conclude it could be notified that these results confirm findings of the literature described above. This results also confirm differences in tolerance of EB-NEL levels with respect to fertility rates between breeds. The study underlines the importance of checking the EB-NEL level of the animals before performing an insemination. It was observed that this approach could be used for further studies, aiming to develop alarm systems based on MIR predictions of EB-NEL MJ/day for management strategies and to improve the success of inseminations and to reduce respective financial losses. The future developments that could be carried out at all levels are: optimizing the insemination period at farm level by selecting the EB-NEL, reports and applications for efficient insemination period, MIR-based applications for reducing economic losses based on fertility rate.

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List of references

Becker, V. A. E., Stamer, E., and Thaller, G. 2021. Liability to diseases and their relation to dry matter intake and energy balance in German Holstein and Fleckvieh dairy cows. J. Dairy Sci., 104(1):628-643.

Brand, W., Wells, A. T., Smith, S.L., Denholm, S.J., Wall, E., and Coffey, M.P. 2021. Predicting pregnancy status from mid-infrared spectroscopy in dairy cow milk using deep learning.J. Dairy Sci., 104(4):4980-4990.

Dale, L.M., Drössler, K., Gollé-Leidreiter, F.,Spiekers, H., Hertel-Böhnke, P., Stamer, E.,Onken, F., and Werner, A. 2020.Blood parameters predictions based on milk MIR spectral data. EAAP – 71th Annual Meeting, Porto. p.506.

Dale L.M., A. Werner, H. Spiekers, P. Hertel-Böhnke, E. Stamer, F. Gollé-Leidreiter, M. Au, and F. Onken. 2019. Prediction of evaluated energy balance (NEL and ME) in dairy cows by milk mid-infrared (MIR) spectra. ICAR Technical Series 2019: 137-141

Delhez, P., Ho, P.N., Gengler, N., Soyeurt, H., and Pryce, J.E. 2020. Diagnosing the pregnancy status of dairy cows: How useful is milk mid-infrared spectroscopy? J. Dairy Sci., 103(4): 3264–3274.

GfE. 2001. Empfehlungen zur Energie- und Naehrstoffversorgung der Milchkuehe und Aufzuchtrinder. Heft 8, DLG-Verlag Frankfurt a.M.

Grelet, C., J.A. Fernández-Pierna, H. Soyeurt, F. Dehareng, N. Gengler, and P. Dardenne. 2014. Creation of universal MIR calibration by standardization of milk spectra: example of fatty acids. EAAP – 65th Annual Meeting, Copenhagen. p.108.

Grelet, C., C. Bastin, M. Gelé, J.B. Davière, M. Johan, A. Werner, R. Reding, J.A. Fernandez-Pierna, F.G. Colinet, P. Dardenne, and N. Gengler. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, beta-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. J. Dairy Sci., 99(6): 4816-4825.

Gomez, N.A. Conley, A.J., and Robinson, P.H. 2018. Effects of long-term, near-term, and real-time energy balance, and blood progesterone concentrations, on the pregnancy rate of contemporary dairy cows. Anim Reprod Sci, 189(1): 136-145.

Kammer, M., Duda, J., and Baumgartner, C. 2015. Phenotyping cows – new tools by combining MIR-spectra and DHI-data. Biotechnologie, Agronomie, Société et Environnement 19.

Lainé, A., Dale, L.M., Bel Mabrouk, H., Bastin, C. and Gengler, N. 2015. Monitoring the pregnancy status of dairy cows using mid-infrared spectra of milk collected from milk recording. BASE 19: 109-110.

Lainé, A., Bastin, C., Grelet, C., Hammami, H., Colinet, F.G., Dale, L.M., Gillon, A., Vandenplas, J., Deharend, F. and Gengler, N. 2017. Assessing the effect of pregnancy stage on milk composition of dairy cows using mid-infrared spectra. J. Dairy Sci., 100(4): 2863–2876.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres, and N. Gengler. 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. J DairySci. 89(9): 3690-3695.

Susenbeth, A. 2018. Der Energiebedarf von Milchkühen heutiger Rassen. Tagungsband optiKuh, LfL-Schriftenreihe 2: 40- 43.

Torres, E., García, J. E., Véliz, F. G., Leyva, C., Macías-Cruz, U., Hernández-Bustamante, J. D., and Mellado, M. 2019. Influence of blood metabolites and body condition score at parturition on fertility and milk yield in Holstein cows. Revista Colombiana De Ciencias Pecuarias.

Enhancing metabolic monitoring during early lactation using NEFA in blood as additional reference indicator

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Abstract

Monitoring the metabolic situation of cows during early lactation is an important part of herd management. One parameter is the occurrence of hyperketonemia - defined as the concentration of beta-hydroxybutyric acid (BHBA) in blood above a certain threshold, e.g. ≥1.2 mmol/l. Another indicator for risk of hyperketonemia from milk analysis is the fat-protein-ratio in milk. Blood BHBA with a threshold of ≥1.2 mmol/l and a fat-protein-ratio with values ≥1.5 indicating risk of hyperketonemia were combined to one reference.

This combined reference was used to build a linear discriminant analysis (LDA) prediction model on milk mid-infrared (MIR) spectral data and information about the cow. The model predicts the risk of hyperketonemia expressed in three alert levels: Green (Low risk), yellow (medium risk) and red (high risk).

Recent research has shown that BHBA in blood is not the only important indicator for metabolic stress in early lactation. The proposed separation values for non-esterified fatty acids in blood (NEFA) of <0.39 mmol/l and ≥0.7 mmol/l were used to create a reference with three classes of low, medium and high risk of poor metabolic adaptation. An LDA model with milk MIR-spectra and cow information and this reference was built.

Farmers in Bavaria are provided with information from both models for cows in the first 50 day of the lactation with the report for each herd test day. This system helps farmers to detect potential problems and allows to intervene earlier.

New reference data for the three breeds Simmental, Brown Swiss and Holstein has recently become available from the Q Check project (q-check.org) and further data collection in Bavaria. This provided the opportunity to enhance the original models and to better evaluate their performance.

Keywords: FTIR spectra, metabolic monitoring, prediction model.

Introduction

Milk testing by a Dairy Herd Improvement Association provides the farmer with the fat-protein-ratio (FER) as a basic tool to evaluate the metabolic situation of dairy cows.

Availability of the MIR spectra for milk samples gives the opportunity to look into models which use more information as predictors for dangerous metabolic situations of dairy cows. The focus of our research was on the early lactation, i. e. 5 to 50 days in milk.

Two references were used: The concentration of for non-esterified fatty (NEFA) acids in blood and the concentration of beta-hydroxybutyric acid (BHBA) in blood combined with the FER. Blood samples were used because they are a reliable analytic standard.

High NEFA values are associated with the body fat mobilization due to negative energy balance and are often a precursor for hyperketonemia. Research also shows that high NEFA levels alone are an indicator for dangerous metabolic stress (Tremblay, 2018).

Since 2019 Bavaria uses a dual traffic light warning system with an early warning based on the NEFA reference and alerts based on the combined BHBA and FER reference.

This paper compares the initial models with the new models based on a much larger dataset.

Materials and methods

Blood and milk samples were collected weekly from 5 to 50 days in milk (DIM). The initial dataset contains data from 26 farms. 359 Simmental cows, 1038 with samples collected 2015-05-05 to 2016-02-11. Two projects collected data using the same protocol: Q Check (2018-01-02 – 2018-12-20) and Metalarm (2019-10-14 – 2021-02-18). A new dataset combined all data from 103 farms, 4058 Simmental, Holstein and Brown Swiss cows and 16923 samples in total.

Milk samples were analyzed on FOSS MilkoScan^{TM} Analyzers (FOSS GmbH, Hamburg, Germany) at the Bavarian Association for raw milk testing (Milchprüfring Bayern e.V.).

Blood samples were analyzed at the laboratory of the Clinic for Ruminants in Oberschleissheim for BHBA and NEFA.

For the NEFA models reference classes were low (green NEFA <0.39 mmol//l), medium (yellow, $0.39 \leq$ NEFA ≤ 0.7 mmol/l) and high risk (red, NEFA >0.7 mmol/l), For the BHBA/FER model reference classes were low (green, BHBA <1.2 mmol/l and FER <1.5), medium (yellow, BHBA $\geq$1.2 mmol/l or FER $\geq$1.5) and high risk (red, BHBA $\geq$1.2 mmol/l and FER $\geq$1.5).

MIR-spectra were restricted to wavenumbers 980 – 1580, 1728 – 1800 and 2810 – 2980 cm^{-1} to eliminate water signals and remove areas with no or redundant information. All spectra were offset corrected. For the BHBA/FER model the dataset was balanced for the reference classes with the ROSE algorithm (Menardi, 2014).

Linear Discriminant Analysis was selected as method and model quality was assessed using 10 fold cross validation. All samples from each farm were assigned to the same fold. The cross validation was run 10 times with random assignment of the data to the folds. For assignment of the predicted classes the a posteriori probability to belong to the green reference class with custom thresholds was used.

There is a large difference in the observed prevalence for the red NEFA reference, while the observed prevalence for the BHBA/FER classes are about the same in the initial and new dataset (Table 1). There are also distinct differences in the overserved NEFA reference prevalence for the breeds in the new dataset, while the differences in the BHBA/FER reference prevalence are much smaller (Table 2).

Table 1. Observed prevalence for the reference classes in the datasets.

Reference Class	NFEA Reference				BHB/FER Reference			
	Initial Dataset		New Dataset		Initial Dataset		New Dataset	
	Samples	%	Samples	%	Samples	%	Samples	%
Green	551	53	13038	78	782	75	12514	74
Yellow	277	27	2671	15	214	21	3708	22
Red	210	20	1214	7	42	4	701	4
All	1038		16923		1038		16923	

Table 2. Observed prevalence for the NEFA (left) and BHBA/FER reference (right) classes in the new dataset for each breed.

Ref. Class	NEFA Reference			BHBA/FER Reference		
	Simmental	Holstein	Brown Swiss	Simmental	Holstein	Brown Swiss
Green %	70	83	86	74	73	78
Yellow %	19	13	11	22	24	18
Red %	10	5	3	5	3	4
Samples	8604	6076	2243	8604	6076	2243

Results

Final model quality was evaluated using the percentage of correct predictions for the green reference class (prediction and reference green) and the percentage of the false red predictions (prediction red and reference green). We think these are the most important measures for the farmer because the farmer sees the predictions and not the references and needs to get an idea how reliable the predictions are.

The NFEA models build with the new dataset shows an improvement in the correct green status messages and a worsening in the false red status messages compared with the evaluation of the initial models. There are clear differences between breeds although breed specific thresholds were used.

For the BHBA/FER there are only minimal changes and the quality is the almost the same across breed due to the breed specific thresholds.

Percentage of red status messages per month

The following results use status message instead of predicted class/predictions. The figures show the percentage of red status messages for each month from 2019 to 2020 with the initial models on the left and the new models on the right.

Status messages and culling rate

To investigate the connection between status messages and culling the last milk testing day with status messages was used with a mean day in milk of 33. Culling within 30 days after the testing date counted towards the culling rates in Table 6. For evaluation of significance the table includes 99.9 % confidence for the culling rate.

Table 3. Probality thresholds and percentages of correct status messages for the NEFA model

	Samples	Reference green %	Reference yellow %	Reference red %
Predicted Class	**Simmental initial dataset (yellow < 80 %, red < 5 %)**			
Green	503	73	21	6
Yellow	401	41	32	27
Red	134	13	33	53
	Simmental new dataset (yellow < 77 %, red < 16 %)			
Green	6122	85	13	2
Yellow	1277	50	35	15
Red	1205	20	36	44
	Holstein new dataset (yellow < 77 %, red < 10 %)			
Green	5026	92	7	1
Yellow	635	51	36	13
Red	415	21	40	40
	Brown Swiss new dataset (yellow < 67 %, red < 4 %)			
Green	1924	92	7	1
Yellow	224	53	35	12
Red	95	25	46	28

Table 4. Probality thresholds and percentages of correct status messages for the BHBA/FER model.

	Samples	Reference green %	Reference yellow %	Reference red %
Predicted Class	**Simmental initial dataset (yellow < 38 %, red < 5 %)**			
Green	756	93	6	0
Yellow	179	42	50	8
Red	102	0	73	26
	Simmental new dataset (yellow < 29 %, red < 3 %)			
Green	6352	94	6	0
Yellow	1863	26	65	9
Red	389	0	65	35
	Holstein new dataset (yellow < 30 %, red < 2 %)			
Green	4433	95	5	0
Yellow	1431	17	76	7
Red	212	0	69	31
	Brown Swiss new dataset (yellow < 37 %, red < 7 %)			
Green	1734	94	6	0
Yellow	328	30	59	11
Red	181	0	65	35

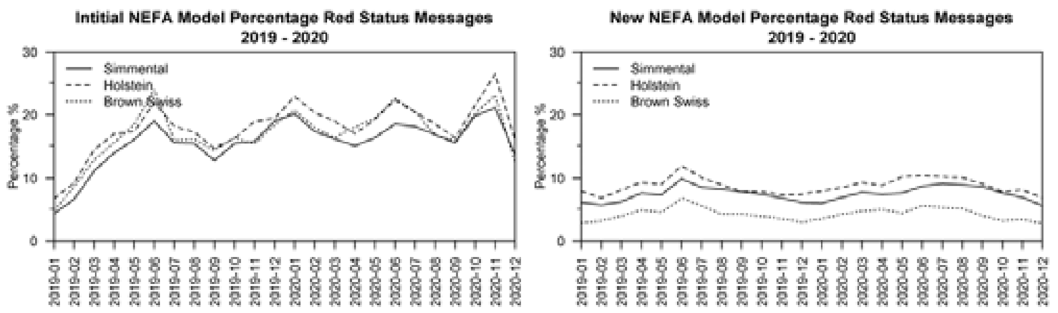

Figure 1. Percentage of red status messages of the NEFA model for each month 2019 - 2020, initial model left and new model right.

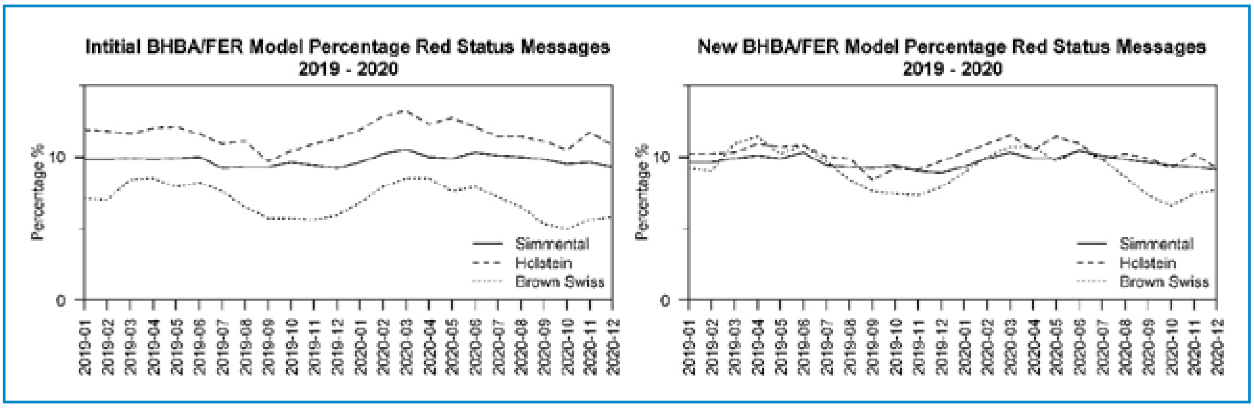

Figure 2. Percentage of red status messages of the BHBA/FER model for each month 2019 – 2020, initial model left and new model right.

Discussion

The NEFA model needed more adjustment than the BHBA/FER model. For all breed more red status messages than seem necessary were generated. Both effects can be explained by the large differences in observed prevalence. Another effect is a much less variation in the percentage over time with the model based on the new dataset. The changes in the percentage of red status messages of the BHBA/FER show the necessary adjustment for the different breed. The initial models generated more red status messages than seems necessary considering the new data, but not excessively so. As the status messages from the NEFA model were promoted as early warning we expect little impact on the farmer.

Looking at culling after the last status messages shows a significantly increased culling rate for cows with red status messages which indicates a connection between the metabolic status predicted by the models and culling.

Conclusion

New data improves our models and because of the changes in the observed prevalence also drives our model evaluation. We think that the percentage of correct predictions/status messages is a good final evaluation measure because it gauges what the farmer will see at makes it easier to communicate. In the near future we will evaluate new model algorithms to replace linear discriminant analysis.

Table 5. Last status messages with the new models for each cow 2019-2020 and culling within 30 days.

Status message	NEFA			BHBA/FER		
	Cows	%	CI 99.9%	Cows	%	CI 99.9%
Green	989350	2.6	2.6 – 2.7	896576	2.8	2.8 – 2.9
Yellow	141826	3.4	3.2 – 3.6	213425	2.9	2.8 – 3.0
Red	84972	8.4	8.1. – 8.7	106147	6.1	5.8 – 6.3
All	1216148	3.1	3.1 – 3.2	1216148	3.1	3.1 – 3.2

Acknowledgments

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List of references

Tremblay M, Kammer M, Lange H, Plattner S, Baumgartner C, Stegeman JA, Duda J, Mansfeld R, Döpfer D. 2018. Identifying poor metabolic adaptation during early lactation in dairy cows using cluster analysis. Journal of Dairy Science 101: 7311–21

Menardi, G., Torelli, N. 2014. Training and assessing classification rules with imbalanced data. Data Mining and Knowledge Discovery 28: 92–122.

Chip-size, low cost near infrared sensors for milk analysis with lab grade performance

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Abstract

In 2019, dairy market was valued at more than $ 600 Billion, and it is projected to exceed 1 trillion $ by 2024, making it one of the largest food sectors worldwide. Milk is also considered one of the most foods consumed worldwide. Therefore, milk composition and quality are two crucial factors to be measured. Milk components are measured by various technologies; most common is ultrasonic in addition to spectroscopic techniques like MIR technology. The issue with these two techniques are mainly accuracy in case of ultrasonic and very high price in case of MIR spectroscopy. In this work, the robustness and efficiency of the chip-size, low cost near-infra red sensors NeoSpectra® to accurately determine the milk components are demonstrated. Compared to other competing technologies in the market as well as some benchtop instruments, NeoSpectra is showing a much better performance and exceeding ICAR on farm standards and even close to lab standards.

The prediction models are built using cow milk taken from various regions/herds. 110 raw, fresh cow milk samples were collected from 3 different breeds and 5 different herds. Chemical analysis for fat, protein and lactose is performed using official methods of analysis of AOAC international 19th edition. Milk measurements using NeoSpectra sensors are collected using milk samples in off the shelf glass beakers and milk is filling at least 1 cm from the bottom of the beaker.

As a summary of the results, fat is measured using NeoSpectra sensors and prediction models with a coefficient of determination ($R^2 = 0.984$) and (RMSE=0.158) and testing results (R^2=0.991, RMSE=0.167, SD (standard deviation) =0.147). Protein is measured with ($R^2 = 0.891$) and (RMSE=0.078) and testing results (R^2=0.979, RMSE=0.127, SD=0.131). Lactose is measured with (R^2= 0.818) and (RMSE=0.105) and testing results (R^2=0.767, RMSE=0.111, SD= 0.114). These results show that NeoSpectra sensors and prediction models surpassed ICAR's standard for on farm analyzer which requires SD (standard deviation) = 0.25 for fat, protein and are very close to ICAR's standard for lab analyzer which requires SD = 0.1(1, 2).

In conclusion, the NeoSpectra® NIR sensors showed the ability to enable low cost milk analyzers that not only complies with ICAR standard for on-farm milk analyzers, but also exceeds it to a performance level that is very close to ICAR lab analyzer requirements.

Introduction

By 2050 the world population is expected to grow to 9.8 billion according to the UN, with that fast-growing population the demand for securing a continuous food supply at reasonable cost is pushing the farmers to the edge, so far dairy farming industry has done a remarkable progress in terms of selecting and tracking the best breeds, cow genetics improvements, artificial insemination and feed management. Farmers also spend time, effort and money on applying these high standard measures expecting an optimum outcome in terms of reproduction and milk quality and quantity. However, without a reliable proper feedback system the farm stays vulnerable to a revenue leak incidents that might not be observed until it is too late.

Among the required feedbacks for the farm managers are the real-time status of the individual animal health, the quality of milk produced per cow and the cow group performance. The industry is fully aware of the importance of these feedbacks and hence on organizational level the milk recording organizations (MRO) do a periodical farm visits. Milk samples from the milking herd are collected and analyzed. This process takes place every six weeks for the MROs' member farms. Also, on milking equipment manufacturing level, the industry tries to deploy the possible sensory solutions to afford such in-field feedbacks for its customer farms.

The MROs send the farm milk samples to a central lab and get back to the farmer with a results report within 48 hours after sampling. The report mainly involves milk constituents of fat, protein and lactose in addition to somatic-cell count (SCC) analysis. Through such data the MRO can advise the farm about a potential or existing metabolic disorder, feed diets and concentrates for cow groups and also clinical or sub-mastitis in specific cows. However, in such cases the lack of real-time data could cause the farmer to endure late decision losses.

As for automatic milking systems (AMS) manufacturers the endeavors for developing in-line and on-line measurements to achieve such real-time monitoring has been ongoing for a few years. Most of these on-line systems use reagents within its analysis process which makes it an expensive solution and complex for installation in parlors. There are other inline solutions that depend on spectroscopy technology in the near infrared range which makes it reagent-less which counts as an advantageous point, but those systems did not achieve the required accuracy yet.

Speaking of the accuracy of milk constituents' analysis, the ICAR organization have set a defining measurements maximum permissible deviation for in-line and at-line systems to be certified [1, 2]. The ICAR organization did not certify according to the author's knowledge any on-farm milk analyzers yet indicating that the industry with its different parties need to work on fulfilling this gap. A feedback solution based on real-time milk constituents' analysis to be optimum should fulfil ICAR standards, prove cost effectiveness for the farmer to deploy it and be appropriate for working in on-farm or parlor conditions. These three parameters should be realized all along in one solution to be a reliable one for the farmer.

Infrared spectroscopy has been a cornerstone for milk analysis for a long time. In fact, some mid-infrared spectrometer (MID-IRS) analyzers are currently certified according to the ICAR laboratory standards but the MID-IRS is an expensive technology to be deployed at the farm and bulky to fit in field. Nowadays, miniaturizing spectrometers and specifically near-infrared (NIRS) using Micro-electro-mechanical (MEMS) techniques has made the technology more cost affordable and more diminished in size to fit in field use. NIRS miniaturized systems successfully achieved two out of the three parameters required for being a reliable system but in a seek of cost and size reduction the accuracy of measurement is slightly sacrificed, balancing those three parameters at such micro size is a very slick iterative work.

Si-ware Systems Company has developed its own version of miniaturized Fourier Transform Near-Infrared Spectrometer (FT-NIRS) called NeoSpectra. Among the market of miniature NIRSs, NeoSpectra offers the widest measuring spectral range of 1,350 – 2,500nm. At such spectral range Si-ware has put an effort and resources to examine, iterate and improve its sensor for the dedicated use of milk constituents' analysis to reach the optimum balance of accuracy, speed and cost effectiveness requirements.

Building-up on the study presented in ICAR conference in Prague 2019 showing the performance of different NIRSs versus ICAR standards [3] we present in this work the NeoSpectra measurements, models used, reference methods applied and standard deviation (SD) resulted of raw farm milk analysis for fat, protein, lactose. Then, a conclusion of the results is presented comparing the proposed solution to the available market solutions demonstrating what the industry have accomplished to fill in the gap of presenting a real-time milk analysis reliable feedback solution for dairy farmers.

Near infrared spectroscopy theory

Spectroscopy is the study of physical and/or chemical properties of materials by analyzing their response to light. Knowing that each chemical component has a unique spectral pattern, the analysis of the spectral response of matters tells a lot about their chemical composition and/or concentration. Today, spectrometer instruments can be found in labs and industrial environments for material identification and/or quantification in different application areas. There are many conventional topologies for spectrometry instrumentation including Fourier Transform InfraRed (FT-IR) that offers several performance and cost advantages. (FT-IR) spectroscopy has evolved over several decades because of its necessity for various applications throughout the physical, chemical, and biological domains. Spectroscopy is used for identification of different kinds of materials (qualitative analysis), or in quantifying the amount of materials which is called quantitative analysis. Compared to the other technologies like dispersive instruments, FT-IR devices provide multiple advantages such as the wide spectral range, the multiplex (Felleget) advantage, higher optical throughput, the use of a single photodetector in addition to wavenumber range flexibility and measurement speed [4]. Example applications include medical analysis [5,6], food quality control [7, 8, 9] and soil analysis [10]. Near infrared (NIR) spectral instruments are having great advantage due to the lower cost of its optical components and also good performance compared to the mid infrared (MIR) range [11]. In addition, samples do not require special preparation, which preserves the sample integrity after completing the analysis. The sample can be measured either in transmission mode or in diffuse reflectance mode, where the latter is the method of choice for most solid samples or liquid samples containing scattering sites. A good comparison about different spectroscopy solutions for milk analysis shows the advantages of different ranges and the disadvantages [12].

Since every material has a unique response to light, the analysis of the light interacting with a certain material can reveal a lot of information about its composition. The analysis of light to determine the properties of materials is what spectroscopy is all about. Light interacting with materials is essentially a spectrum of electromagnetic waves with different wavelengths. Fundamentals of bands absorbed by materials are found in Mid-Infrared (MIR) region of light (wavelengths >3,500 nm). The Near Infrared (NIR) region of light is absorbed by the energy bands related to the overtones and combinations of the fundamental bands.

The unique information from the vibrational absorption bands of a molecule is reflected in the NIR spectrum, but some spectral numerical processing and statistical analysis are required to "unlock" this information. The application of statistical methods to the analysis of experimental data is known as chemometrics. NIR spectroscopy has long been used as a material analysis tool. It provides various advantages over other analysis methods including:

- Tests are non-destructive.
- Almost no sample preparation is required.
- Different parameters can be measured simultaneously.
- Ability to obtain results instantly.

Conventional FT-IR spectrometers are bulky, expensive, and sensitive to vibrations limiting their usage to the lab or controlled environments. There is a huge demand for a portable device that can be used for inline or field applications [13]. That is why many new emerging technologies have gained a lot of attention in the past decade in different industries. One of the main enabling technologies is the micro-electro-mechanical system (MEMS) [14, 15, and 16]. There are many MEMS based spectrometers reported in literature based on different technologies, including diffraction gratings [17], micro-mirror devices (DMDs) [18], multimode interference (MMI) interferometers [19], linear variable filters with photo-detector array and tunable Fabry-Pérot filters [20]. These solutions are either limited in spectral range or not scalable enough to meet the growing need of the spectral sensing market. The adoption of NIR analysis instruments has been mostly limited to labs or in-line process analyzers. This limitation was mostly due to the fact that those instruments are very bulky. Recently, portable NIR analyzers have become commercially available. However, their high price tags limited their adoption to limited use cases, or their limited performance limited the application they could enable.

NeoSpectra solutions based on MEMS FTIR technology introduces a real lab performance compared to benchtop devices while at the same time having instance and cost effective solutions for in field applications. They are based on Fourier Transform Infrared (FT-IR) technology that offers a wide spectral range for the best qualification and quantification of materials. NeoSpectra solutions operate in the Near-Infrared (NIR) from 1,350 to 2,500 nm. This wavelength range goes up to the highest point of the NIR and is the widest range versus comparable solutions.

Looking on the milk analysis industry, the most commonly commercially available milk analysis solutions are either based on wet chemistry, Ultrasound technologies, or Mid Infrared Spectroscopy Technology.

Wet chemistry provides highly accurate results. However, it requires analytical lab professionals to perform lengthy analysis processes. Hence, they can only be performed in labs and it does not enable quick analysis checks. Ultrasound technology comes at relatively low cost and is relatively easy to use, making them more adoptable across the supply chain but their results are not reliable. The accuracy of the results is highly dependent on sample composition and conditions. Mid IR technologies enable highly accurate analysis and quickly. However, their costs and size limited their adoption to labs and large stakeholders in the supply chain, leaving the rest of the supply unchecked.

In the last decade new technologies that allow miniaturizing NIR based solutions have been emerging. While these solutions are quite promising in potentially enabling ubiquitous, easy to use and quick milk analyzers, achieving the accuracy level that is acceptable across the milk supply chain has been a major challenge. To date, to the best of the author’s knowledge, there has been no commercially available solution based on NIR technology that satisfies ICAR standards for in-farm or in-lab use.

This raises the questions: How to bring the advantages of the NIR spectroscopy to the field for instant, non-destructive, easy to use, and cost effective milk analysis solution. NeoSpectra spectral sensing solutions presents a great answer for this question. The technology combines the advantages of MEMS technology, FTIR spectroscopy and artificial intelligence to enable a chip-sized, cost effective, and scalable solutions. The solutions offer performance comparable to laboratory-based spectrometers, and at the same time with instant and cost-effective performance.

Experimental results

In order to demonstrate the capability of the technology in milk analysis, NeoSpectra solutions are used in testing different milk samples in the following experiment. In order to make sure that different variations of milk samples were covered, milk was lactated from different cow types, different locations, and different farms that use different farm management systems. In numbers: 110 cows from 3 different breeds and 5 different herds were lactated to provide 110 milk samples from four different villages in two different states. Upon lactation, milk samples were stored in an ice-box until it reached the lab and stored at -20C in the lab. Before collecting measurements from stored milk samples, the milk samples are pre-warmed at 30C for 20-30 minutes. To homogenize the samples, gentle stirring (10 times clockwise and 10 anti-clockwise) is performed before each measurement. The steps for developing a model that can be used in predicting the different milk parameters is shown in Figure 1. After milk collection the milk samples are splitted in to two identical groups. First group is used to build the reference database using the standard chemical analysis methods while the other group is used to measure the spectral reference measurements using NeoSpectra solutions.

The spectral data and their corresponding reference values can be used to build mathematical data models or train AI algorithms. Those models and algorithms can be used to predict the chemical composition of samples with unknown reference values. The following steps are required in order to build the analysis models that predicts the chemical composition of milk samples:

1. Collecting various milk samples. The collected samples should cover the different variations that are expected to be found in the real use-case. The number of samples to be collected usually vary depending on the expected variations in the sample to be analyzed. Typically, a sample set of around 100 samples is good enough to have a robust analysis model.
2. Measuring reference values for the parameters of interest using reliable reference methods.
3. Measuring the spectral data of the collected samples.
4. Splitting the collected data sets to 2 parts: A calibration set that is used to build the chemometrics model, and a validation set that is used to test how well the model

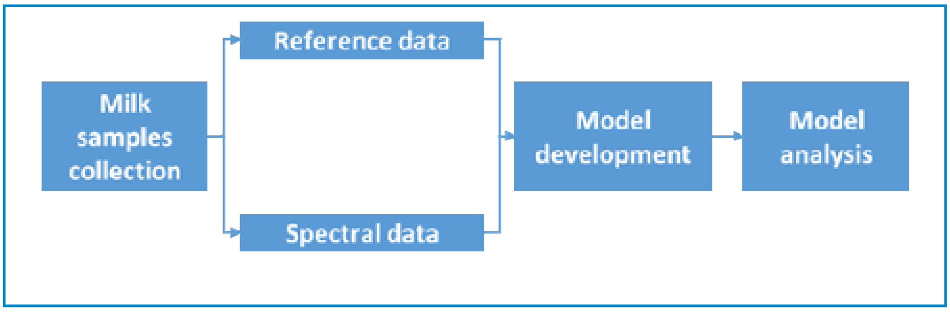


Figure 1. Model development steps.

can predict the values of the parameters for samples that were not part of the training data.

5. Data pre-treatment: Data are preprocessed to eliminate any variabilities that may affect the accuracy of the models to be developed. This includes removing the effect of any sample presentation irregularities and normalizing the different samples. [21]
6. Developing AI models using the calibration set.
7. Analyzing results by comparing the predicted values with the pre-known reference value. A study of the prediction error and bias evaluates the model performance.

Reference database building

In order to have high confidence in the prediction of NIR, it is important to make sure that accurate methods were used to collect the reference value used in building the analysis models. In other words, garbage input data to the modeling process would result in garbage output. Therefore, according to Procedure 1 of Section 12 of ICAR Guidelines - Protocol for the Evaluation of Milk Analysers for ICAR Approval the reference methods used should comply to standardized methods of at least one of the international guidelines (ISO, IDF, AOAC). In this study, chemical methods used to measure milk components, which complies with AOAC (Association of Official Analytical Chemists) official methods No. 990.20 (2012), as follows:

Total Protein Analysis – Kjeldahl

The total milk proteins were measured chemically according to Kjeldahl method. Briefly, solutions of 15.00 g K_2SO_4, 1 ml $CuSO_4$ catalyst solution and 5ml of warm milk (38°C) were mixed and weighed and immediately place in digestion flask. 25 ml H_2SO_4 were added, and digestion was conducted over heating device. Acid digest was cooled to room temperature. After digest, the mixture was cooled to room temperature, and 300 ml H_2O was added to flask and mixed by swirling. For distillation, the condenser water was turned on and 50 ml H_3BO_3 solution was added with indicator to graduated 500 ml Erlenmeyer titration flask and flask placed under condenser trip. 75 ml 50% NaOH was added to the diluted digest down sidewall of Kjeldahl flask with no agitation. Immediately flask was connected to distillation bulb on condenser. The ammonia formed is distilled into a boric acid solution containing the indicators bromocresol green and methyl red ($\geq$ 200 ml total volume). H_3BO_3 receiving solution was titrated with standard 0.1 N HCL solution until the first trace of a pink color. In order to identify the amount of the total protein, the following equation was used.

Moles of HCl = moles of NH_3 = moles of N in the sample.

A reagent blank should be run to subtract reagent nitrogen from the sample nitrogen.

$$\%N = \frac{\text{NHCl} \times \text{Corrected acid volume}}{\text{g of sample} \times 1000} \times 14 \times 100$$

where:

NHCl = normality of HCl, in mol/1000 ml. Corrected acid vol. = (ml std. acid for sample) - (ml std. acid for blank). 14 = atomic weight of nitrogen. A factor is used to convert percent N to percent crude protein = 6.38. % Protein = % N * 6.38.

Total fat analysis – Gerber method

The total fat analysis is performed using the Gerber method. The procedure is as follow : Total of 10 ml of H_2SO_4 at 15–21 °C were added into a Gerber milk bottle, and using a Gerber pipette, 11 ml milk sample 11 ml were accurately added into the Gerber bottle, and 1ml of isoamyl alcohol was added to the bottle. The mixture was shaken and centrifuged briefly for 5 minutes. The mixture was then placed in a water bath at 60–63 °C and the fat content was measured from the graduations on the bottle neck. To get an accurate measure of the fat content, the previous steps were repeated three times for the same milk sample and the fat content was determined by taking the average of the three measurements.

Lactose content determination - Fehling's Test

The lactose content is measured using the Fehling test. The procedure is as follow: 25 ml of milk was transferred to a standard 250 mL flask and 50 ml of distilled water was added in addition to 5 ml of ferrocyanide potassium solution and 5 ml zinc acetate (with flipping after each addition), and filtrated. 10 ml of Fehling's solution A ($CuSO_4.5H_2O$) was added into a conical flask followed by Fehling's solution B (sodium potassium tartrate solution) and 25 ml of the filtrate was added into the conical flask containing solutions A and B and heated to boil for two minutes. Then, three drops of methylene blue indicator were added into the boiling solution and filtrated drop wise at intervals of 10 seconds until the blue color of the methylene blue indicator disappears. The volume of filtrate used at the end point of the reaction (red color) was measured and Lactose % was calculated from *Lane-Eynone Table*.

Spectra data collection

The spectral data collection procedure should follow the same procedure that shall be used in the prediction of real use-case measurements. Therefore, it is important to keep in consideration that the lab setup used in the experiment shall enable an easy to use, stable, and repeatable sample handling and measurement procedure.

The collection of spectral data consists of two main steps:

- Background measurements: This step is important to calibrate the spectrometer to a reference spectral response and minimize any contribution from the instrument on the output spectrum. To do so, a disc of a white reference material "Spectralon" that has a flat spectral response over the NIR range is placed on top of the NeoSpectra sensor, and a background measurement is collected. This step, despite its importance, doesn't necessarily have to be done every time a new spectrum is collected, but should be done in the beginning of the measurements session or whenever any variations in the setup or the environment in which the experiment is taking place.
- Sample measurement: The samples are poured in a beaker that has a thin, flat bottom surface. An off the shelf beaker is used in this experiment which is commercially available and the material of the beaker should be transparent in the NIR range. This makes sample handling simple and an easy procedure which does not need any sample preparation. The user should only make sure that the sample has a height or thickness larger than 10 mm inside the beaker. This is to ensure that the sample thickness is larger than the penetration depth of NIR light in the milk. The beaker is then simply placed on top of the NeoSpectra sensor and a measurement is acquired. The selected scan time for each scan is 5 s.

The output spectrum consists of the ratio of sample measurement and background measurement. For each sample, 3 measurements are collected and the position of the beaker is changed after each measurement.

Data analysis and modeling

The collected spectral data for all samples were tabulated with their corresponding reference values. Data was split into two groups:

- 90% samples for calibration set.
- 10% samples for validation set.

In order to ensure the representativeness of the data used to build and validate the analysis models, the selection of the data sets was made in a way that ensures that reference values of the different parameters are well distributed across the value ranges.

To check the accuracy of the prediction models a comparison is performed between the predicted values with the reference values. This is done by plotting the reference value against the predicted value. Ideally, this plot should be an identity line. In practice, the predicted values are fitted to a regression line and model is characterized by using different statistical parameters

- **Coefficient of determination or R^2**: This parameter describes how well the predicted values fit the regression line. An ideal model has R^2 of 1. A model with R^2 of 0.75 means that the fit describes 75% of the variability of the target value being predicted [21, 22].
- **Root Mean Square Error of Prediction or RMSEP**: This parameter measures the average accuracy of the prediction. It is considered that 2 times the RMSEP represents a 95% confidence interval for the real value. For instance, if the fat prediction model has an RMSEP value of 0.1%, and the predicted value of fat content is 4%, then there is a 95% chance that the reference value of fat content for this sample is between 3.8% - 4.2%.
- **Bias**: This parameter represents the average difference between predicted values and reference values. Ideally, the value of the bias is 0. Higher values mean that the model tends to overestimate the composition, and lower values mean that model tend to underestimate the composition of the material.
- **Standard error of prediction or SEP**: This parameter measures the precision of prediction which means the difference between different measurements for the same sample. When Bias tends to 0, the SEP tends to have the same value as the RMSEP.

Modeling results

First, it is important to make sure that range of values cover the ranges to be expected in the field. In the calibration set, fat content ranged from 1.03 to 4.93, protein content ranged from 2.89 to 4.11, and lactose content ranged from 4.18 to 5.31. In the validation set, fat content ranged from 1.03 to 4.52, protein content ranged from 2.89 to 3.96, and lactose content ranged from 4.36 to 5.06. The reference values of both the calibration and validation sets were scattered across the range of the corresponding milk constituent to have a good representation of the population of the study in both sets. The figures below show the histogram of the fat, protein and lactose values of the samples collected in this study.

Regression models based on non-linear neural networks architecture [23] were built for each parameter and were tested using the validation set. Dimensionality reduction was first carried out on the spectral data to build the model based on the independent variables that best represent the dataset.

- For the fat content, the calibration model shown in figure (3) (a) achieved R^2 of 0.98 and RMSE of 0.158. The model was then validated using the validation set where it scored the following: R^2=0.99 and SEP=0.147. this is shown in figure (3) (b).
- For the protein content, the calibration model shown in figure (4) (a) achieved R^2 of 0.89 and RMSE of 0.078. The model was then validated using the validation set where it scored the following: R^2 =0.98 and SEP=0.131, this is shown in figure (4) (b).
- For the lactose content, the calibration model achieved R^2 of 0.82 and RMSE of 0.105. The model was then validated using the validation set where it scored the following: R^2 =0.77 and SEP=0.114, this is shown in figure (5) (b).
- Models of the three milk constituents show high correlation with the chemical references. Results show a high generalization to the validation set assuring the regression model robustness to new samples.

Table 1 summarizes the different results achieved for the three parameters. Biases of the three modeled parameters are all close to zero showing no shifts in predictions from actual values. SEPs of the three modeled parameters are below 0.15.

In order to judge on how those results are, they are compared to other results reported in literature [3, 24] for experiments conducted using miniaturized NIR technologies for in-field use. The results are also compared to ICAR standard for in-field and in-lab use as shown in figure (6). It can be seen that results generated using NeoSpectra are the only ones exceeding the ICAR standards for in-field use for all parameters, and their performance is very close to those of in-lab standards.

Table 1. Fat, protein, and lactose modeling results summary.

	Calibration		**Validation**			
	R2	**RMSE**	**R2**	**RMSE**	**SEP**	**Bias**
Fat	0.984	0.158	0.991	0.167	0.147	0.096
Protein	0.891	0.078	0.979	0.127	0.131	-0.033
Lactose	0.818	0.105	0.767	0.111	0.114	0.033

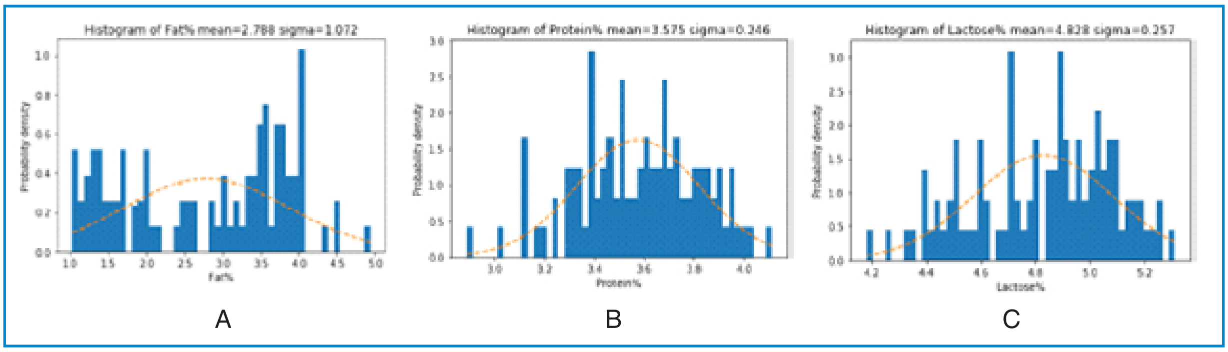
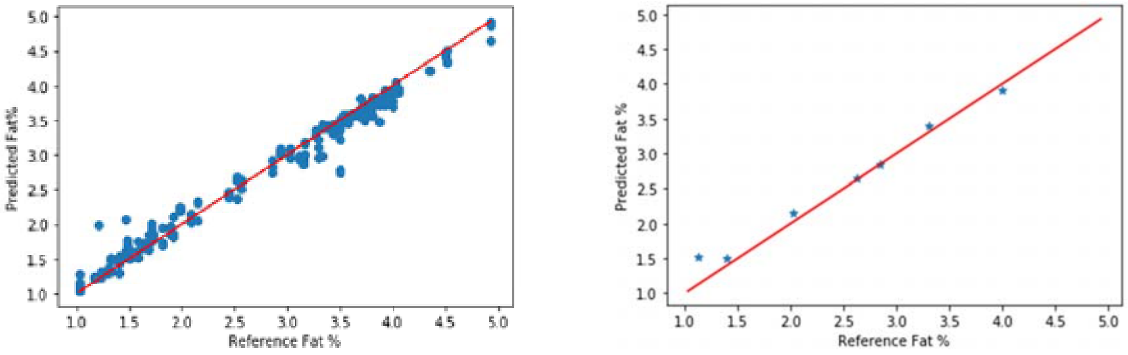


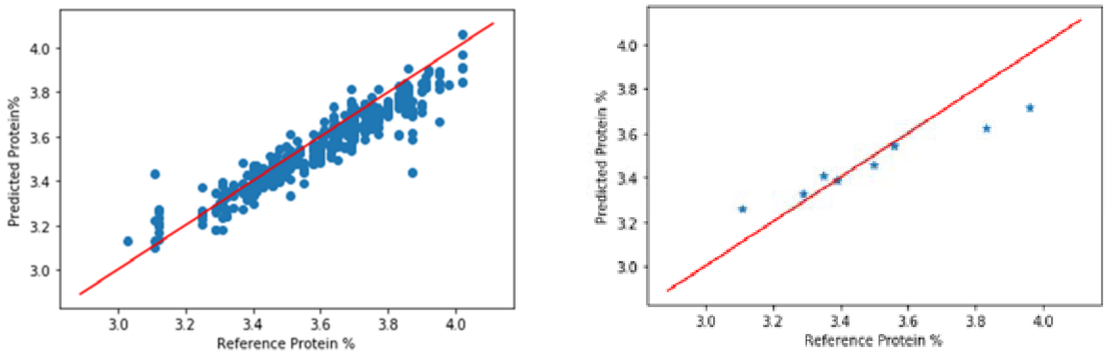
Figure 2. (a) Histogram for fat samples (b) Histogram for protein samples (c) Histogram for lactose samples



a

b

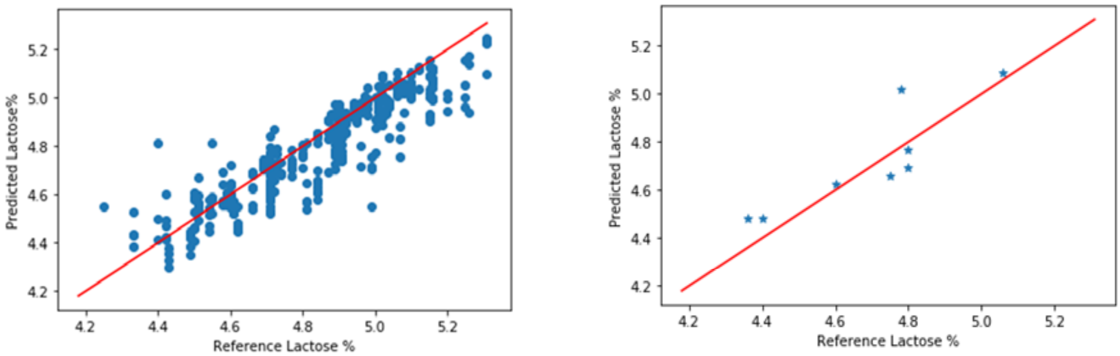
Figure 3. (a) Fat Calibration model with R2 of 0.984 and RMSE of 0.158. (b) Fat Validation results with R^2=0.991, RMSE = 0.167, Bias=0.096 and SEP=0.147



a

b

Figure 4. (a) Protein calibration model with R^2 of 0.891 and RMSE of 0.078. (b) Protein validation results with R^2 = 0.979, RMSE = 0.127, Bias = -0.033 and SEP=0.131



a

b

Figure 5. (a) Lactose calibration model with R^2 of 0.818 and RMSE of 0.105. (b) Lactose validation results with R^2 =0.767, RMSE=0.111, Bias = 0.033 and SEP=0.114

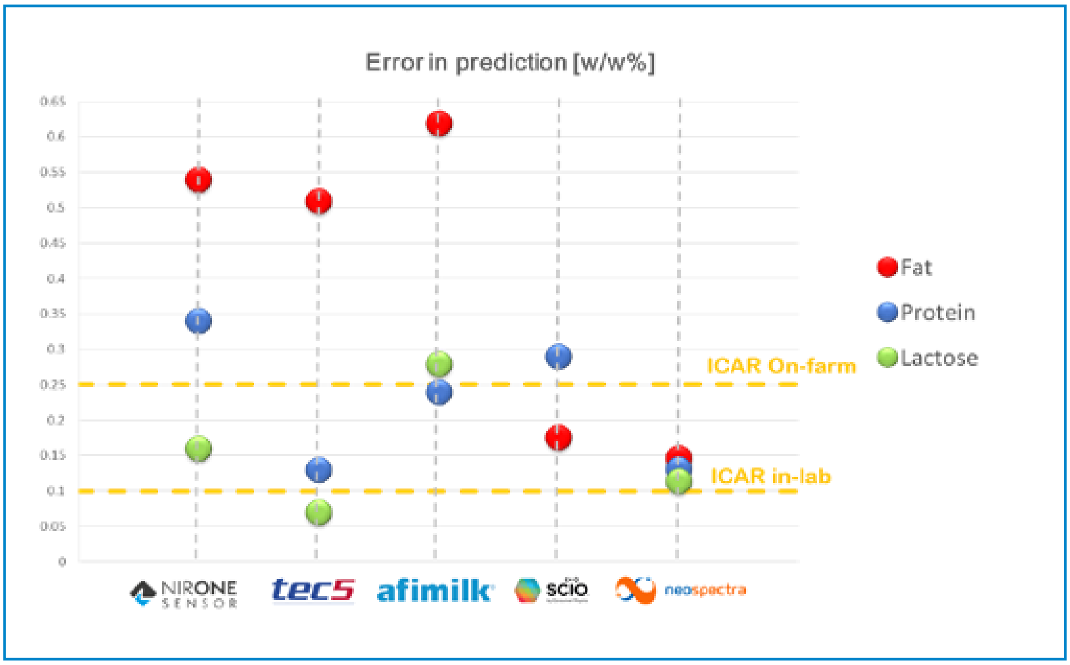


Figure 6. NeoSpectra achieved results compared to different technologies reported in literature and ICAR standards.

Conclusion

The results presented in this work indicates that NeoSpectra NIR spectral sensor has the potential to become the first commercial milk analysis solution that complies with ICAR standard for in-use applications and at the same time democratizes the adoption of milk analysis across the whole milk supply chain. Unlike other conventional and emerging technologies for milk analysis, NeoSpectra is a solution that uniquely combines a set of features that makes it possible to enable cost-effective, quick, ubiquitous, easy to use, and accurate milk analysis. Further experimentations are also conducted to assess the ability of NIR analysis to detect milk adulteration and somatic cell count (SCC). Combining milk accurate in-field milk composition analysis, with adulteration detection and SCC in one solution can disrupt the way we qualify, produce, and trade milk across the whole milk supply chain from cow to cup.

List of references

1. ICAR. 2017a. Guidelines for Testing, Approval and Checking of Milk Recording Devices.

2. ICAR. 2017b. Guidelines for On-Line Milk Analysis.

3. S. Uusitalo, B. Aernouts, J. Sumen, E. Hietala, M. Utriainen, L. Frondelius, S. Kajava, and M. Pastell , Comparison of milk analysis performance between NIR laboratory analyser and miniaturised NIR MEMS sensors, Proceedings ICAR Conference 2019, Prague

4. P. R. Griffiths and J. A. de Haseth, Fourier Transform Infrared Spectrometry, 2nd edition, Hoboken, NJ: John Wiley and Sons, 2007.

5. Yang, H., Yang, S., Kong, J., Dong, A. and Yu, S. Obtaining information about protein secondary structures in aqueous solution using Fourier transform IR spectroscopy. Nat. Protoc. 10, 382 (2015).

6. Martin, F. L. *et al.* Distinguishing cell types or populations based on the computational analysis of their infrared spectra. Nat. Protoc. 5, 1748 (2010).

7. de Voort, F. R., Sedman, J., Emo, G. and Ismail, A. A. A rapid FTIR quality control method for fat and moisture determination in butter. Food Res. Int. 25, 193–198 (1992).

8. Shiroma, C. and Rodriguez-Saona, L. Application of NIR and MIR spectroscopy in quality control of potato chips. J. Food Compost. Anal. 22, 596–605 (2009).

9. Das, A. J., Wahi, A., Kothari, I. and Raskar, R. Ultra-portable, wireless smartphone spectrometer for rapid, non-destructive testing of fruit ripeness. Sci. Rep. 6, 32504 (2016).

10. Madejova, J. FTIR techniques in clay mineral studies. Vib. Spectrosc. 31, 1–10 (2003).

11. Erfan, M. *et al.* On-chip micro-electro-mechanical system Fourier transform infrared (MEMS FT-IR) spectrometer-based gas sensing. Appl. Spectrosc. *https://doi.org/10.1177/0003702816638295* (2016).

12. Gastélum-Barrios, G. M. Soto-Zarazúa, A. Escamilla-García, M. Toledano-Ayala, G. Macías-Bobadilla, and D. Jauregui-Vazquez, “Optical Methods Based on Ultraviolet, Visible, and Near-Infrared Spectra to Estimate Fat and Protein in Raw Milk: A Review,” Sensors, vol. 20, no. 12, p. 3356, Jun. 2020.

13. Crocombe, R. A. Portable spectroscopy. Appl. Spectrosc. 72, 1701-1751 (2018).

14. B. Saadany, H. Omran, M. Medhat, F. Marty, D. Khalil, T. Bourouina. ‘‘MEMS Tunable Michelson Interferometer with Robust Beam Splitting Architecture’’. Paper presented at: IEEE/LEOS International Conference on Optical MEMS and Nanophotonics 2009. Clearwater, FL; August 17–20 2009.

15. Y. Nada, M. Medhat, M. Nagi, F. Marty, B. Saadany, T. Bourouina. ‘‘Mechanical Displacement Multiplier: 250 mm Stable Travel Range MEMS Actuator Using Frictionless Simple Compliant Structures’’. Paper presented at: IEEE International Conference on MEMS 2012. Paris; January 29–February 2 2012.

16. Yasser M. Sabry, Khaled Hassan, Momen Anwar, Mohamed H. Alharon, Mostafa Medhat, George A. Adib, Rich Dumont, Bassam Saadany, Diaa Khalil, “Ultra-compact MEMS FTIR spectrometer”, Proceedings Volume 10210, Next-Generation Spectroscopic Technologies X; 102100H (2017).

17. Pügner, T., Knobbe, J. and Grüger, H. Near-infrared grating spectrometer for mobile phone applications. Appl. Spectrosc. 70, 734–745 (2016).

18. Pedro Gelabert, Eric Pruett, Gavin Perrella, Sreeram Subramanian, and Aravind Lakshminarayanan “DLP NIRscan Nano: an ultra-mobile DLP-based near-infrared Bluetooth spectrometer”, Proc. SPIE 9761, Emerging Digital Micromirror Device Based Systems and Applications VIII, 97610B (15 March 2016).

19. B. Mortada, M. Erfan, M. Medhat, Y. M. Sabry, B. Saadany, and D. Khalil, "Wideband Optical MEMS Interferometer Enabled by Multimode Interference Waveguides," J. Light. Technol., 2016.

20. Sabry, Y. M. *et al*. In-plane deeply-etched optical MEMS notch filter with high speed tunability. J. Opt. 17, 125703 (2015).

21. Naes, T., A User-friendly Guide to Multivariate Calibration and Classification, NIR Publications, (2004).

22. Allen B. Downey. Think Stats - Probability and Statistics for Programmers , 2011

23. Hopfield, J. J. (1982). ”Neural networks and physical systems with emergent collective computational abilities”. Proc. Natl. Acad. Sci. U.S.A. 79 (8): 2554–2558.

24. Riu J, Gorla G, Chakif D, Boqué R, Giussani B. Rapid Analysis of Milk Using Low-Cost Pocket-Size NIR Spectrometers and Multivariate Analysis. Foods. 2020; 9(8):1090. *https://doi.org/10.3390/foods9081090*

Interpretation of residual feed intake by phenotypic recursiveness in dairy cattle: A simulation study

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Abstract

There has been an increasing interest in the use of residual feed intake (RFI) as a measure of net feed efficiency in recent years. RFI is estimated by the residual from a linear regression, which treats feed intake as a linear regression function of key energy sinks. Re-arranging the linear regression for RFI suggested an alternative interpretation of RFI, which postulates the RFI phenotypes as resulting from the recursive effects of energy sinks on dry matter intake. This leads to a Bayesian recursive structural equation model for a direct genetic evaluation of RFI. A simplified algorithm was proposed to compute the Bayesian recursive model. Persepectives on the recursive model were taken from a simulation study.

Keywords: Dry matter intake, Feed efficiency, milk, SNP, structural equation model.

Introduction

Feed efficiency is an important trait for dairy cattle because feed costs comprise almost half of the total costs associated with dairy production. Residual feed intake (RFI), which was initially proposed by Koch *et al*. (1963), is becoming increasingly popular as a measure of net feed efficiency in the past decade. The original idea of genetic evaluation on RFI consists of two stages (Berry and Crowley, 2013). In the first stage, dry matter intake (DMI) is fitted by single-trait linear regression (LR) encompassing energy sinks with or without relevant factors, and the residuals are taken to be the RFI phenotypes (Løvendahl *et al*., 2018; Templeman *et al*., 2015). In the second-stage, RFI is fitted by a mixed-effects model, which includes additive individual genetic effects for genetic evaluation. Combining both stages leads to the one-step model, eliminating the need to specifically estimate the residuals as the RFI phenotypes (Templeman *et al*., 2015).

Fitting phenotypes as regressor variables is criticized because standard regression models assume that regressor variables have been measured precisely or observed without error (Lu *et al*., 2015). In reality, however, phenotypes are subject to measurement errors. Multiple-trait models have been used, which bypass the above critics, but they represent indirect methods because RFI's genetic values are obtained through a follow-up partial regression procedure based on the estimated variance-covariance componets (Kennedy *et al*., 1993; Lu *et al*., 2015; Tempelman and Lu, 2020). By re-arranging the regression models for RFI, we came with an alternative, causative interpretation of RFI. This led to a Bayesian recursive structural equation model (RSEM) which allows for directly predicting genetic values and estimating genetic parameters for RFI and all the involving traits jointly.

Bayesian recursive structural equation model

Consider a single animal, say *i*. Following Løvendahl *et al.* (2018), we let the energy sink model include metabolic body weight ($MBW=BW^{0.75}$), energy-corrected milk (ECM), and change in body weight (ΔBW). That is

$$y_{i1} = \mu_1 + \lambda_{12} y_{i2} + \lambda_{13} y_{i3} + \lambda_{14} y_{i4} + r_i \qquad (1)$$

Here, μ_1 the overall mean and λ_{1i} qualifies the rate of the change with DMI (denoted by y_1) with respect to the three energy sink traits, denoted by y_2 (MBW), y_3 (ECM), and y_4 (ΔBW), respectively. The residual (r_i) as the RFI phenotype is then described by a mixed-effects model:

$$r_i = \mathbf{x}'_{ir}\boldsymbol{\beta}_r + \mathbf{z}'_{ir}\boldsymbol{a}_r + e_{ir} \qquad (2)$$

where β is a vector of “fixed” effects of an appropriate length; a_r is a vector containing the “random” additive genetic effects for all individuals; x_{ir} and x_{zr} are the corresponding incidence vectors relating the RFI phenotypes to the fixed and random effects, and e_{ir} is an error term. Note that “random” permanent environmental effects are not considered in model (2), but they may be relevant in real applications.

Combining equations (1) and (2) and moving all the y variables to the left-hand side leads to the following one-step model:

$$y_{i1} - \sum_{j=2,3,4} \lambda_{1j}\, y_{ij} = \mu_1 + \mathbf{x}'_{ir}\boldsymbol{\beta}_r + \mathbf{z}'_{ir}\boldsymbol{a}_r + e_{ir} \qquad (3)$$

The above is recognized as a recursive structural equation between DMI and the three energy sink traits (MBW, ECM, and ΔBW) for the ith individual. It postulates that the RFI phenotypes result from the recursive effects from energy sinks to DMI, but the feedback or simultaneous effects are assumed to be non-existent. Because the model parameters in (3) pertain to RFI, not DMI, hence it can provide a direct evaluation of RFI.

To complete the recursive structural equation model, we define mixed-effect models for the energy sinks as follows

$$y_{ij} = \mu_j + \mathbf{x}'_{ij}\boldsymbol{\beta}_j + \mathbf{z}'_{ij}\boldsymbol{a}_j + e_{ij},\ \text{for } j = 2, 3, 4. \qquad (4)$$

The recursive structural equation model that combines equations in (3) and (4) for the ith individual is the following:

$$\boldsymbol{\Lambda}\mathbf{y}_i = \boldsymbol{\mu} + \mathbf{X}_i\boldsymbol{\beta} + \mathbf{Z}_i\boldsymbol{\alpha}_i + \mathbf{e} \qquad (5)$$

Here, we have

$y_i = (y_{i1}, y_{i2}, y_{i3}, y_{i4})'$;

$\mu = (\mu_1, \mu_2, \mu_3, \mu_4)'$, and

$e = (e_r, e_2, e_3, e_4)'$;

The vectors for the fixed and random effects were sorted by traits within animals. For example, we have

$\beta = (\beta_{11}\ \ \beta_{12}\ \ \beta_{13}\ \ \beta_{14} \ldots\ldots \beta_{\kappa 1}\ \ \ \beta_{\kappa 2}\ \ \ \beta_{\kappa 3}\ \ \beta_{\kappa 4})'$, where k is the total number of fixed effect levels.

Assuming a full loading of each fixed effect on all the four traits, we have

$X_i = x'_i \otimes I$, where $x'_{i1} = \ldots = x'_{i4} = x'_i$, **I** is a 4×4 identity matrix, and $\otimes$ is the Kronnecker product operator.

Similarly, we have $\alpha = (\alpha_{11}\ \ \alpha_{12}\ \ \alpha_{13}\ \ \alpha_{14} \ldots\ldots \alpha_{\nu 1}\ \ \ \alpha_{n2}\ \ \ \alpha_{n3}\ \ \alpha_{n4})'$

where n is the total number of animals, and $Z_i = z'_i \otimes I$, where $z'_{i1} = \ldots = z'_{i4} = z'_i$.

Finally, the structural matrix (Λ) defines the phenotypic relationships between DMI and energy sinks:

$$\boldsymbol{\Lambda} = \begin{pmatrix} 1 & -\lambda_{12} & -\lambda_{13} & -\lambda_{14} \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} \qquad (6)$$

Bayesian modeling of structural equation models via Markov chain Monte Carlo (MCMC) implementation is described by Gianola and Sorensen (2004) and Wu et al. (2017, 2018). Here, we briefly describe a simplified algorithm to compute the recursive model for RFI. Assume zero genetic and residual covariances, and hence zero phenotypic covariances, between RFI and energy sinks, the posterior inferring of structural coefficients does not involve any unknown parameters for the energy sink traits. Assign a multivariate normal prior distribution for all unknown structural coefficients,

$$\lambda \sim MVN(1\lambda_0, I\sigma^2_\beta), \text{ where } \lambda = (\lambda_{12}\ \ \ \lambda_{13}\ \ \ \lambda_{14}\ \ \ \lambda_{15})'.$$

Then, given the sampled fixed and random effects (β_r, a_r, and p_r) for RFI and the residual variance-covariance matrix (R_0), the conditional posterior distribution of λ is a multivariate normal distribution:

$$\lambda|else \sim MVN(\hat{\lambda}, \mathbf{V}_\lambda)$$

(7)

where

$$\hat{\lambda} \quad \mathbf{V}_\lambda\left(\sum_{i=1}^{n}\mathbf{Y}'_i\mathbf{R}_0^{-1}(\mathbf{y}_i-\mathbf{X}_i\beta_r-\mathbf{Z}_i\mathbf{a}_r)+\mathbf{1}\tau_\lambda^{-2}\lambda_0\right)$$

$$\mathbf{V}_\lambda \quad \left(\sum_{i=1}^{n}\mathbf{Y}'_i\mathbf{R}_0^{-1}\mathbf{Y}_i+\tau_\lambda^{-2}\mathbf{I}\right)^{-1}$$

In the above, $\boldsymbol{y}_i = (y_{i1} \quad y_{i2} \quad y_{i3} \quad y_{i4})'$ and Y_i is a working 4×(4-1) matrix constructed by noting that $\Lambda y_i = y_i - Y_i\lambda$ for each individual.

That is,

$$\boldsymbol{Y}_i = \begin{pmatrix} y_{i2} & y_{i3} & y_{i3} \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$$

Furthermore, the posterior inference of the model parameters for RFI, given the structural equation coefficients, does not involve any unknown paramters for the energy sinks. Thus, a simplified algorithm for computing the recursive structural equation model is proposed as follows:

> The fixed effects, random effects (β_{r} and a_{r}), and variance-covariance matrices G_{r} and R_{r} for the energy sinks are estimated based on a standard multiple-trait mixed-effects model, independent of the computed RFI phenotypes. Computing the submodel for the energy sinks can be implemented through Markov chain Monte Carlo sample by iteratively sampling unknown paramters from their conditional posterior distributions, or implemented by REML.
>
> The unknown structural coefficients were sampled iteratively from the multivariate normal distribution (7). Then, given the sampled structural coefficients and the computed RFI phenotypes ($r_i = y_{i1} - \sum_{j=2,3,4}\lambda_{1j}y_{ij}$), the fixed and random effects and the variance components $(\sigma^2_{a_r}, \cdot \sigma^2_{p_r}, \cdot \text{and} \cdot \sigma^2_{e_r})$ for RFI are sampled from their respective conditional posterior distributions based on a standard single-trait mixed effects model.

Note that MCMC simulation was only necessary for sampling the structural coefficients and unknown parameter for RFI only. This drastically simplified the model computing when dealing with a large dataset. Also note that the covariances between RFI and energy sinks are fixed to zeros, but the covariances between DMI and energy sinks are not, which are computed as follows:

$$\mathbf{G}_0^* \quad \Lambda^{-1}\mathbf{G}_0\Lambda'^{-1}, \text{and } \mathbf{R}_0^* \quad \Lambda^{-1}\mathbf{R}_0\Lambda'^{-1}, \text{where } \mathbf{G}_0 = \begin{pmatrix}\sigma^2_{a_r} & 0' \\ 0 & \mathbf{G}_{-r}\end{pmatrix}, \mathbf{R}_0 = \begin{pmatrix}\sigma^2_{e_r} & 0' \\ 0 & \mathbf{R}_{-r}\end{pmatrix}.$$

Simulation results

We simulated standardized phenotypes of DMI, MBW, ECM, and ?BW based on an actual pedigree for 908 cows. These animals were progenies derived from 125 sires and 477 dams and raised in the USDA Beltsville Agricultural Research Center (BARC) Dairy Herd (Beltsville, Maryland, USDA). For simplicity, the fixed effects included only the overall mean (μ_j=0) for each trait, and the random effects included individual additive genetic effects plus the residuals. The simulated genetic and residual variance-covariance components were shown below:

$$G\ \begin{pmatrix} 0.399 & 0.205 & 0.191 & 0.012 \\ 0.205 & 0.521 & 0.033 & 0.037 \\ 0.191 & 0.033 & 0.287 & -0.005 \\ 0.012 & 0.037 & -0.005 & 0.048 \end{pmatrix};\ R\ \begin{pmatrix} 0.581 & 0.236 & 0.311 & 0.179 \\ 0.236 & 0.534 & 0.142 & 0.144 \\ 0.311 & 0.142 & 0.671 & -0.022 \\ 0.179 & 0.144 & -0.022 & 0.96 \end{pmatrix}$$

We compared four models: (1) one-step linear regression (LR1), (2) two-stage linear regression (LR2), (3) recursive structural equation model (RSEM), and (4) multiple-trait mixed-effects model (MT). To set up an accurate bench model for comparison, the (co) variance components in the MT model were assumed to be known and took directly from the actual values. RSEM was implemented via MCMC simulation. Ten parallel chains (or replicates) were run for each model, each of 2000 iterations, after a burn-in of 1000 iterations and thinned every two draws. The means and standard deviations of parameter estimates were obtained as averages across the ten replicates.

All the MCMC chains coverged well after 1,000 iterations. The estimated structural coefficients from RSEM corresponds closely to the partial regression coefficients from the one-step LR. The partial regression coefficients from two-stage LR agreed perfectly with the phenotypic partial regression from the MT model, but differed from the genetic partial coefficient based on the MT model. These results indicates that both RSEM and the single-trait linear regression approaches depicts RFI by the phenotypic relationships between DMI and energy sinks. Although there were some differences in partial regression coefficients between two-stage LR and one-step LR (or RSEM), the estimated additive genetic values are highly comparable between these models (Spearman's correlation was 0.999). The correlations of the estimated RFI genetic were also highly comparable values between RSEM and MT, but the correlation was slightly lower than that beween RSEM and a LR model. Thus, we concluded that RSEM resembled the LR model more than the MT. This was because both RSEM and the LR approaches inferred phenotype recursive relationshiops. For the MT model, RFI genetic values were inferred based on genetic relationships between DMI and energy sinks, which can differ from the phenotypic relationship. This, however, does not mean

Table 1. Estimated partial regression coefficients (structural coefficients) from linear regression (LR), recursive structural equation model (RSEM), and multi-trait, mixed-effects model (MT).

Energy sink	Two-stage LR	One-step LR	RSEM	MT	PMean	PSD	b.COVP	b.COVG	b.COVE
MBW	0.327	0.026	0.313	0.027	0.312	0.027	0.327	0.349	0.300
ECM	0.498	0.027	0.470	0.027	0.469	0.027	0.498	0.629	0.404
∆ LW	0.127	0.026	0.137	0.025	0.137	0.025	0.127	0.049	0.151

SD = standard deviation; PSD = posterior standard deviation; PMean = Posterior mean.
b.COVP, b.COVG, b.COVE = partial regression coefficient based on phenotypic, genetic, and residual (co)variances, respectively.

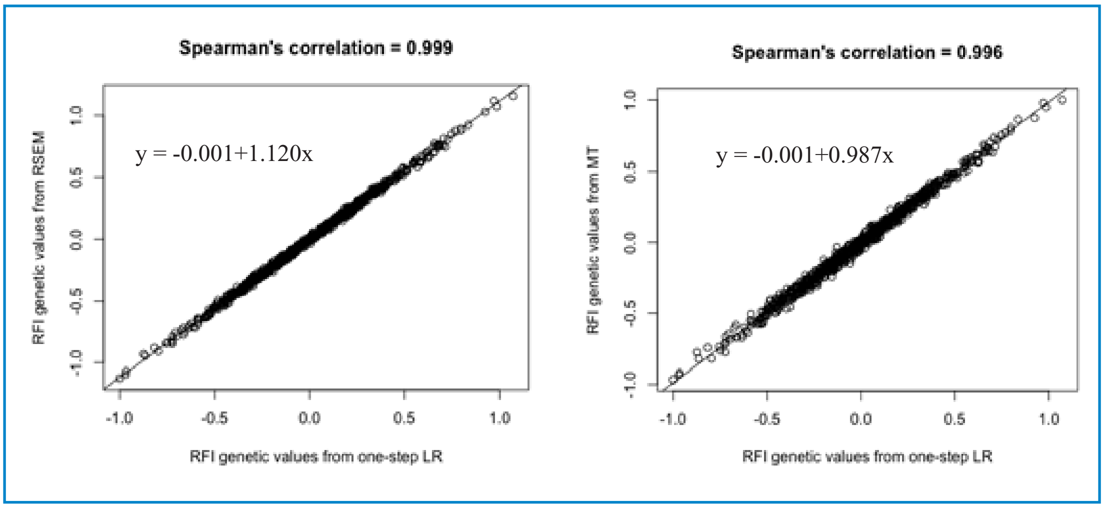

Figure 1. Comparing the estimated RFI genetic values from one-step linear regression (LR) and those from the recursive model (RSEM, left) or the multiple trait model (MT, right).

that RSEM is more accurate than the MT model, because the true RFI gentic values were unknown. It is possible to simulate RFI directly, but the simulated relationshiops depends on the assumptions, whether it favors one model or the other. Estimated heritability for RFI was 0.339 (two-stage LR), 0.348 (one-step LR), and 0.324 (RSEM).

In conclusion, we proposed RSEM as a direct genetic evaluation of RFI. Concerning the estimated RFI genetic values, RSEM was equivalent to the single-trait linear regression model, but RSEM expended the analytical capability to multiple traits with causative relationships assumed. RSEM can also provide the estimates of genetic paramters for RFI and all the involving traits, which are not discussed due to the page limitation. Finally, the recursive model extends very naturally to deal with heterogeneous structural coefficient matrices (Wu et al., 2007). Extending RSEM for RFI to genomic selection is just as straightforward, e.g., by replacing the additive relationship matrix with the genomic relationship matrix (VanRaden, 2008).

List of references

Berry, D. P., and J. J. Crowley. 2013. Cell Biology Symposium: genetics of feed efficiency in dairy and beef cattle. J. Anim. Sci. 91, 1594-613.

Gianola, D., and D. Sorensen. 2004. Quantitative genetic models for describing simultaneous and recursive relationships between phenotypes. Genetics. 167, 1407-1424.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22, 486–494.

Identification of cows and individual feed intake records using a 3D camera system in commercial farms

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Abstract

A system has been developed to make identification of cows and individual feed intake records in commercial farms using a 3D camera system. Cameras are installed in the lock after milking where the RFID eartag is identified and images of the cows back is stored to generate reference images. These reference images are used to predict a given cow when she is eating. Individual feed intake is estimated at any visit during the day. The last image of the feed before a visit is stored together with the first image of the feed after a visit. These 2 surfaces is subtracted to estimate an intake for a visit. Based on feed density the volume is transformed to kilos. All visits are summed to daily intake and later on to weekly mean of daily intake and reported throughout lactation. The system is installed in 7 herds (5 Jersey, 1 Holstein and 1 Red dairy cows) measuring in 1292 Jersey, 536 Holstein and 222 Red Dairy cows in a two-year period.

Mean daily feed intake measured was 54.7 kilo for Jersey, 60.6 for Holstein and 59.7 for Red dairy cattle. Corresponding standard deviation was 8.5, 11.2 and 9.5 kilos respectively.

A mixed linear model was used to analyse the data for each breed separately. The model contained a fixed effect of herd (for Jersey), week of year, week in lactation, year, a random effect of animal and a residual term. Repeatabilities (animal variance divided by total variance) of daily individual feed intake as a weekly mean were moderate to high across the three breeds. For Jersey repeatability was 56%, for Holstein 60% and for Red Dairy cattle 61%.

The results suggests that data from 3D cameras can be used to make large-scale individual records for feed intake in indoor-housed dairy cattle. This data can be used to make genetic evaluations and management decisions. Further data over a longer time period and from more herds is needed to confirm these results. Therefore, the system will be installed in new herds in 2021 and more than 3000 cows will be added to the CFIT registration system.

The data is measured throughout lactation, which opens up for estimation of genetic correlation between efficiency and health traits in early lactation. Using 3D camera data opens up for individual measures of other phenotypes. This includes weight but also health, welfare and behavioral traits.

Keywords: Feed intake, identification, artificial intelligence, Holstein, red dairy cattle, Jersey.

Introduction

Feed cost is up to 70% of the running cost for a farmer, so only a marginal save of feed will make a huge impact on the return on farm. A part of the variation in feed intake and efficiency is heritable, which make selection for improved feed efficiency possible (Løvendahl *et al.*, 2018). A limitation to implement this is lack of data on an individual level which is recorded in commercial settings throughout lactation. So far, equipment for making individual feed intake records has primarily been on research farms and based on scale system that are expensive and time consuming to manage (Seymour et al., 2019). Feed intake measured in research farms is repeatable (0.66) as well as heritable (0.34) (Berry *et al.*, 2014), so selection can be performed in order to change the trait in the preferred direction. The ideal system measures, controls, and monitors individual feed intake of the free-housed cow while not interfering with feeding habits and not introducing additional work or inhibiting workflow on the farm (Halachmi *et al.*, 1998).

A 3D camera system to identify cows and make individual feed intake records have been developed (WO 2017/001538; WO 2014/166498). The system works without disturbing daily behavior of either the cows or the farmer. The cameras records data around the clock and based on image analysis cows are identified at the feeding table (Thomasen *et al.*, 2018) and the amount of feed eaten is quantified (Lassen *et al.*, 2018). In these studies, the data was collected in a limited time period and identification percentage and repeatabilities of the feed intake between days and weeks were reported and showed promising results. Other camera-based systems have been initiated to make individual feed intake records. Bezen *et al.* (2020) used the CNN approach to quantify feed intake and showed a MSE of 0.119 kg^2 feed pr meal based on 63 meals recorded on 6 cows in 36 hours. Identification relied on observing digits related to the cow ID on collars on the neck of the cow.

The aim of this study was to analyze individual measures of feed intake recorded in commercial farms using 3D cameras. This was studied by estimating the repeatability of the phenotypes recorded in three different breeds.

Materials and methods

To make individual feed intake measures 3D cameras from Kinect was used (Microsoft). Each camera is placed 2.5 meter apart and 4.5 meter from the empty feeding table covering the entire feeding table. In addition, an ear tag reader and a 3D camera is placed outside the milking parlor in the exit corridor. Three types of images are recorded from the 3D camera: normal pictures, IR pictures and depth pictures indicating the distance from the camera to the object that is within the range of the camera.

Data was recorded in seven commercial dairy herds in Denmark with Jersey cows. Data was recorded from 1st of February 2019 to 1st of March 2021. In 5 of the herds there were Jersey cows, in one there were Holstein cows and in 1 herd there were red dairy cattle. In all herds cows were kept indoors year around.

When cows leave the milking system their electronic ear tag was read and at the same time a 3D picture is taken of the back of the cow. These pictures were stored and used as a reference to predict the same cow based on the contours, color and patterns of the back of the cow, when eating at the feeding table based on the MASK-CNN algorithm (He *et al.*, 2018; Thomasen *et al.*, 2018; WO 2017/001538). This approach was tested in a validation study where cows id was manually annotated to visits at the feeding table over a 14 day period. The validation was conducted in comparing the real ID of the cow with the predicted id based on the MASK-CNN algorithm.

When a cow enters the head to the feeding table the last image of the feeding pile before she put in her head and began eating was stored from vbiruel boxes defined in the feeding table. When the cow has finalized the meal and takes the head out again the first new image of the feeding pile is stored. The height in each pixel from two stored images are now subtracted from each other and the removed feed is quantified on pixel level. From each specific visit 5 variables are stored: the ID of the cow, the placement in the barn, time when the meal was initiated, time when the meal was finalized, and the amount of feed eaten. During a meal feed will be allocated to a specific cow from 5 virtual boxes. The virtual box right below the cow as well as he two virtual boxes to the left and the right. If two cows share a virtual box during a feeding visit, they will also share the feed taken from this box during the two cow specific visits.

The feed intake approach been validated in a controlled experiment where 325 liters of feed where put in a pile below the cameras. Feed were then removed as bits from various places of the pile to mimic how a cow would remove feed 19 liters at the time with 2 minutes interval. The standard error on how well the sum of the pixels predicted the feed removed was then estimated to see how well the algorithm worked.

In order to obtain repeatability, data was analyzed using the mixed procedure in SAS. The model used to estimate the repeatability, looked as follows:

$$FI= h + w + y + wil(dim) + dim + lac + animal + res$$

where h is the fixed class effect of herd, w is the fixed class effect of week, y is the fixed class effect of year, wil(dim) is a wilmink regression on days in milk, dim is a regression on days in milk, lac is a fixed class effect of lactation, animal is the random animal variation and res is the random residual variation.

Results and discussions

A total of 2050 cows were measured. This was 1292 Jersey cows (JER), 222 Red Dairy Cows (RDC) and 536 Holstein cows (HOL). These cows were distributed in 5 JER herds, 1 RDC herd and 1 HOL herd. Mean feed intake in kilo with corresponding standard deviation and minimum and maximum value is presented in table 1.

This is done both overall for each breed and for the 7 herds. Mean intake was higher for RDC (59.7 kg) and HOL (60.6 kg) than for JER (54.7 kg). The standard deviation of the mean was also higher in HOL (11.2 kg) and RDC (9.5 kg) than for JER (8.5 kg).

Repeatability estimates for daily feed intake was 0.56, 0.61 and 0.60 for JER, RDC and HOL respectively (table 2).

These estimated were based on animal variances of 30.55, 46.41 and 58.53 for JER, RDC and HOL, respectively. The magnitude of these estimates corresponds to the difference in magnitude seen in the standard deviation of the mean of the feed intake. The JER animal variance might also be somewhat lower due to the larger number of animals in the study. The measured repeatability was at the same level as several other studies have reported in the past based on research farm data (Berry *et al*.,

Table 1. Data description including breed, number of cows, mean feed intake in kilos, and standard deviation.

Breed	# of cows	Mean	Std
Jersey	1292	54.74	8.53
Red Dairy Cattle	222	59.72	9.53
Holstein	536	60.59	11.20

Table 2. Breed, animal variance, residual variance and repeatability.

Breed	V(a)	V(e)	rep
Jersey	30.55	24.01	0.56
Red Dairy Cattle	46.41	29.67	0.61
Holstein	58.53	39.02	0.60

2014). The feed intake in comparable with feed intake measured in a research farm with Jersey cows (Li *et al*., 2016), RDC cattle (Liinamo *et al*., 2012; Li *et al*., 2016) and HOL cows (Li *et al*., 2016).

The identification algorithm was tested in a validation study over a 2-week period for all three breeds. Results showed that id was correct in more than 99% of the visits independent on breed. The system is not dependent on freeze marking the cows, various neck collars or physical installations in the stable.

A major priority for developing this system was to obtain data throughout lactation. In relation to genetic analysis several studies have shown that genetic correlation change during lactation between feed intake, milk yield and body weight (Manzanilla-Pech *et al*., 2014; Li *et al*., 2017). Estimates for the genetic correlation between feed intake and milk yield varies from -0.80 in early lactation up to 0.8 in mid and late lactation (Manzanilla-Pech *et al*., 2014). Selection for improved efficiency in early lactation where feed intake specifically needs to be improved based on records obtained in mid lactation then might lead to even lower feed intake in early lactation. From a management perspective also feed intake records are interesting throughout lactation. In early lactation the absolute majority of health problems occurs both related to mastitis, reproduction and nutrition. In mid lactation the farmer wants to know which cow are most efficient to optimize culling strategy and in late lactation the feed intake is interesting in order to optimize strategies in relation to drying off cows. With this system all information will be available for the farmer.

The system will be installed in herds covering more than 7000 cows during 2021.

Conclusions

Individual feed intake measures can be made using a 3D camera system that both identifies the cow and quantifies the amount of feed eaten by the cow. Repeatability was between 0.56 and 0.61 for daily feed intake measured as a weekly average over a time period of more than two years.

List of references

Berry, D. P., M. P. Coffey, J. E. Pryce, Y. de Haas, P. Løvendahl, N. Krattenmacher, J. J. Crowley, Z. Wang, D. Spurlock, K. Weigel, K. Macdonald, and R. F. Veerkamp. 2014. International genetic evaluations for feed intake in dairy cattle through the collation of data from multiple sources. J Dairy Sci 97(6):3894 3905.

He K., G Gkioxari, P. Dollar and R Girshick 2018 Mask R-CNN. arXiv:1703.06870v3 [cs.CV] 24 Jan 2018.

Bezen, R., Y Edan, I. Halachmi 2020 Computer vision system for measuring individual cow feed intake using RGB-D camera and deep learning algorithms Computers and Electronics in Agriculture 172:105345

Halachmi, I., Y. Edan, E. Maltz, U. M. Peiper, U. Moallem, and I. Brukental. 1998. A real-time control system for individual dairy cow food intake. Comput. Electron. Agric. 20:131–144.

Lassen, J., J. R. Thomasen, R. H. Hansen, G. G. B. Nielsen, E. Olsen, P. R. B. Stentebjerg, N. W. Hansen, and S. Borchersen. 2018. Individual measure of feed intake on inhouse commercial dairy cattle using 3D camera technology, Proceedings of the World Congress on Genetics Applied to Livestock Production, vol. Technologies Novel Phenotypes, p. 635, 2018.

Li, B., W. F. Fikse, J. Lassen, M. H. Lidauer, P. Løvendahl, P. Mäntysaari, and B. Berglund. 2016. Genetic parameters for dry matter intake in primiparous Holstein, Nordic Red, and Jersey cows in the first half of lactation. J. Dairy Sci. 99 (9): 7232-7239.

Li, B., B. Berglund, W. F. Fikse, J. Lassen, M. H. Lidauer, P. Mäntysaari, and P. Løvendahl. 2017. Neglect of lactation stage leads to naive assessment of residual feed intake in dairy cattle. J Dairy Sci 100 (11), 9076 9084.

Liinamo A-E, Päivi Mäntysaari, EA Mäntysaari 2012 Genetic parameters for feed intake, production, and extent of negative energy balance in Nordic Red dairy cattle J Dairy Sci 95:6788-6794.

Løvendahl, P, GF Difford, B Li, MGG Chagunda, P Huhtanen, MH Lidauer, J Lassen, P Lund 2018 Selecting for improved feed efficiency and reduced methane emissions in dairy cattle Animal 12:336-349.

Manzanilla Pech CIV, RF Veerkamp, MPL Calus, R Zom, A Van Knegsel, JE Pryce, Y De Haas 2014 Genetic parameters across lactation for feed intake, fat- and protein-corrected milk, and liveweight in first-parity Holstein cattle J. Dairy Sci 975851-5862.

Seymour DJ, A Cánovas, Christine Francoise Baes, T.C.S. Chud, V.R. Osborne, J.P. Cant, L.F. Brito, B. Gredler-Grandl, R. Finocchiaro, R.F. Veerkamp, Y. de Haas, F. Miglior 2019 Invited review: Determination of large-scale individual dry matter intake phenotypes in dairy cattle. J Dairy Sci 102:7655-7663.

Thomasen, J. R., J. Lassen, G.G.B. Nielsen, C. Borggard, P.R.B. Stentebjerg, R.H. Hansen, N. W. Hansen, and S. Borchersen. 2018. Individual cow identification in a commercial herd using 3D camera technology, Proceedings of the World Congress on Genetics Applied to Livestock Production, vol. Technologies Novel Phenotypes, p. 613, 2018.

WO 2017/001538 System and method for identification of individual animals based on images of the back.

WO 2014/166498 System for determining feed consumption of at least one animal.

Towards secure digital farming: security model and risks associated to machine learning

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Abstract

In digital farming, machine learning is already widely used to optimize the production using sources such as genomics, health, welfare, production, and environmental data. However, this increasing use of machine learning has led to the emergence of multiple types of confidentiality and integrity breaches targeting both the models and the data they have been trained on. Our main objective in this paper is to discuss possible security issues that could arise in digital farming due to the use of machine learning techniques and the urgency to implement innovative countermeasures to prevent them. First, we propose a security model dedicated to the specific settings and threats of the digital farming context. In this model, we identify the resources at risk, define the different classes of actors, determine the risk vectors, and propose some realistic attack scenarios. Afterwards, we use this model to put in perspective the machine learning induced risks and show how they may adversely affect digital farming. The considered attacks encompass model theft, model inversion, membership inference, data poisoning and adversarial examples. For each of these threats, we also briefly revied possible mitigation means, such as differential privacy, prediction access control and robust statistics.

Keywords: Digital farming, cyber security, security model, machine learning.

Introduction

German Agricultural Society defines digital farming as the evolution of smart farming to better emphasize that nearly all aspects of farming now heavily rely on digital means (DLG Committee for Digitization, Work Management and Process Technology *et al*., 2018). Collecting data massively from a wide variety of sources has allowed to take smart farming to a new level, leveraging big data to further improve the power of the decision-making system. To do so, various kind of data are collected such as environmental, production, health, welfare, genomics, and management. Machine learning (ML) is the core concept behind decision-making system, in which a sample of data called training dataset is used to generate a predictive model. ML is widely used across many industries and as ML techniques become, cybersecurity threats emerge (Papernot *et al*., 2016) putting data confidentiality and production system integrity at risk.

Many actors have stated that cybersecurity is a concern in agriculture. For instance, a 2019 report from the U.S. Government Accountability Office (Dodaro, 2019), has indicated that improving cyber security should be one of the main priorities for actors in the agriculture sector. Geil *et al.* (2018) presented a survey in which they show that farmers are being directly affected at large scale. Window (2019) has conducted a study that presents major issues concerning data privacy, data ownership and level of attention given to cyber security in agriculture and all those points are requirements provided by the German Agricultural Society as well in their recent position paper.

A literature review allows us to conclude that working specifically on ML induced cybersecurity risks is a missing gap in the literature. Indeed, several reports focus on networking and Internet of Things (IoT) related risks (Gupta *et al.*, 2020; West, 2018) and several others on Big Data (Sykuta, 2016; Wolfert *et al.*, 2017). However, only a few works have been produced to study risks introduced by data analysis techniques in digital farming particularly in dairy industries. The U.S. Department of Homeland Security (Champion *et al.*, 2018) has also released a report in which they mention machine learning and at the regulatory level, farm data ownership is often present in the specialized literature (Sykuta, 2016; Window, 2019; DLG Committee for Digitization, Work Management and Process Technology *et al.*, 2018).

To study ML induced threats to digital farming, we first propose a security model adapted to this context of dairy farms, particularly in Canada, before studying the data life cycle and its interactions with the different resources and actors. Secondly, we propose an adversarial model to determine realistic threat vectors to ML systems in digital farming before proceeding with the investigation of the risks associated to ML, looking at five known vulnerabilities of ML systems and three possible practical mitigation strategies. Finally, we discuss another ML related security topics that should be investigated along ML induced threats.

Security model

Data chain: resources and actors

ML applications rely mainly on two assets: the training dataset and the learnt model. We will use the CIA framework to understand the impact of potential compromises on these resources. Confidentiality of the training dataset may be critical for privacy reasons, as for example valuable data such as genomics are used in digital farming, but also because it is part of the intelligence developed by ML application developers. Integrity of the training dataset is key to build reliable ML model and availability seems to only be a concern at the operational level. In addition, the confidentiality of the ML model is important in settings in which it is a monetizable resource such as ML as a Service (MLaaS), which is a form of pay-per-request service that could be compromised if the ML model was to be stolen. Furthermore, the ML model is a statistical representation of the training dataset as its confidentiality impact directly the confidentiality of the dataset. The integrity of the ML model is a concern for situations in which the ML predictions are used in a sensitive context such as farm management. Availability is a concern in time sensitive settings and for systems that cannot be substituted.

Wolfert *et al.* (2017) proposed a data chain in their framework for big data in smart farming. We adapted it to exclude network/infrastructure-based risk and focus on machine learning induced threats to assets described earlier. The principal node is data processing in which the ML model is developed. This node has two interfaces, the upstream data acquisition node in which the training dataset is being constituted and the downstream marketing node in which the end user is presented with a tool to query the model and obtain the associated predictions.

In Figure 1, we annotate the data chain to integrate actors found at each stage of the data lifecycle. The data provider is the main actor at the data acquisition stage, which is often the farmer but could also be laboratories in some cases (*e.g.* sample analysis on milk). We refer to the data collector to formalize the intermediary step consisting in centralizing and storing the data. The data processor is the actor found at processing stage, who pre-process data to form the training dataset and train the model. Once

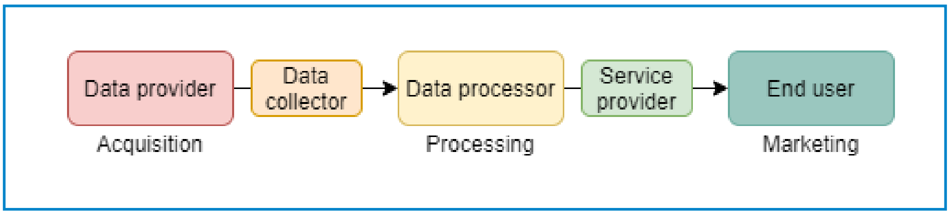

Figure 1. Actors involved in the data chain.

the model is trained, the service provider refers to the actor in charge of wrapping the model in a product that can be distributed. Finally, the end user is the actor present at marketing stage that makes requests and applies predictions using the model through the marketed tool.

Adversarial model and threat vectors

We adopt the point of view of the data processor who forms the training dataset (preprocessing) and engineers the ML model (processing) because he is the actor having full and direct access to the resources that we aim to protect. We assume that the attacker could be or could impersonate a data provider (upstream) or an end user (downstream). For instance, in the case of Machine Learning as a Service (MLaaS), the attacker could target the model confidentiality for financial gain. In addition, if training dataset contains valuable information (e.g., genomics and/or production data), the attacker could target training dataset confidentiality. Finally, when the ML system is used for critical applications (food supply or seed production), the attacker could target integrity/availability of training dataset, ML model or prediction.

For the specific context of dairy digital farming, there can be a wide range of adversaries (insiders to the context of digital farming or not), thus leading to various levels of risks, ranging from a farmer seeking financial gain to eco-terrorists aiming to disrupt the food supply. As a result, the adversary is likely to have detailed knowledge about the digital farming and could have weak to strong technological skills. To study the attack surface and related threat vectors, we look at the interfaces of the ML system leaving aside all security concerns that are not inherently tied to ML (network, access control…).

Looking at the data chain, we have the upstream interface in which data is collected and pre-processed to form the training dataset and the downstream interface in which the trained model generates predictions upon user requests. At data collection stage, an attacker can craft and provide malicious data points to compromise the ML model and its predictions. At model interaction stage, malicious requests can lead to leak the ML model and the training dataset. More precisely, in controlled access settings, the attacker will have to compromise the model through a distant API whereas in model sharing settings the attacker is free to access both the program containing the model and the API locally, thus making the prediction access control harder and raising new concerns like reverse engineering.

Risks to machine learning

Confidentiality of data and model

Membership inference attacks aim to deduce if a given data point is present in the training dataset or not. The first membership attack against a ML system was realized by Shokri *et al.* (2017). They targeted black box models in a context of MLaaS and were able to differentiate member data points only by sending requests to the model.

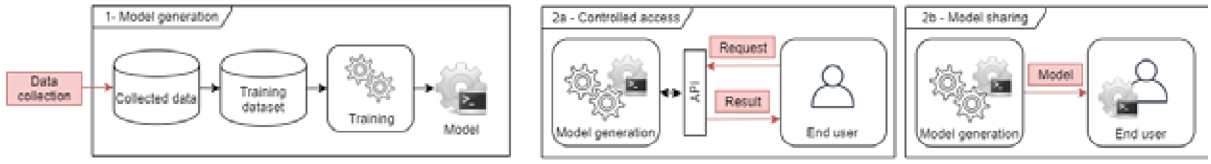

Figure 2. Attack vectors - (1) Upstream, (2) Downstream.

Salem *et al.* (2018) have built upon this work to relax assumptions and extend the attack scenario. Others have studied membership on Generative Adversarial Network (Hayes *et al.*, 2019) and even on robust Deep learning techniques (Song *et al.*, 2019).

Model inversion attack aims to reconstruct information about a data point present in the training dataset. This type of vulnerability was introduced by Fredrikson *et al.* (2014) and demonstrated for a ML system used for personalized warfarin dosing. They were able to show that an attacker possessing the ML model and demographic information about a patient would be able to infer their genetic markers. Until recently, attacks failed to inverse higher complexity model such as neural networks but Y. Zhang *et al.* (2020) presented a technique that uses a small amount of auxiliary knowledge against neural network in white-box settings.

Finally, model theft attack aims to gain knowledge about a black box or grey box model such as type of algorithm, hyper-parameters or trained model parameters. Tramer *et al.* (2016) shows that model parameters at risk in a context of MLaaS, even when attacker does not have knowledge about the training data set or model algorithm. They use equation-solving attack to extract highly accurate model with a little number of requests. In their work presented earlier for membership inference Shokri *et al.* (2017) actually use a shadow model (*i.e.*, a model mimicking the target model behaviour) as a step to mount their attack, essentially stealing a black-box version of the model.

Integrity of model and prediction

Data poisoning attack enables an adversary to influence the predictive power of a model by injecting malicious data points into the training data set. For example, Chen *et al.* (2017) presents a scenario in which a back door is installed on a deep learning authentication system. Recently, the particular case of sequentially generated data for continuous learning system has been studied by X. Zhang *et al.* (2019). Finally, adversarial learning is a class of attacks in which an attacker exploits a predictive system by finding an input that induces an abnormal behaviour of the system. For example, Al-Dujaili *et al.* (2018) successfully crafted adversarial example on malware binaries that allowed them to evade detection systems. Papernot *et al.* (2017) showed that they can instantiate such an attack in black-box settings targeting MLaaS.

Practical means of mitigation

Differential privacy is a designed privacy model to share information about a computation made on a dataset without compromising the privacy of each unique element. A possible implementation of differential privacy is through the addition of noise to the result to render unnoticeable the presence or absence of a particular profile in the data set. Differential privacy is a defence technique that is often used to counter membership inference and model inversion attacks. Since it can help generalizing the model, it can also be used to mitigate adversarial example and data poisoning attacks. Several attacks that we described take advantage of the accuracy of the predictions or use confidence levels shared along with the prediction. Controlling how the end user accesses the results of the predictive system (*e.g.*, by removing confidence levels) can help mitigating attacks such as membership inference and model inversion. Finally, robust models are designed to be more resilient to data perturbation both at training and prediction stage, helping mitigate adversarial crafting of data both at training stage (data poisoning) and at prediction stage (adversarial example). Several techniques can be employed to increase model robustness such as robust statistics, which are a class of estimation techniques that can be used to minimize impact of high diversity in statistical data distribution (*e.g.* outliers or small subgroups). Adversarial training is another technique in which adversarial examples are purposefully crafted and inserted in the training dataset to increase robustness against adversarial examples.

Discussion

In this paper, we have focused exclusively on threat vectors present at the data collection and the prediction interfaces. However, the security and privacy of ML systems security and privacy are also impacted by other concerns, which we briefly review in this section. In the data chain, in most cases the data collector (mostly the farmers) hand their data to data-processors. In addition, some of the data collected may be critical (*e.g.*, genomics) and considered as business secret (*e.g.*, production data). For this reason, the data collector might be reluctant to share its data which would break the first link of the data chain. In this scenario, homomorphic encryption is a cryptographic technique that could allow the data processor to train the ML system without the need for the data collector to divulge its valuable data, effectively maintaining our data chain functional. Data collectors have formulated concerns about the privacy of their data, and lack of cooperation between actors of the data chain have led to tensions within digital farming ecosystem. Doing our research, we have found multiple threat scenarios where the adversary is an insider to the digital farming context. Releasing tensions between actor would thus in itself help mitigate all ML induced risk to digital farming by lowering the likelihood of scenarios where the adversary is part of the data chain. A data trusts is a regulatory tool (a contract) designed to ensure that the management of a resource benefits each shareholder such as the resource provider (*i.e.* data-collector), resource processing agent (*i.e.* data-processor) and resource beneficiary (*i.e.* end-user).

Conclusion

As mentioned by organizational actors and looking at the context, it appears that security is a very concerning topic for digital farming that has been overlooked until now. We evaluated that ML was the left aside in terms of security and data privacy and dedicated our effort to help raise the attention as it is being used extensively. We have designed a Security model that helps framing the problem and investigated technical vulnerabilities and practical ways to mitigate them. During our study we also have found that related concern such as data ownership are directly impacting ML security and data privacy. Technical and regulatory tools such as homomorphic encryption and data trusts are available to help with these related concerns, effectively helping mitigating ML induced risks. Agriculture has always been a technophile ecosystem and up to this day it has taken the most out of available technologies, leading to the digitalization of farming. We believe that digital farming sector should learn from other industries and take the opportunity to be ahead of the curve on security and data privacy concerns.

List of references

Abdullah Al-Dujail, Alex Huang, Erik Hemberg and Una-May OReilly, 2018. Adversarial deep learning for robust detection of binary encoded malware. 2018 IEEE Security and Privacy Workshops (SPW): 76-82.

Scott Champion, Linsky, Peter Mutschler, Brian Ulicny, Thomson Reuters, Larry Barrett, Glenn Bethel, Michael Matson, Thomas Strang, Kellyn Ramsdell and Susan Koehler, 2018. Threats to precision agriculture. U.S. Department of Homeland Security.

Xinyun Chen, Chang Liu, Bo Li, Kimberly Lu and Dawn Song, 2017. Targeted backdoor attacks on deep learning systems using data poisoning.

DLG Committee for Digitization, Work Management and Process Technology, DLG

Committee for Technology in Crop Production, DLG Committee for Technology in Animal Production, DLG Work Group Information Technology, Hans W. Griepentrog, Norbert Uppenkamp and Roland Hörner, 2018. Digital agriculture: A DLG position paper.

Gene L. Dodaro, 2020. Priority open recommendations: U.S. department of agriculture. U.S. Government Accountability Office.

Matthew Fredrikson, Eric Lantz, Somesh Jha, Simon Lin, David Page and Thomas Ristenpart,2014. Privacy in pharmacogenetics: An end-to-end case study of personalized warfarin dosing. In 23rd USENIX Security Symposium (USENIX Security 14): 17-32.

Andrew Geil, Glen Sagers, Aslihan D. Spaulding and James R. Wolf, 2018. Cyber security on the farm: An assessment of cyber security practices in the United States agricultural industry. International Food and Agribusiness Management Review (3): 317-334.

Maanak Gupta, Mahmoud Abdelsalam, Sajad Khorsandroo and Sudip Mittal, 2020. Security and privacy in smart farming: Challenges and opportunities. IEEE Access (8):34564-34584.

Jamie Hayes, Luca Melis, George Danezis and Emiliano De Cristofaro, 2019. Logan: Membership inference attacks against generative models. Proceedings on Privacy Enhancing Technologies 2019(1): 133-152.

Nicolas Papernot, Patrick McDaniel, Arunesh Sinha and Michael Wellman, 2016. Towards the science of security and privacy in machine learning. Arxiv 1611.03814.

Nicolas Papernot, Patrick McDaniel, Ian Goodfellow, Somesh Jha, Z. Berkay Celik and Ananthram Swami, 2017. Practical black-box attacks against machine learning. Proceedings of the 2017 ACM on Asia Conference on Computer and Communications Security: 506-519.

Ahmed Salem, Yang Zhang, Mathias Humbert, Pascal Berrang, Mario Fritz and Michael Backes, 2018. Ml-leaks: Model and data independent membership inference attacks and defense son machine learning models. Network and Distributed Systems Security (NDSS) Symposium 2019.

Reza Shokri, Marco Stronati, Congzheng Song and Vitaly Shmatikov, 2017. Membership inference attacks against machine learning models. 2017 IEEE Symposium on Security and Privacy (SP).

Liwei Song, Reza Shokri and Prateek Mittal, 2019. Membership inference attacks against adversarially robust deep learning models. 2019 IEEE Security and Privacy Workshops (SPW): 50-56.

Michael Sykuta, 2016. Big data in agriculture: Property rights, privacy and competition in ag data services. The International Food and Agribusiness Management Review (19): 57-74.

Florian Tramèr, Fan Zhang, Ari Juels, Michael K. Reiter and Thomas Ristenpart, 2016. Stealing machine learning models via prediction apis. In Proceedings of the 25th USENIX Conference on Security Symposium (SEC'16): 601–618

Jason West, 2018. A prediction model framework for cyber-attacks to precision agriculture technologies. Journal of Agricultural and Food Information 19 (4):307-330.

Marc Window, 2019. Security in precision agriculture: Vulnerabilities and risks of agricultural systems. Luleå University of Technology - Department of Computer Science, Electrical and Space Engineering.

Sjaak Wolfert, Lan Ge, Cor Verdouw and Marc-Jeroen Bogaardt, 2017. Big data in smart farming - a review. Agricultural Systems (153):69-80.

Yuheng Zhang, Ruoxi Jia, Hengzhi Pei, Wenxiao Wang, Bo Li and Dawn Song, 2020. The secret revealer: Generative model-inversion attacks against deep neural networks. 2020 IEEE/CVF Conference on Computer Vision and Pattern Recognition (CVPR): 250-258.

Xuezhou Zhang, Xiaojin Zhu and Laurent Lessard, 2020. Online data poisoning attack. Proceedings of the 2nd Conference on Learning for Dynamics and Control, PMLR (120): 201-210.

SMARTER – Which novel traits to improve feed efficiency?

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Abstract

The SMARTER (SMAll RuminanT breeding for Efficiency and Resilience) H2020 project aims to develop and implement innovative strategies to improve resilience and efficiency (RandE) related traits in sheep and goats. Regarding feed efficiency, the objective is to identify novel traits that would be relevant, easy to measure and cheap enough to be collected in many animals to identify the most efficient individuals. In practice, feed efficiency can be assessed by different criteria such as residual feed intake and feed conversion ratio. Both criteria require feed intake to be measured for each individual which remains an expensive trait to get and hardly feasible under farm conditions. Thus, our objective is to identify novel traits related to feed efficiency and use them as proxies for feed intake and/or feed efficiency.

First, novel phenotypes are identified and studied in experimental farms where feed intakes of concentrate and forage are recorded for each animal. Different novel phenotypes are being considered to study feed efficiency in sheep and goats, including

1. Biomarkers (from blood or milk metabolomics),
2. Differentially expressed genes in targeted tissues,
3. Genomic polymorphisms,
4. Ruminal microbiota,
5. Faecal nir spectra, (
6. Greenhouse gas emissions and body composition traits.

These novel traits will be recorded either under classical feeding or under nutritional restriction to quantify, for example, the impact of a shortage of concentrate inputs.

Then, the most promising novel traits will be measured in commercial populations. From these larger datasets, we will estimate heritabilities of the novel traits and genetic correlations to other traits in the breeding goal.

Finally, for some case studies, we will quantify GxE interactions, particularly by considering the same breed under different breeding systems or regions.

Keywords: Small ruminants, sheep, goat, resource use efficiency, novel phenotypes.

Introduction

Feed efficiency is the ability of livestock to transform feed into food edible by humans. Breeding feed-efficient livestock is of high interest both at environmental and economic levels. Among livestock, ruminants are the only ones able to transform fibers into proteins. There is growing interest in breeding ruminants mainly fed with increasing amounts of forage and decreasing quantities of concentrates. Breeding programs are willing to include feed efficiency in their breeding objectives. Residual feed intake (RFI) is currently one of the most common criteria used to improve feed efficiency. RFI has been shown to be variable and moderately heritable in meat sheep (Cammack et al., 2005; Johnson et al., 2018; Paganoni et al., 2017; Snowder and Van Vleck, 2003; Tortereau et al., 2020) and dairy goats (Desire, S. et al., 2017). Selecting on RFI is thus possible, but it requires feed intake to be recorded, which is the major limit to the deployment of this recording on a broader scale, particularly in small ruminants. Therefore, there is an increasing interest in identifying proxies for feed intake and/or feed efficiency directly. In order to be widely recorded, proxies must be easy to collect and low in cost. In the SMARTER project, we do not only focus on such new phenotypes, but also on others that are more difficult to collect and/or more expensive to acquire. The overall objective is to understand the biological pathways underlying feed efficiency in small ruminants, which will help in identifying proxies.

The objective of this work is to gather data from different experimental and commercial farms of small ruminants to dissect feed efficiency and to propose proxies that could be widely collected to estimate their genetic parameters and their ability to predict feed intake or feed efficiency.

Material and methods

Animals

Two categories of animals are considered: animals from experimental farms and animal from commercial farms. In dairy sheep, experimental protocols rely on 4 different breeds : Chios (n=8), Lacaune (n=62), Frizarta (n=8) and Assaf (n=47), and in commercial farms, ewes from 5 breeds are being phenotyped : Chios (n=250, in 2 farms), Frizarta (n=500, in 2 farms), Lacaune (n=3,972 in 8 farms), Pyrenean breeds (n=876, including ewes from Manech Tête Rousse and Basco-Béarnaises breeds, in 7 farms). An experiment rely on a nutritional challenge in 40 Assaf ewe replacement lambs: half of the animals are subjected, during their prepuberal stage, to a diet contained 42% less crude protein than controls. In both control and restricted group, animals have been chosen from each of the extremes of the distribution of genetic values (paternal average) for milk production. An experiment in Lacaune ewes (n=55) relies on divergent lines selected on milk persistency.

In dairy goats, two experiments are conducted, the first in the Alpine breed (n=110) and the second in a mixed breed (n=3,421) based on Alpine, Saanen and Toggenburg breeds. A total of 11 commercial farms are involved in the collection of fine phenotypes of goats: 6 farms with Saanen (n=1,678 goats) and 5 farms with Alpine (n=1,176 goats). The experiment in Alpine breed involves divergent lines on longevity. Goats are daughters of bucks selected according to their extremely high/low EBV on that trait.

In meat sheep, experiments are based on 7 different breeds: Romane (n=277), Merino (n=1002), Corriedale (n= 303), Dohne (n= 360), Texel and Texelx Lleyn(n= 2,340) and Scottish BlackFace (n~2,000). In commercial farms, data are being collected in 5 breeds: Mouton Vendéen (n=1,500 ewes in 5 farms), Rouge de l'Ouest (n= 800 ewes in 5 farms), Blanche du Massif Central (n=2,000 ewes in 5 farms), Norwegian White Sheep (n=1,600 ewes in 15 farms) and Texel (n~6,000 animals in 154 flocks). The experiment in the Romane breed relies on two divergent lines on Residual Feed Intake (RFI): male lambs that are phenotyped are selected for their extremely high/low EBV on this trait.

Table 1. Phenotypes recorded on experimental and/or commercial dairy ewes, dairy goats and meat sheep.

Group of traits	Trait	Production	Population	Comment
Intake	Total Feed intake (forage or concentrate)	Meat Milk	Experimental mainly	During given control periods, under different diets
Intake	Concentrate intake	Milk	Commercial Experimental	Concentrate supplied in milking parlour
Body composition	Live weight	Meat Milk	Commercial Experimental	Collected at different ages per animal
Body composition	Chest width	Meat Milk	Commercial	At different production stages
Body composition	Chest depth	Meat Milk	Commercial	At different production stages
Body composition	Shoulder height	Meat	Commercial	At different production stages
Body composition	Muscle depth (ultrasound)	Meat Milk	Commercial Experimental	Only in experimental farms for dairy animals and at different production stages
Body composition	Back Fat Thickness (ultrasound)	Meat Milk	Commercial Experimental	Only in experimental farms for dairy animals and at different production stages
Body composition	Body condition score	Meat Milk	Commercial Experimental	At different production stages
Udder composition	Udder conformation	Milk	Commercial Experimental	
Production	Milk Production (MY, FY, PY, FC, PC)	Milk	Commercial Experimental	
Production	Carcass Traits	Meat	Commercial	
Gas emissions	GHG emissions (CH_4, CO_2)	Meat	Commercial Experimental	
milk samples	Gene expression (RNA-seq)	Milk	Experimental	
milk samples	Epigenetic marks	Milk	Experimental	
milk samples	MIRS	Milk	Commercial Experimental	
milk samples	Metabolomic pattern	Milk	Experimental	
rumen samples	Fatty Acids (volatile and long)	Meat Milk	Experimental	Under different diets
rumen samples	NMR	Meat Milk	Experimental	Under different diets
rumen samples	16S – microbial abundances	Meat Milk	Experimental	Under different diets
Blood samples	Targeted metabolites*	Meat Milk	Commercial Experimental	
Blood samples	NMR	Meat Milk	Experimental	Under different diets
Blood samples	Genotypes	Meat Milk	Commercial Experimental	
Faeces samples	NIRS	Meat Milk	Experimental	Under different diets

MY: Milk Yield, FY: Fat Yield, PY: Protein Yield, FC: Fat Content, PC: Protein Content, MIRS: Mid Infra-Red Spectra, NMR: Nuclear Magnetic Resonance NIRS: Near Infra-Red Spectra
* targeted metabolites : glucose, non-esterified fatty acids, beta-hydroxybutyrate, insulin

Phenotypes

Different sets of phenotypes and biological samples are being collected, depending on the production type (meat vs. milk) and the farms (experimental vs. commercial). All the phenotypes directly collected in the animals or obtained after analysis of biological samples are described in table 1.

Other phenotypes are calculated from these elementary traits: Average daily gain (ADG), Feed Conversion Ratio (FCR), Residual Feed Intake (RFI). Differences in BCS between two successive physiological stages will also be computed. Milk fine compositions from MIRS were also estimated from previous equations. Microbiota abundances were get from 16S sequencing and metabolome abundances from NMR.

Feed intakes are obtained with automatic feeders that record each visit of any animal. Feeding behavior traits were calculated from these datasets.

Not all the phenotypes are available for all individuals. However, live weights and body composition traits obtained through ultrasounds are recorded for almost all meat sheep, and milk production traits are recorded for all dairy sheep and goats. Blood samples are obtained for almost all individuals with phenotypes, at least for genotyping purposes.

Statistical analyses

First, each trait will be analysed independently within each experiment. Fixed effects and covariates will be selected according to each trial. For example, for divergent lines experiment, the line will be considered as a fixed effect. Each trait will be considered as a proxy for feed intake or feed efficiency. Combinations of elementary traits listed in table 1 will also be considered as proxies. Proxies will be identified in each protocol and will be tested in the other trials when possible. The quality of a proxy will be assessed through different methodologies, adapted to the different protocol dimension. Here, we analysed an experiment in 30 dairy ewes from 4 breeds (8 Chios, 7 Lacaune, 8 Frizarta and 7 Assaf), with sensitivity/sensibility approach. The receiver operating characteristic analysis was used to define thresholds for changes in body composition traits as predictors of negative energy balance. Moreover, mixed linear models were used to test the association of blood biomarkers with fat and muscle reserves and their mobilization.

Multivariate approaches will be applied for large datasets such as microbiota and metabolomics matrices. The MixOmics package implements such methods and will be used (Le Cao, et al., 2016).

In commercial populations, the higher numbers of phenotyped individuals will allow genetic parameters to be estimated in each population. Pedigrees are recorded in the frame of national genetic evaluation programs. These data are currently under collection, so no results are available yet.

Results and discussion

In dairy ewes from the Chios, Assaf, Lacaune and Frizarta breeds (total=30 ewes), NEFA were used to analyse energy balance: an increase in NEFA being synonymous with a negative energy balance. Association analyses highlighted that a NEFA status of more than 0.3 mmol/L could be predicted by a decrease in Back Fat Thickness (BFT) and in the sum of BFT and *Longissimus Dorsi* muscle Thickness (LDT) of more than 0.075 mm and 0.350 mm, respectively. Likewise, a NEFA status of more than 0.7 mmol/L could be predicted by a decrease in BFT, in LDT and in their total sum of more than 0.15 mm, 0.065 mm and 0.350 mm, respectively. In the same 30 ewes, a significant positive association of serum albumins with LDT was found. Specifically, a change of serum albumins by 1 g/dL was associated with a change in LDT by 1.27

mm. Such an association could be explained by the fact that serum albumins and LDT are both deposits of amino acids in the tissues.

In meat sheep, Romane from the 3rd generation of divergent selection on RFI exhibited significant differences (p-value<0.05) in feed intake, with more efficient lambs eating 195g less of concentrate than less efficient lambs, and RFI, with a difference of 1.9 genetic standard deviations between both groups. Live weight differences were observed, with inefficient individuals being heavier (+1.75 kg at 5 months old). Ruminal microbiota analyses highlighted differences in composition, and plasmatic amino acids were also different between both groups: more efficient lambs have lower plasmatic levels than less efficient lambs.

Conclusion

The collection of phenotypes is still on-going. However, first analyses of experimental datasets highlight that some phenotypes are linked to feed intake, feed efficiency or energy status and can be proposed as promising candidates for proxies of those traits.

List of references

Cammack, K.M., Leymaster, K.A., Jenkins, T.G., and Nielsen, M.K. (2005). Estimates of genetic parameters for feed intake, feeding behavior, and daily gain in composite ram lambs. J. Anim. Sci. 83, 777–785.

Desire, S., Mucha, S., Mrode, R., Coffey, M., Broadbent, J., and Conington, J. (2017). Genetic parameter estimations for feed intake in UK dairy goats. (Tallinn), p. 152.

Johnson, P.L., Knowler, K., Wing, J., Hickey, S., and Johnstone, P. (2018). Preliminary estimates of genetic parameters for residual feed intake in New Zealand maternal sheep. In WCGALP, Auckland (New Zealand).

Le Cao, Kim_Anh, Rohart, Florian, Gonzalez, Ignacio, Dejean, Sebastien, and with key contributors Benoit Gautier, Francois Bartolo, contributions from Pierre Monget, Jeff Coquery, FangZou Yao and Benoit Liquet (2016). mixOmics: Omics Data Integration Project. R package version 6.1.1.

Paganoni, B., Rose, G., Macleay, C., Jones, C., Brown, D.J., Kearney, G., Ferguson, M., and Thompson, A.N. (2017). More feed efficient sheep produce less methane and carbon dioxide when eating high-quality pellets. J. Anim. Sci. 95, 3839.

Snowder, G.D., and Van Vleck, L.D. (2003). Estimates of genetic parameters and selection strategies to improve the economic efficiency of postweaning growth in lambs. J. Anim. Sci. 81, 2704–2713.

Tortereau, F., Marie-Etancelin, C., Weisbecker, J.-L., Marcon, D., Bouvier, F., Moreno-Romieux, C., and François, D. (2020). Genetic parameters for feed efficiency in Romane rams and responses to single-generation selection. Animal 14, 681–687.

Feasibility of a genomic selection approach for gastro-intestinal nematodes resistance based on single-step evaluation and a female informative population in Sarda breed sheep

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Abstract

Gastrointestinal nematodes are a major health problem in grazing animals. In Sardinia, sheep farming systems are based on grazing natural pastures and forage crops where infection is unavoidable. However, the inclusion of nematode resistance in the current breeding scheme is difficult due to the laboriousness of the fecal egg counting at Herd Book scale. The aim of this work was to assess the feasibility of a genomic selection approach based on a female nucleus as reference population and young Herd Book rams as selection candidates by using ssGBLUP. The female nucleus consisted of 5386 ewes from 10 F1 Sarda x Lacaune and 208 Sarda rams. From 2000 to 2020, 16,977 individual records were collected in 25 dates on 4,304 ewes. All recorded ewes, their sires and available male ancestors were genotyped as well as 734 Herd Book rams. The weights of A and G matrices when building the H matrix in ssGBLUP were varied from 0 to 1 to detect the model showing the highest heritability and predictive ability and then using it for the BV prediction of the selection candidates. The highest heritability estimate (0.27) was obtained when the G weight was between 0.20 and 0.40 whereas predictive ability was more or less constant across the different G weights. The average expected accuracies of 245 young Herd Book rams depended on the amount of information on relatives in the female nucleus. Overall results show that in our population high weights of the pedigree information are needed when building the relationship matrix in ssGBLUP to improve heritability and predictive ability. It is likely that pedigree information adjusts for genomic relationships which are partly due to IBS rather than IBD genome sharing. Moreover, recording pedigree information in the Herd Book is crucial also to calculate the amount of information on relatives in FRP of selection candidates and predict their expected accuracies.

Keywords: Genomic selection, female nucleus, nematodes resistance, ssGBLUP.

Introduction

Gastrointestinal nematodes (GIN) are major health problems in grazing animals (Kaplan *et al.*, 2012). GIN infections determine important yield reductions and increased production costs due to medical treatments and higher culling rates (Mavrot *et al.*, 2015). Moreover, anti-helminthics resistance is more and more an issue in several countries (Sargison *et al.*, 2007). Fecal egg count (FEC), i.e., the number of parasite eggs per gram of faeces has been largely used as proxy trait to measure resistance to GIN. In Sardinia, sheep farming systems are based on grazing natural pastures and forage crops where GIN infection is unavoidable. Thus, most farmers administrate anti-helminthics often with an empirical approach in terms of individual diagnosis, doses and frequency of treatments (Sechi *et al.*, 2010). Genetic variation between individuals and breeds has been documented (Assenza *et al.*, 2014; Bouix *et al.*, 1998; Sechi *et al.*, 2009). However, the inclusion of GIN resistance in current breeding schemes is difficult due to the laboriousness of the FEC recording at Herd Book (HB) scale.

The aim of this work was to assess the feasibility of a genomic selection approach based on a female nucleus as reference population and young HB rams as selection candidates by:

1. Using single-step GBLUP to improve the genomic predictions accuracy of HB rams by exploiting both genomic and pedigree information;
2. Detecting the optimal weights of **A** and **G** matrices into **H** matrix using heritability and predictive ability as indicators;
3. Evaluating the expected accuracies of selection candidates.

Material and methods

Female reference population

The generation of the female reference population (FRP) started in 1999 when 10 Lacaune × Sarda F1 sires were mated to Sarda ewes to produce 928 back-cross female lambs. Subsequently, only HB Sarda rams were used to produce the yearly replacement of FRP. Until 2009, the average size of the sire families was 43 daughters whereas, from 2010, it was reduced to 9 daughters to increase the number of bloodlines represented in the FRP. Sarda sires were chosen among those with high genetic impact in HB from the Artificial Insemination Center of the breed. In total, 5,386 ewes from 218 rams (10 F1 and 208 SA) were generated until 2020. The farming system was similar to that commonly applied in Sardinia with most of the adult ewes lambing in autumn and yearlings lambing between January and March. The feeding regime was based on grazing of natural pastures and forage crops, supplemented by hay and concentrates in winter and late spring.

Phenotyping

FEC was the proxy trait used to assess GIN resistance under natural conditions of infection in the experimental flock. Periodically, a sample of around 50 ewes representative of the different management groups was monitored to evaluate the percentage of infected animals and decide whether to sample the whole flock and possibly administrate anthelmintic.

FEC was determined using a copro-microscopic exam conforming to the McMaster technique on individual samples. When the number of infected animals and the level of infestation was considered sufficient to appreciate individual variability, individual FEC were measured on the whole flock. From 2000 to 2020 individual FEC were recorded from 1 to 3 times per year, more frequently in September and July. Due to the low level of infection found, no individual measures were realised between June 2006 and November 2007. FEC recording for a QTL detection experiment was closed in 2012 and restarted in 2015 for the new cohorts with the perspective of implementing MA or genomic selection.

Table 1. Distribution of FEC records (NR), dates (ND) and animals (NA) per year (Y).

Y	2000	2001	2002	2003	2005	2006	2008	2009	2010	2011	2012	2016	2017	2018	2019	2020
NR	1813	1775	1703	1631	717	1803	1270	748	1190	636	588	931	540	599	640	393
ND	2	2	2	2	1	3	2	1	2	1	1	2	1	1	1	1
NA	914	906	853	988	717	879	918	748	792	636	588	740	540	599	640	393

Finally, 16,977 FEC measurements were recorded in 25 dates on 4,304 animals (Table 1).

FEC presented skewed distribution and were log-transformed using lnFEC=ln(FEC+14).

Genotyping

All 4,304 ewes of FRP, 194 sires and 78 available male ancestors (272 rams) as well as 734 HB Sarda rams were genotyped with the Illumina Inc. OvineSNP50 Beadchip. SNP editing was performed using call rate and MAF thresholds of 90% and 1%, respectively. After quality control, 43,293 SNP across 26 autosomes were retained for the analysis.

Single-step genomic BLUP

Single-step genomic BLUP was used to estimate variance components and breeding values with a repeated animal model including the permanent environment and additive genetic random effects. Environmental fixed effects were the date of sampling, the age of the animal (from 1 to 4 years) and the interaction between the number of lambs and the days from lambing. The additive genetic effect was modeled using a realized **H** relationship matrix, which combines pedigree and genomic relationships (Legarra *et al.*, 2009). Compared to BLUP, in ssGBLUP, the inverse of the numerator relationship matrix $\mathbf{A}^{-1}$ is replaced by $\mathbf{H}^{-1}$ defined as follows:

in which **G** is the genomic relationship matrix. The **G*** matrix used for blending was obtained according to VanRaden (2008) and Aguilar (2010) as:

$$\mathbf{G^*} = [w\mathbf{G}^{-1} + (1-w)\mathbf{A}_{22}^{-1}]^{-1}$$

were the weight (*w*) ranged between 0.0 and 1.0 by 0.2. Heritability (h^2) and repeatability (r) were computed as

$$\sigma^2_a / (\sigma^2_a + \sigma^2_{pe} + \sigma^2_e) \text{ and } (\sigma^2_a + \sigma^2_{pe}) / (\sigma^2_a + \sigma^2_{pe} + \sigma^2_e) \text{ respectively}$$

where s^2_a is the additive genetic variance, s^2_{pe} is the permanent environment variance and s^2_e is the error variance. The parameter *w* scales differences between genomic and pedigree-based information. (Christensen and Lund, 2012; Meyer *et al.*, 2018; Lourenco *et al.*, 2020). In several studies, different values of *w* were used to construct the relationship matrix without estimating variance components and h^2 (Aguilar *et al.*, 2010; McMillan and Swan, 2017). Usually variance component estimation has been performed just for traditional AMBLUP, GBLUP and ssGBLUP with a *w* between 0.95 and 0.99 (Aldridge *et al.*, 2020; Cesarani *et al.*, 2019; Forni *et al.*, 2011; Hidalgo *et* *al.*, 2020). In our study, *w* was varied from 0.0 to 1.0 to detect the blending weights of **A** and **G** matrices showing the highest h^2 and PA and then using them for the BV prediction of the selection candidates.

Single step GBLUP was performed using the package AIREMLf90 included in the BLUPf90 suite programs (Misztal *et al.*, 2002). The following options where provided to AIREMLf90 to built **H** matrix and blending **A** and **G**:

- the pedigree file was provided to built the $\mathbf{A}^{-1}$ internally including inbreeding;
- the genomic relationship matrix was created as in VanRaden (2008);

The pedigree file included 6,242 animals tracing back to three generations of FRP ewes. Further 2,323 animals were added to the pedigree file when HB Sarda rams were included in the analyses.

The predictive ability (PA) of future phenotypes was calculated for each tested *w* as the correlation between the breeding values predictions and the individual average yield deviation of 235 last generations ewes. Breeding values and their expected accuracies (r_{BV}) were calculated with a training dataset in which the 421 records of the 235 ewes were masked.

Expected accuracy of selection candidates HB rams

The model showing the highest h^2 and PA estimates was used for the GEBV estimation of 1006 genotyped rams born from 1993 to 2020 collected in different frameworks of genotyping activities and classified according to their age and level of relationship with FRP:

- 272 sires or ancestors of FRP ewes;
- 489 old HB rams born from 1993 to 2015 with daughters in HB flocks
- 245 young HB rams (including 27 son of FRP ewes) born from 2016 and 2020 without daughters in HB flocks and considered as selection candidates.

Expected accuracy of breeding values of animal i were estimated as:

$$r_{BVi} = \sqrt{1 - \frac{SEP_i^2}{h_{ii} \times \sigma_a^2}}$$

where SEP$_i$ is the standard error of prediction derived from the diagonal element of the left-hand side inverse of the mixed model equations and h$_{ii}$ is the diagonal element for the animal *i* of the **H** matrix.

The overall results for FEC were compared with those obtained for milk yield (MY) in the same population using the same approach (unpublished results).

Results and discussion

The overall mean and standard deviation of FEC and lnFEC were 332 ± 727 and 4.82 ± 1.43, respectively.

Estimated variance components are shown in Table 2. The highest values of σ^2_a were reached when *w* is between 0.2 and 0.4 and then decreases as *w* increases. Moreover, σ^2_{pe} decreases as σ^2_a increases while σ^2_e is quite constant across the models.

Table 2. Estimated variance components (σ^2_a : additive genetic variance; σ^2_{pe}: permanent environment variance; σ^2_e: error variance; σ^2_{tot}: total variance) based on single-step GBLUP varying the weight (wG) of genomic information in blending H.

	*w*G=0.0	*w*G=0.2	*w*G=0.4	*w*G=0.6	*w*G=0.8	*w*G=1.0
σ^2_a	0.416 ± 0.035	0.460 ± 0.035	0.459 ± 0.034	0.438 ± 0.032	0.407 ± 0.030	0.383 ± 0.029
σ^2_{pe}	0.067 ± 0.022	0.029 ± 0.021	0.029 ± 0.020	0.045 ± 0.019	0.070 ± 0.018	0.082 ± 0.017
σ^2_e	1.189 ± 0.015	1.190 ± 0.015	1.191 ± 0.015	1.192 ± 0.015	1.192 ± 0.015	1.193 ± 0.015
σ^2_{tot}	1.672	1.680	1.678	1.675	1.669	1.658

Table 3. Heritability (h^2), repeatability (r), predictive ability (PA) and mean of expected accuracy (r_{BV}) of single-step GBLUP ranging the weight (wG) of genomic information when blending A and G into H.

	wG = 0.0		wG = 0.2		wG = 0.4		wG = 0.6		wG = 0.8		wG = 1.0	
	FEC	MY	FEC	MY	FEC	MY	FEC	MY	FEC	MY	FEC	MY
h^2	0.249	0.413	0.274	0.441	0.273	0.424	0.262	0.392	0.244	0.354	0.231	0.328
r	0.289	0.585	0.292	0.587	0.290	0.585	0.288	0.581	0.286	0.577	0.281	0.573
PA	0.304	0.244	0.317	0.262	0.320	0.260	0.320	0.251	0.319	0.238	0.316	0.221
r_{BV}	0.450	0.479	0.470	0.496	0.493	0.514	0.519	0.535	0.547	0.557	0.575	0.582

In Table 3 are reported the results in terms of h^2, r, PA and r_{BV} in comparison with those obtained for milk yield on the same population with the same approach. For FEC, in agreement with the variance components estimates, h^2 shows the highest value when *w* is between 0.2 and 0.4 and gradually decreases as *w* increases. When *w* assumes values between 0.2 and 0.4, h^2 is 18% higher than when just the genomic information is considered (*w*=1.0). On the other hand, repeatability is quite similar across mo1dels. PA is more or less constant across the models whereas the average r_{BV} of the female validation sample increases as *w* increases.

The same pattern of and r_{BV} is found for MY. However, in this case, PA shows a pattern similar to h^2 *i.e* the model showing the highest h^2 showed also the highest PA estimate.

The r_{BV} estimates of HB rams, obtained using the model with the highest h^2 and PA (*w* = 0.4), ranged between 0.95 and 0.19 with a mean of 0.71 ± 0.18 for sires or ancestors of FRP, 0.33 ± 0.12 for young HB rams and 0.30 ± 0.08 for old HB rams. Among the selection candidates, the highest values of r_{BV} were obtained for the progeny of FRP ewes as shown in Figure 1.

As shown in previous studies (Pszczola *et al.*, 2012; Usai *et al.*, 2018), r_{BV} of selection candidates can be easily and accurately predicted by parameters derived by the numerator relationships matrix which measure their amount of information on relatives in the reference populations. This result is important for designing the flow of animals from and toward the nucleus in order to achieve good accuracies for most selection candidates.

Conclusion

Overall heritability results show that selective breeding may be an option to increase resistance to infection of gastro-intestinal nematodes in sheep. Both traditional progeny testing and genomic selection with a female reference population are realistic options. However, the laboriousness of fecal egg counting at Herd Book scale makes genomic selection based on a female nucleus potentially more profitable in terms of costs-

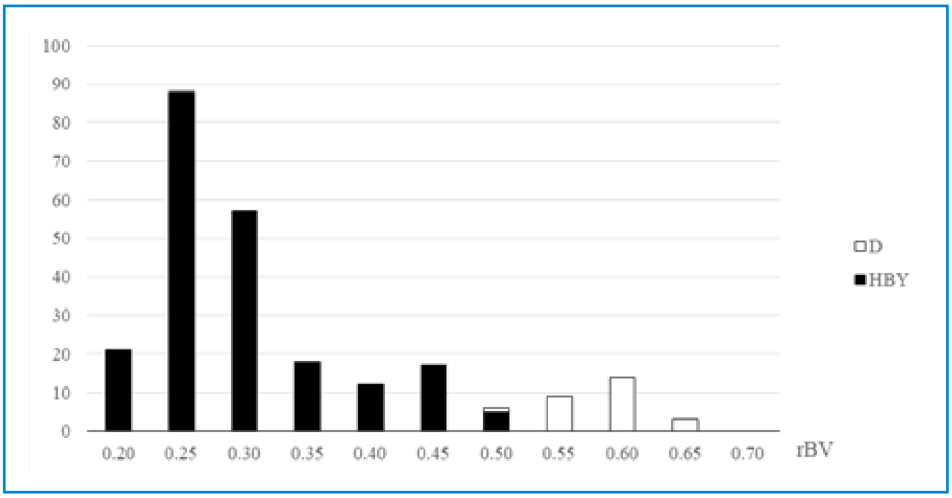

Figure 1. Distribution of the expected accuracies (r_{BV}) of 245 selection candidates (D: descendants of FRP; HBY: young HB rams).

benefits. Single-step GBLUP is essential to manage genomic selection based on female nuclei. In our population, high weights of the pedigree information are needed when building the relationship matrix to improve heritability and predictive ability. It is likely that pedigree information adjusts for genomic relationships which are partly due to IBS rather than IBD genome sharing. Moreover, recording pedigree information in HB is crucial also to calculate the amount of information on relatives of selection candidates in FRP and predict their expected accuracies. A better understanding of genomic predictions accuracies is needed to optimize the flow of animals from and toward the nucleus in order to achieve good accuracies for most selection candidates. As a perspective, accuracies of genomic predictions may be improved including in the evaluation model 10 highly significant SNP identified in QTL detection analysis (Casu *et al*., 2021, submitted). In the short term, genomic predictions for resistance to gastro-intestinal nematodes will be delivered just for the herd book selection candidates well genetically connected with the female nucleus.

List of references

Aguilar, I., I. Misztal, D.L. Johnson. A. Legarra, S. Tsuruta and T.J. Lawlor. 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. J. Dairy Sci. 93, 743–752.

Aldridge, M.N., J. Vandenplas, R. Bergsma, and M.P.L. Calus. 2020. Variance estimates are similar using pedigree or genomic relationships with or without the use of metafounders or the algorithm for proven and young animals. J. Anim. Sci. Vol. 98, No. 3, 1–9

Assenza F, J-M. Elsen, A. Legarra, C. Carré, G. Sallé, C. Robert-Granié, *et al.* 2014. Genetic parameters for growth and faecal worm egg count following Haemonchus contortus experimental infestations using pedigree and molecular information. Genet Sel Evol. Feb 14;46(1):13.

Bouix J, J. Krupinski, R. Rzepecki, B. Nowosad, I. Skrzyzala, M. Roborzynski, *et al.* 1998. Genetic resistance to gastrointestinal nematode parasites in Polish long-wool sheep. Int J Parasitol. 1998 Nov;28(11):1797–804.

Cesarani, A., G. Gaspa, F. Correddu, M. Cellesi, C. Dimauro, and N.P.P. Macciotta. 2019. Genomic selection of milk fatty acid composition in Sarda dairy sheep: Effect of different phenotypes and relationship matrices on heritability and breeding value accuracy. J. Dairy Sci. 102:3189–3203.

Christensen, O.F., and M.S. Lund. 2010. Genomic prediction when some animals are not genotyped. Genet. Sel. Evol. 42, 2.

Forni, S., I. Aguilar, I. Misztal. 2011. Different genomic relationship matrices for single-step analysis using phenotypic, pedigree, and genomic information. Genet. Sel. Evol. 43, 1.

Hidalgo, J., S. Tsuruta, D. Lourenco, Y. Masuda, Y. Huang, K.A. Gray, and I. Misztal. 2020. Changes in genetic parameters for fitness and growth traits in pigs under genomic selection. J. Anim. Sci. 1–12.

Legarra A, I. Aguilar and I. Misztal. 2009. A relationship matrix including full pedigree and genomic information. J. Dairy Sci. 92, 4656–4663.

Kaplan RM and A.N. Vidyashankar. 2012. An inconvenient truth: Global worming and anthelmintic resistance. Vet Parasitol. 186(1–2):70–8.

Mavrot F, H. Hertzberg and P. Torgerson. 2015. Effect of gastro-intestinal nematode infection on sheep performance: A systematic review and meta-analysis. Parasites and Vectors. Oct 24;8(1):557.

McMillan, A.J., and A.A. Swan. 2017. Weighting of genomic and pedigree relationships in single step evaluation of carcass traits in Australian sheep. Proc. Assoc. Advmt. Anim. Breed. Genet. 22:557-560.

Meyer, K., B. Tier and A. Swan. 2018. Estimates of genetic trend for single step genomic evaluations. Genet Sel Evol. 50:39.

Misztal, I., S. Tsuruta, T. Strabel, B. Auvray, T. Druet, and D. H. Lee. 2002. Blupf90 and related programs (BGF90). Commun. No. 28–07 in Proc. 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France.

Pszczola M, T. Strabel, H.A. Mulder, M.P.L. Calus. 2012. Reliability of direct genomic values for animals with different relationships within and to the reference population. J Dairy Sci. 95:389–400.

Sargison N.D., F. Jackson, D.J. Bartley, D.J. Wilson, L.J. Stenhouse and C.D. Penny. 2007. Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. Vet Parasitol. Apr 10;145(1–2):65–76.

Sechi S, M. Giobbe M,G. Sanna, S. Casu, A. Carta and A. Scala. 2010. Effects of anthelmintic treatment on milk production in Sarda dairy ewes naturally infected by gastrointestinal nematodes. Small Rumin Res. Feb;88(2–3):145–50.

Sechi S, S. Salaris, A. Scala, R. Rupp, C. Moreno, SCSC Bishop, *et al.* 2009. Estimation of (co)variance components of nematode parasites resistance and somatic cell count in dairy sheep. Ital J Anim Sci. 8(SUPPL. 2):156–8.

Usai M.G., S. Salaris, S. Casu, T. Sechi, S. Miari, P. Carta, and A. Carta, 2018. Feasibility of genomic predictions of Sarda breed rams using a female reference population. Proceedings, 11th WCGALP. Auckland, New Zealand, 11–16 February.

VanRaden, P.M. 2008. Efficient methods to compute genomic predictions. J. Dairy Sci. 91, 4414–4423.

Selection tools to benefit from international cooperation in small ruminants: a comprehensive work package of the SMARTER project

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Abstract

SMARTER (SMAll RuminanT breeding for Efficiency and Resilience) is an H2020 EU multi-actor project, whose global objective is to develop novel and collaborative strategies to improve resilience and efficiency of the sheep and goat sectors at the animal, population/breed and system/farm levels. A dedicated work package (WP6) aims to contribute to accelerated genetic progress for resilience and efficiency (R&E) through international harmonisation and cooperation in order to deploy genomic evaluations across countries. The goal of this paper is to present the different tasks that are being undertaken to successfully meet the WP6 goals.

SMARTER will generate recommendations on the phenotyping of R&E related traits, which will enrich the ICAR guidelines in the area of small ruminants. SMARTER will also build 3 prototypes (meat and dairy sheep, dairy goat) of across country genetic and genomic evaluations. The activities under development will result in the tools to enable a future routine international evaluation whose business and practical operation model will be established during the project. SMARTER has already proposed and implemented several tools towards the development of across country genomic evaluations. For instance, the file formats to exchange pedigree, phenotype and genotype, have been established based on Interbull and Interbeef experiences. An international codification of the sheep and goat breeds has been initiated and is expected to be consolidated by ICAR. The current breeding programs and genetic evaluation systems have been described, based on a survey distributed to all the partners. A comparison of sheep genotype metrics across breeds and countries should lead to a common genotype platform suitable to all SMARTER populations. Data sharing agreements have been signed between partners to pool large set of historical phenotypic and genomic datasets. Furthermore, a deterministic model has been developed to quantify the long-term benefits and feasibility of international genetic and genomic evaluations has been evaluated. The assessment of the long-term benefit of international evaluation is crucial

to inform countries to endorse the harmonisation of their methods of phenotyping and evaluation and accept to pool together data from different countries.

Keywords: Sheep, goat, guidelines, international evaluation, genomics, harmonisation

Introduction

SMARTER stands for "SMAll RuminanTs breeding for Efficiency and Resilience ». It is an H2020 project running from November 2018 to October 2022 (initially), expanded to 2023 (due to Covid-19) and coordinated by Carole Moreno-Romieux from INRAE (SMARTER project, 2019).

This is a multi-actor project with 27 full partners from 13 countries. Half the partners are non-academic. ICAR is included in the partnership. Various stakeholders are also participating in the project. Most of the members of the Sheep, Goat and Camelid ICAR working group are from organisations which are either partners or stakeholders in SMARTER.

An entire work package (WP6) is dedicated to propose and develop practical tools to achieve the objective of contributing to faster genetic progress for resilience and efficiency traits in sheep and goats through improved international cooperation.

Four tasks are being undertaken, related to:

- The harmonisation of phenotypes, genotypes and pedigree.
- Across country evaluations.
- The practicalities of international evaluations.
- The modelling of the benefits of harmonisation and international cooperation on long-term genetic gain.

This paper aims to describe the work carried out in the work package with a particular focus on the topics of harmonisations and international cooperation. Both of these are part of ICAR's core activities, namely guidelines, evaluation and certification services. In this respect, the outputs from SMARTER should be useful for ICAR community.

Harmonisation of phenotypes, genotypes and pedigree

The first batch of actions, related to harmonisation, has the triple objective of

1. providing the prerequisite for international evaluations;
2. conceiving an optimized and affordable genomic tool and
3. proposing recommendations for recording resilience and efficiency traits.

The following advancements are described in this section: description of breeding programs and evaluation systems in the partners' countries; production of sharing agreements for pooling data; proposition of file formats for exchanging phenotypes, genotypes, pedigree; research on allele frequency across country x breeds; listing of novel traits and their definition; codification of breeds.

Description of breeding programs and evaluation systems in the partners' countries

Genetic evaluation systems and breeding programs have been described and data collated from 45 sheep and goat populations across 12 countries. This was done through surveys distributed to all partners. This work was presented at the EAAP 2020 (Brito *et al.*, 2020). The main conclusions was that there are numerous challenges to be addressed for pooling data from different countries (for example: high heterogeneity of trait recording, SNP panels and statistical models used, average of ~30% of animals with unknown sires). However, there are also many opportunities to use the current resources to optimize selection for resilience and efficiency in small ruminants across countries.

Proposition of file formats for exchanging phenotypes, genotypes, pedigree

A coherent set of formats have been proposed to pool data from different countries, built on principles close to those used in Interbull and Interbeef. An appraisal will be undertaken at the end of the project to possibly update these formats, based on the issues encountered while using them.

Production of sharing agreements for pooling data

A template of a sharing agreement was produced for pooling data. Ten bilateral agreements were signed (between the organisation producing the data and the organisation in charge of the evaluation) covering 3 case-studies (meat and dairy sheep, dairy goats). The agreements detail the data and the purpose of aggregating the data and performing statistical analysis, and give the rights and duty of the providers and the researcher on the use of the data. This template, conceived for the needs of the projects, could serve beyond the project for the routine exchange of data.

Research on allele frequency across country x breeds

The conception of a genomic tool was carried out by pooling allele frequency information acquired from 18 sheep breeds (including 10 meat sheep and 8 dairy sheep populations from 5 countries – the UK, Ireland, Uruguay, Spain and France; some populations were represented in several countries, such as Charollais, Texel and Vendéens). Comparison of genotype metrics across populations enables the detection of the most informative SNPs across populations and breeds. This work could be deepened by adding other breeds and will result in an optimal panel of SNP for the breeds involved that could be exploited in a tool widely used and therefore more affordable.

The main results were presented at the EAAP in 2020 (O'Brien *et al*, 2020). Several metrics were produced from informative SNP, identified where the frequency of the "A" allele was between 0.2 and $\leq$ 0.8 in each of two pair-wise populations compared. In particular, the correlation between minor allele frequencies of the SNPs presents in two populations allows to assess the distance between them.

Listing of novel traits and their definition

An important practical result of this action is to produce ICAR guidelines on phenotyping resilience and efficiency traits. This work is based on the results that will be produced by the work packages dedicated to the novel traits, with a two-step approach: determination and genetic analysis of the more relevant traits in experimental farms, and the proposition of proxies usable on private farms for larger scale for selection. These tasks are on-going. However, a first milestone was produced that described the traits for assessing resilience and efficiency in sheep and goats. The guidelines will be due by the end of the project when all the results are available.

Coding breeds

A first coding of the breeds involved in the work package was proposed. This code is based on 3 letters and could be extended beyond SMARTER. The interest of such a code relies on the existence of data exchanges across country. The extension of the code will be proposed to ICAR. Crossbreed livestock should also be addressed.

Across country evaluation

Once the prerequisites are set, the second general objective of the work package is to assess the interest of an across-country evaluation. This is done through 3 tasks: the implementation of 3 pilot studies on multi country evaluation in meat sheep, dairy sheep and dairy goats; the writing of the organisational and business model of a routine international evaluation; the assessment of the cost-benefit of international sharing of germplasm.

Implementation of across country evaluation in three case-studies

The case-studies concern:

- Ireland, the UK and France in meat sheep (Fitzmaurice *et al*., 2021);
- France and Spain in dairy sheep;
- France, Canada, Switzerland and Italy in goats

The common purpose is to document the practical issues (ID, phenotypes), to assess the connectedness across country, to estimate the genetic correlations across country, and to run a BLUP / SSGBLUP animal model based on raw phenotypes through multi-trait analysis

The work is underway and practical results will be available in late 2021.

Creating an international initiative for across country evaluation in small ruminants

The operation and business model for a possible initiative on across-country evaluation in small ruminants is based on 3 axes:

- The willingness of countries to participate;
- The technical lessons derived from the case-studies, basically the strategy for pooling data (typically, raw phenotypes versus EBVs, with or without genotypes), the way to address the technical issues on the data, the connectedness between population;
- The opportunities and risks of international evaluation. This point was addressed through a survey within SMARTER that will be extended outside SMARTER. An interesting range of opinions and comments were already gathered, that can be summarised as follows.

The main opportunities mentioned in the survey are:

- Faster genetic gain obtainable from a larger reference population, a higher selection intensity and a higher accuracy.
- A way to select for difficult-to-measure traits (i.e. health, welfare, environmental challenges).
- The interest of having EBVs from abroad on the domestic scale.
- The insurance to have fair exchanges across countries.
- As an externality, the benefit from international collaboration and sharing of knowledge, especially through networking amongst stakeholders.
- An incentive to deliver on the harmonisation of phenotype recording.

The main risks mentioned in the survey are:

- The lack of genetic links between countries and the risk of having genotype by environment interactions.
- The promotion a few breeds of wide commercial applicability that might endanger further, some local breeds. This is because it is likely that very few breeds will benefit from the exploitation of genetic improvement to accelerate genetic gain, thereby diverging further away from the performance levels of the marginal breeds.
- The unbalanced benefits among countries, with the risk that the sale of genetics would only be one way.
- The risk that the initiative should be too expensive and too time consuming for the small ruminant sector. The question of cost-benefit balance is clearly raised.
- The loss of independence of genetic evaluations. This risk was highlighted by many respondents, suggesting a preference / need to maintain national research groups and genetics capability in this area.

The survey will be extended to different stakeholders in order to get a greater variety of respondents, and to propose a business plan taking into account the opportunities and risks, and how manage and mitigate them.

Estimating the cost-benefit of international genetic/ genomic evaluations and cooperation

Gene flow models were developed to quantify the impact a specific subpopulation can have over time on the genetic gain or economic benefit of an industry (Fetherstone *et al*, 2020). Importation of foreign germplasm is a widely adopted strategy in some populations. An Ireland-New Zealand case study was simulated in order to quantify the potential gain that could be achieved through the importation of foreign sire contributions (New Zealand) into a domestic sheep industry (Ireland). Within the study, multiple market scenarios were assessed. Results reveal that the maximum genetic (measured as average genetic gain) and economic (measured as an annualised cumulative value) benefit could be achieved by implementing a market scenario which involved shifting market share away from conservative domestic breeders and increasing the selection intensity of rams retained for breeding without the use of foreign genetics.

This developed framework could be used in other case-studies within the project.

Conclusion: practical outputs for ICAR community

Harmonisation and international cooperation are the major objectives of ICAR. The SMARTER project, by balancing academic and non-academic partners, as well as including various stakeholders in its network and by achieving both scientific and practical results, will be useful for the ICAR community involved in sheep and goat production. The findings and achievements of the work package described in this paper should contribute to a framework of future services towards small ruminants breeding organisations.

The guidelines for efficiency and resilience traits in small ruminants will complete the set of recommendations already existing for dairy traits recording in sheep and goats (ICAR, 2020) or on the verge of being published on meat and reproduction traits on small ruminants.

The creation of an international initiative and the preparation of necessary procedures to facilitate, encourage and motivate cooperation in international evaluations in small ruminants, might result in a routine international evaluation.

Finally, in order to coordinate the previous points and provide a support to the breeding organisations on performance recording and genetic evaluation in sheep and goats, the definition of a zootechnical Reference Centre for Small Ruminants will be proposed within SMARTER.

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List of references

Brito L.F., Berry D., Larroque H., Schenkel F., Ciappesoni G., O’Brien A., Tortereau F., Ugarte E., Palhière I., Bapst B., Jakobsen J., Antonakos G., Kominakis A., Clement V., Bruni G., Loywyck V., Massender E., Oliveira H.R., Posta J., and Astruc J.M. Genetic evaluation systems and breeding programs in sheep and goats: an international perspective. In EAAP, Virtual Meeting, Portugal, Book of Abstracts, December 2020.

Fetherstone, N., Hely, F.S., McHugh, N., McGovern F.M., Amer P.R. Genetic and economic benefits of foreign sire contributions to a domestic sheep industry; including an Ireland-New Zealand case study. Genet Sel Evol 53, 5 (2021). *https://doi.org/10.1186/s12711-020-00594-y*

Fitzmaurice, S, Conington, J, McHugh, N, Banos G. Towards future genetic evaluations for live weight and carcass composition traits in UK sheep. Small Ruminant Research 196: 106327 March 2021. *https://doi.org/10.1016/j.smallrumres. 2021.106327*

ICAR, 2020. ICAR guidelines. Section 16 on Dairy Sheep and Goats. https://www.icar.org/index.php/icar-recording-guidelines/

O’Brien A.C., Astruc J.M., Tolkamp A., Berry D.P. Comparison of sheep genotypes metrics across breeds and countries. In EAAP, Virtual Meeting, Portugal, Book of Abstracts, December 2020

SMARTER Project, 2019. Website *www.smarterproject.eu.* Selection tools to benefit from international cooperation in small ruminants: a comprehensive work package of the SMARTER project

Reducing environmental impact in the Dutch dairy sector with ANCA-tool

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Abstract

ANCA or ‘KringloopWijzer’ (in Dutch) is a software instrument that offers Dutch dairy farmers insight in the environmental performances of their farm. The ANCA instrument measures the cycles of nitrogen, phosphorus, greenhouse gases and carbon. Using this instrument will help to reduce mineral losses and to reduce costs on a dairy farm.

The dairy sector, the feed industry, the milk processors and the accountancy organisations made an agreement to reduce the environmental impact in the Netherlands by using ANCA. They agreed that from 2016 onwards using ANCA would be mandatory for all Dutch dairy farmers. ZuivelNL organised the data collection and storage of the data in a central database. This database with information of all Dutch dairy farms enables to establish a benchmark for relevant indicators. In 2020 data analyses about greenhouse gases were performed with more than 34.000 farm records. The average green house gas emission allocated to milk yield in the Netherland from 2016 – 2018 was 1226 g CO_2-equivalents per kg FPCM. The results can be used for improving the mineral management on a dairy farm, to save costs. On the other hand, the results can be used as indicators in sustainability programs. Depending on the scores of the indicators, farmers can be rewarded with financial bonusses.

Introduction

In the main dairy exporters, Oceania, EU-28 and North America, dairy farming is under pressure to reduce greenhouse gas emissions, ammonia emissions, and nitrogen en phosphate losses. This is also the case for the Netherlands. Therefore WUR developed an instrument that calculates the greenhouse gas emission, ammonia emission, nitrogen and phosphate losses for each dairy farm individually (de Vries et al., 2020). Eventually to avoid pollution. This instrument (ANCA, Annual Nutrient Cycling Assessment or ‘KringloopWijzer’ in Dutch) uses detailed farm specific input that is measured on farm and collected from governmental and industrial data sources. Examples are animal numbers, feed stocks, manure exports and nitrogen and phosphorus contents of feed and manure. This paper shortly describes the greenhouse gas emissions in ANCA, the ANCA introduction in the Dutch dairy sector and its greenhouse gas emissions. Closing with the (potential) benefit for the individual dairy farmer.

ANCA or KringloopWijzer

The ANCA instrument measures the cycles of nitrogen (together with ammonia and nitrous oxide), phosphorus, greenhouse gases and carbon. It subsequently quantifies the excretion of nitrogen and phosphorus of the herd for individual farms, the uptake of nitrogen and phosphorus with crops and the total crop yield as well (Aarts et al., 2015). The greenhouse gas emissions are calculated according tot de PEFCR guidance (PEFCR, 2018a, b, c). This is chain approach where the following aspect are taken into account:

- The production of purchased feed.
- The production of all inputs to the farm, such as fuels, fertilizers and machines.
- Machine use by contract workers.
- The land use change associated with the cultivation of crops outside the farm

Methane is an important part of the greenhouse gas emissions. The ration fed to the herd and characteristics of the feed components are important for methane production from enteric fermentation and manure. ANCA is able to determine the rations and uses the mentioned characteristics for calculation the methane emission. The greenhouse gas nitrous oxide is calculated according to IPCC (2006) and depends on the total nitrogen application (de Vries et al., 2020). The other greenhouse gas that is calculated concerns carbon dioxide. This is not only about fuel and electricity but purchased feed and materials as well (de Vries et al., 2020). To be able to sum different gases, the greenhouse effect of methane and nitrous oxide is expressed in CO2 equivalents. 1 kg methane from biological processes corresponds to 34 kg CO2, 1 kg CH4 from fossil fuel corresponds to 36.75 kg CO2 and 1 kg N2O corresponds to 298 kg CO2 (PEFCR, 2018a).

Scaling up to national level

The ANCA-tool provides useful management information about feeding and crops. The farmer and his adviser get signals if feeding and crop yields can be improved. This helps to reduce mineral losses and to reduce costs for feed, fertilizer and manure. In 2013 the dairy sector recognized this potential positive effect of the ANCA tool. So in that year the dairy sector, the feed industry, the milk processors and the accountancy organisations made an agreement to reduce the environmental impact from Dutch dairy farms by using ANCA. They agreed that from 2016 onwards it is mandatory for Dutch dairy farmers to use the ANCA tool and to store the input and output data in the central database that is owned by ZuivelNL (DairyNL). ZuivelNL is an organisation of the Dutch dairy supply chain. ZuivelNL has developed a database system to connect available input data to the ANCA tool to determine the environmental performance for each farm individually. Using available data connections with dairy processors, feed companies, governmental institutes (animal numbers, manure and land) and analysing laboratories reduce the effort from farmers and advisors to fill out the ANCA tool. However, using these data sources will also help to ensure the correct data input values. This database with input and output results enables to establish a benchmark for relevant indicators and thus room for improvement. In the past the data are used for analysing trends in nitrogen use efficiency (Oenema and Oenema, 2021). Recently the performances for all individual dairy farms in the Netherlands were analysed (Mollenhorst en de Haan, 2021). The database not only gives insight in the performances about nitrogen surpluses, ammonia emissions but gives insight in the average greenhouse gas emissions as well, see table 1. However (almost) all dairy farms had to use the ANCA instrument, far less records were used for analysing (Table 1) than the number of dairy farms in the Netherlands. Because, when screening

Table 1 Greenhouse gas emission (g CO_2-equivaltents/kg FPCM[1]) from dairy farms recorded in the central database in the years 2016, 2017 and 2018 (calculated with ANCA version 2019). Subsequently are the average GHG-emissions for 2016, 2017, 2018 and the three year average shown, together with the division into 5 different sources, the average performance of the 25% farms with the lowest GHG (Q1) and 25% farms with the highest GHG (Q3).

	2016	2017	2018	Overall	%	Q1 overall	Q3 overall
# records	10802	10799	12560	34161			
Total GHG (average, allocated to FPCM)	1208	1213	1254	1226	100%	1105	1312
- Enteric Methane	495	484	500	493	40%	452	527
- Stored Manure	139	140	148	143	12%	130	157
- Feed Production	134	135	141	137	11%	79	151
- Imports	375	391	402	390	32%	340	432
- Energy sources					5%		

[1]fat and protein corrected milk yield

the data, about 12 – 33% of all the records appeared to be incorrect. Still the number of records used for analysing is considerably high. Table 1 shows that the average production of greenhouse gasses allocated to dairy of a Dutch dairy farm is 1226 g CO_2-equivalents per kg FPCM. 40% is enteric methane, 12% comes from the manure storage, 11% is nitrous oxide coming from the fields and 32% of the emissions is due to imports (eg concentrates, artificial fertilizer). Emission from energy sources are a rather small part and not analysed.

Taking advantage of using ANCA

The results can be used for improving the mineral management on a dairy farm. On the other hand, the results can be used as indicators in sustainability programs. Organized by dairy processors or other organisations that want to improve sustainability on farm level. Greenhouse gas emission is usually an indicator used in sustainability programs. Depending on the scores of the indicators farmers can be rewarded with financial bonusses. Several dairy processors already implemented indicators from the ANCA tool in their sustainability programs, where dairy farmers can earn extra income. Quite well known is the ‘on the way to planet proof’ certificate (managed by SMK[1]), where collaborating dairy farmers can earn up to 2 cents extra per kg milk.

List of references

Aarts, H.F.M., M.H.A. de Haan, J.J. Schröder, H.C. Holster, J.A. de Boer, J.W. Reijs, J. Oenema, G.J. Hilhorst, L.B. Sebek, F.P.M. Verhoeven and B. Meerkerk (2015). Quantifying the environmental performance of individual dairy farms - the Annual Nutrient Cycling Assessment (ANCA). In: Grassland Science in Europe, Volume 20 pp 377 – 380.

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[1] Stichting milieukeur: https://www.smk.nl/761/about-smk/smk—partner-for-sustainability.html

IPCC, 2006. IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, Eggleston H.S., Buendia L., Miwa K., Ngara T. and Tanabe K. (eds). Published: IGES, Japan. *http://www.ipcc-nggip.iges.or.jp/public/2006gl/vol4.html*.

Mollenhorst, H. & M.H.A. de Haan, 2021. Analyse Kringloopwijzer data 2016-2018. Report 1305, Wageningen Livestock Research, Wageningen, The Netherlands, 51pp.Oenema, J. & O. Oenema, 2021. Intensification of grassland-based dairy production and its impact on land, nitrogen and phosphorus use efficiencies. Front. Agr. Sci. Eng. 2021, 8(1): 130–147

PEFCR, 2018a. Product Environmental Footprint Category Rules Guidance Version 6.3 – May 2018. *http://ec.europa.eu/environment/eussd/smgp/pdf/PEFCR_guidance_v6.3.pdf*

PEFCR, 2018b. Product Environmental Footprint Category Rule Feed for food-producing animals. *http://ec.europa.eu/environment/eussd/smgp/pdf/PEFCR_feed.pdf*

PEFCR, 2018c. Product Environmental Footprint Category Rule for Dairy Products. *http://ec.europa.eu/environment/eussd/smgp/pdf/PEFCR-DairyProducts_2018-04-25_V1.pdf*

Vries, de, M, W. van Dijk, J.A. de Boer, M.H.A. de Haan, J. Oenema, J. Verloop & L.A. Lagerwerf, 2020. Calculation rules of the Annual Nutrient Cycling Assessment (ANCA) 2019; Background information about farm-specific environmental performance parameters. Report 1279, Wageningen Livestock Research, Wageningen, The Netherlands, 112pp.

Practical tools for assessing and improving a farm's enviornmental footprint: an example from the United States

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Abstract

In 2017, the National Milk Producers Federation (NMPF) initiated a new component of the Farmers Assuring Responsible Management (FARM) Program: FARM Environmental Stewardship (ES). The FARM ES program tracks and communicates a farm's environmental achievements. The tool uses a model that is scientifically robust – it explains 98% of the variability in total GHG footprint across farms – while only requiring a limited amount of farm data. Over 1,800 dairy farms have completed a FARM ES assessment through the end of 2020. FARM ES supports farms in identifying opportunities to improve efficiency and productivity; the farm's environmental footprint; and internal management systems. Cooperatives and dairy processors can use FARM ES to collect on-farm GHG emissions data in a consistent and streamlined way; helping dairy farmers and the entire U.S. dairy value chain demonstrate its commitment to environmentally responsible production.

Introduction

Translating the concept of circular economy into the practical and actionable requires farmer-friendly programs and tools. In 2017, the National Milk Producers Federation (NMPF) initiated a new component of the Farmers Assuring Responsible Management (FARM) Program: FARM Environmental Stewardship (ES). The FARM ES program tracks and communicates a farm's environmental achievements. The online tool combined with the program's resources assist farms with pursuing continuous improvement in ways that align with business goals. Some of the program's key features include:

- Strong scientific basis with periodic updates.
- Implemented by a network of trained, 2nd party evaluators.
- Offers farms resources for continuous improvement.
- Enables supply chain reporting and collaboration.

Methods

Enrollment and training

Dairy cooperatives and proprietary processors in the United States are eligible to enroll in FARM Environmental Stewardship (ES). Their staff complete a training program to become certified, second-party evaluators. The training program is self-paced and online. It is composed of 7 discrete modules. The purpose of the training is to equip evaluators with knowledge on how to collect the data inputs and interpret results. The training encourages consistent program implementation across organizations involved in FARM ES.

Selecting farms

Dairy cooperatives and processors can use the FARM ES Random Sampling Protocol to enroll a representative sample of their farms in the program. The protocol is based on a stratified random sampling method. The stratifying factors used in this protocol are: 1) quartile of Fat and Protein Corrected Milk (FPCM) produced on a dairy in a day, and 2) geographic region defined as the first 3 digits of the dairy zip code. In order to streamline the selection process for dairy cooperatives and milk marketing organizations, a Microsoft Excel spreadsheet has been created that performs the necessary calculations to select farms according to the protocol

Evaluation process

FARM is implemented through a network of trained, second-party evaluators. Evaluations can be conducted on-site, remotely, or a combination of both approaches. The steps of an evaluation are generally:

1. Pre-Visit: The evaluator schedules a visit or phone call with the farm to conduct the evaluation. The evaluator provides the farm with resources and information to prepare, such as the FARM ES Evaluation Prep Guide.
2. Evaluation: Data is collected from the farm. The data is entered into the FARM ES platform through either the web portal or the app.
3. Results: The FARM ES platform provides the farm's carbon and energy use footprints. The evaluator reviews the results with the farm and discusses considerations for improvement.

Resources

Several resources have been developed and released as part of the FARM ES program to support evaluators and farmers during evaluations:

- The FARM ES User Guide helps evaluators and individual farmers input their data into the FARM ES platform. It explains which resources may contain the data on their farm, as well as answers common questions about how to interpret each measure.
- The FARM ES Evaluation Prep Guide is a resource for farmers to learn about the program and prepare for an evaluation.
- The FARM ES Data Gathering Sheet facilitates process of collecting information by capturing all of the required data input into an Excel sheet or fillable PDF.

Model

FARM ES estimates farm-level GHG emissions and energy intensity using a scientific, peer-reviewed model. The FARM ES model was generated out of a life cycle assessment (LCA) published in April 2013 (Thoma *et al.*, 2013). The study collected data from 536 farms covering a wide range of management practices. Authors of the study published another paper that proposed using the lifecycle assessment results and narrowing down the inputs into a simplified model for on-farm use (Asselin-Balençon *et al.*, 2013). The model is intended to create an accurate and representative tool for farmers to determine and potentially reduce their cradle to farm gate carbon footprint. The simplified model strongly reduces the farm specific data requirement from 162 animal-rations in the detailed LCA survey to 12 feed rations for lactating cows, while explaining 91% of the variability in feed print and 98% of the variability in total footprint.

Inputs

The FARM ES evaluation includes key data inputs that highlight circular economy concepts – including ration composition and manure management strategies. The full list of data inputs is located at nationaldairyfarm.com. The following summarizes model inputs:

- Milk Production: total annual milk production, annual average protein and fat content.
- Herd Profile: average herd size, average % dry, calves / heifers raised on and off farm, cows.
- Energy Use: annual total energy used by category (electricity, diesel, gasoline, etc.), solar / wind generated.
- Feed: time spent on pasture, average daily DMI, ration breakdown on dry matter basis.
- Manure and Nutrient Management: types of manure management systems, use of digester or solid-liquid separation.

Outputs

The evaluation results are life cycle based, representing all the GHG emissions and energy use associated with the farm's milk production from cradle to farmgate. The emissions footprint is broken down by source: feed production, on-site manure management, on-site enteric fermentation, and on-site energy use. The energy results are divided into feed production and on-site energy.

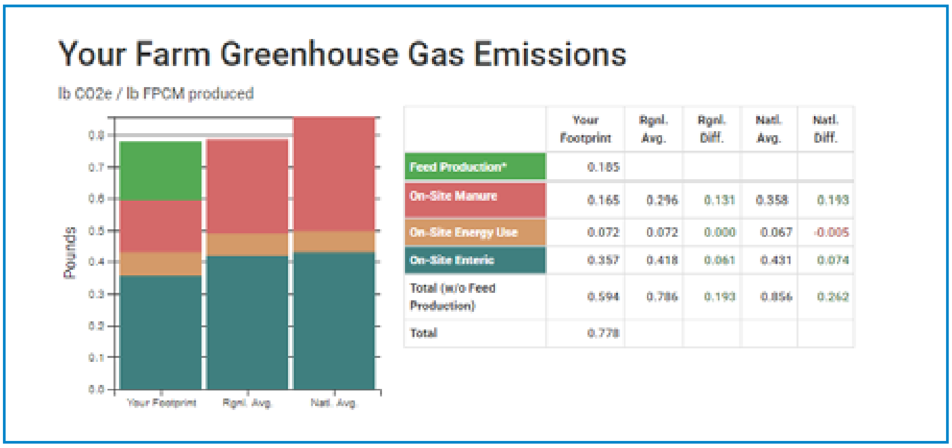
"On-site" refers to dairy activities on the farm. If the operation purchases feed and does not engage in feed production activities, the output will still generate an estimate for the environmental impacts of the purchased feed.

Results are compared to regional and national averages. These averages come from the industry's LCA research. Benchmarks for feed production emissions are not available in the current version (Version 2.0) of the tool.

Results

Farm benefits

FARM ES supports farms in improving their environmental footprint in ways that align with business goals. The primary resource to aid in this effort is the FARM ES Reference Manual. The FARM ES Reference Manual offers science-based considerations on ration formulation, forage quality and concentrate management, manure management, energy efficiency as well as animal health, nutrition and cow comfort to achieve gains



Your Farm Greenhouse Gas Emissions

lb CO2e / lb FPCM produced

	Your Footprint	Rgnl. Avg.	Rgnl. Diff.	Natl. Avg.	Natl. Diff.
Feed Production*	0.185				
On-Site Manure	0.165	0.296	0.131	0.358	0.193
On-Site Energy Use	0.072	0.072	0.000	0.067	-0.005
On-Site Enteric	0.357	0.418	0.061	0.431	0.074
Total (w/o Feed Production)	0.594	0.786	0.193	0.856	0.262
Total	0.778				

Figure 1. Sample FARM ES output.

in productivity, feed efficiency, and GHG emissions intensity. In other words, the FARM ES Reference Manual offers ideas for management practices, technologies and other considerations that can help reduce on-farm GHG emissions and energy use *in ways that make business sense*. For example, improving herd health and optimizing ration formulations are key opportunities.

The FARM ES Reference Manual benefited from independent review and input by a panel of subject matter experts – the Technical Review Panel – led by the World Wildlife Fund (WWF). The panel's goal was to ensure FARM Environmental Stewardship provides a best in class guide to support farmers in understanding the FARM ES results and to identify opportunities for improvement. The panel was comprised of farmers, academics, processors, and conservationist.

Additionally, use of FARM ES can lead to improved internal management systems**.** Farms must gather documents and records to complete the FARM ES evaluation: nutrient management plans, milk production records, crop production data, and more. Collecting and reviewing these records in one place is a chance to think about on-farm management in a new way. Farms report creating or improving systems to track data as a result of the FARM ES evaluation, which can enable better management over time.

Finally, FARM ES is an opportunity for a farm to demonstrate its commitment to natural resource stewardship. U.S. dairy farms improved their carbon footprint by 19% between 2007 and 2017 (Capper and Cady, 2020). And they continue to innovate by adopting new technologies and management practices. FARM ES captures these efficiency gains and helps identify opportunities to continue the path toward continuous improvement. By addressing a topic that society views as an urgent natural resource risk – GHG emissions – the program helps strengthen consumer confidence and maintain U.S. dairy's leadership position in the global marketplace.

Participation and sharing our progress

Since program inception, the FARM ES evaluation has been implemented on more than 1,800 farms by 38 different cooperatives and processors (Figure 2). With each FARM ES evaluation, farmers, cooperatives and processors can assess change over time, identify areas of operational improvement, and report progress to their customers. Indeed, dairy processors, retailers, and food service customers are making public commitments to

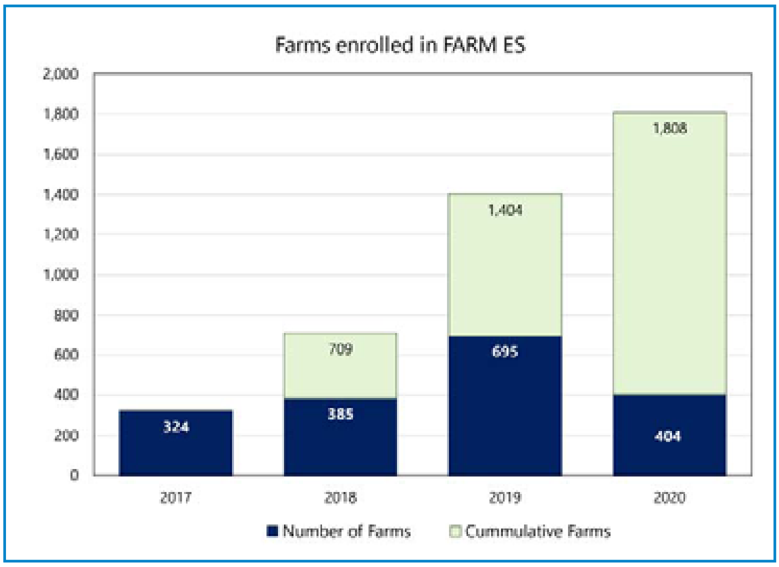


Figure 2. Farm enrollment in FARM ES by year of first evaluation.

reduce the environmental footprint of their products. These commitments include targets to reduce the GHG emissions of their entire supply chain beginning at the farm level. To meet those targets, dairy buyers are asking cooperatives and dairy processors to provide aggregated farm-level data on GHG emissions.

Cooperatives and dairy processors can use FARM ES to collect on-farm GHG emissions data in a consistent and streamlined way; helping dairy farmers and the entire U.S. dairy value chain demonstrate its commitment to environmentally responsible production.

List of references

Asselin-Balençon, A.C., Popp, J., Henderson, A., Heller, M., Thoma, G. and Jolliet, O., 2013. Dairy farm greenhouse gas impacts: A parsimonious model for a farmer's decision support tool. International Dairy Journal, *31*, pp. S65-S77.

Capper, J.L. and Cady, R.A., 2020. The effects of improved performance in the US dairy cattle industry on environmental impacts between 2007 and 2017. Journal of animal science, *98*(1), p.skz291.

Thoma, G., Popp, J., Nutter, D., Shonnard, D., Ulrich, R., Matlock, M., Kim, D.S., Neiderman, Z., Kemper, N., East, C. and Adom, F. 2013. Greenhouse gas emissions from milk production and consumption in the United States: A cradle-to-grave life cycle assessment circa 2008. International Dairy Journal, 31, pp. S3-S14.

A new approach to predicting the fat content in 2x milking

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Abstract

Traditionally, the estimation of the 24-hour fat content from a one-milking sample has required conducting a large study with multiple samples during the recording day from thousands of cows from different herds and milking intervals. In this study, we show how a large amount of practical data can be used for creating a local calculation method without additional samples. In the Finnish case, some 7.5 million samples with data on preceding milking intervals were used, combined with what we know about the analysed milk composition and about the individual cows. The results are also compared from the point of view of how well they alleviate the differences between milking times and intervals.

Summary

This paper describes the current status with 24-hour yield calculations in the Finnish milk recording system, as well as the historical reasons behind the choices made. The results of each method are also shortly analysed. The methods are found to be working reasonably well, but not perfectly. New correction factors were calculated out of the local data set.

Keywords: Milk recording, conventional milking, 24-hour yield calculation.

Introduction

In the Finnish milk recording, owner sampling has been common practice since the 1980's, and appr 95% of all herds now record by that method, with their own private recording equipment. At the same time, 90% of all samples are reportedly taken from one milking only. Farmers are also responsible for some 90% of milk recording data capture. In September 2019, 40.0% of all recordings during the previous rolling 12-month period came from automatic milking. This article presents the 24-hour yield calculation methods currently in use.

Historical overview

Up until 2003, the only available sampling method in Finland was proportional sampling. 24-hour yields were simply calculated by adding up milk weights and using the analysed values as they come.

This approach, however, started to be problematic due to three main reasons. Firstly, in a farmer-recording system it became evident that many samples were in fact not exactly proportional between the evening and morning milkings. Secondly, the results showed that a number of farmers were taking samples from one milking only. And the third reason was the advent of automatic milking which made it impossible to continue the old way.

For these reasons, it was decided to allow one-milking sampling starting April 2003.

Currently used methods

In traditional milking systems, milk weights are measured at two consecutive milkings (or three, if the cows are milked thrice per day). Some 10% of all herds claim to take proportional samples, and no correction is applied to their laboratory analyses. For fat content in one-milking samples from traditionally milked herds, the Delorenzo and Wiggans (1986) correction is applied with the received factors.

In automatic milking systems, the milk weights are collected during a 96-hour period and these results are used for calculating a 24-hour yield for each cow (Lazenby *et al*., 2002). In this calculation, the preceding intervals are taken into account to adjust to uneven individual cow measurement periods.

The fat and protein yields, however, are estimated on the basis of a one-milking sample, using data of only two preceding milkings (Peeters and Galesloot,2002). This method was also tried for milk weights but some herds with irregular cow traffic had a lot of problems with that, so the approach was changed in 2016. Also, the original Peeters and Galesloot method was found to produce slightly underestimated fat contents when compared to dairy deliveries, so in 2017, the method was updated by the corrections suggested by Roelofs *et al*. (2006), adding factors like stage of lactation, parity, and hour of the day to the equation.

Evaluation of current methods

To evaluate how the methods are working, a very simple comparison was made with average

24-hour yields produced by each method. The results for the whole Finnish dairy cow population are shown in table 1. In general, the differences between the methods are small.

However, we notice here that the calculation does not entirely cover the difference between fat contents in morning and evening milk. The correction factors used are already 33 years old and are based on data from a significantly lower yield level. Typical Finnish feeding also produces high milk fat contents which is maybe not entirely in keeping with the data used for making these formulae.

Automatically milked cows also tend to obtain a lower 24-hour fat content than cows from conventional milking systems. This was presumed to be due to higher milk yield, but due to the fact that the automatic milking herds have a significantly higher proportion of Holsteins, the results were recalculated for Holstein breed only (Table 2). Here the difference between morning samples and samples from automatic milking was slightly smaller.

Table 1. Corrected and recorded 24-hour averages by method, all cows.

		Average result			
Sampling scheme	**Nr of samples**	**Milk, kg**	**Butterfat, %**	**Protein, %**	**Cell count**
One-milking sample (Z), milking time 4-10 AM	255,461	29.8	4.30	3.58	157
Z sample, milking time 2-8 PM	309,974	30.2	4.51	3.61	187
Proportional (P) sample	112,620	29.6	4.41	3.61	167
Z sample, automatic milking	370,908	33.4	4.23	3.56	214

Table 2. Corrected and recorded 24-hour averages by method, Holstein cows only.

		Average result			
Sampling scheme	**Nr of samples**	**Milk, kg**	**Butterfat, %**	**Protein, %**	**Cell count**
One-milking sample (Z), milking time 4-10 AM	116,009	31.3	4.17	3.52	161
Z sample, milking time 2-8 PM	142,204	31.9	4.36	3.56	184
Proportional (P) sample	45,191	31.4	4.25	3.55	167
Z sample, automatic milking	231,346	34.8	4.14	3.52	216

The accumulated data set

Since 2003, a data set of 7.5 million recordings has accumulated with data on the time of the sampled and preceding milking as reported by the farmer, the lab analysis results, and the 24-hour milk yield. Grouped according to the preceding interval, the analysed fat content gives a nice sigmoid curve with the highest fat content found after a 540 to 630 minutes' interval (9 to 10.5 hours) and the lowest at 810 to 930 minutes (13.5 to 15.5 hours).

The results were also divided into subgroups according to lactation number, phase of lactation, and breed. The effect of the preceding milk interval on milk fat seems to be bigger with older cows and in the beginning of lactation. It was also bigger with Ayrshire cows as compared with Holsteins. At this point, however, the decision was made not to take these factors into account when calculating new correction factors.

Table 3. Average analysed milk fat by preceding interval class, 2003 - 2020

Interval before sampling, minutes	Number of samples	Average interval in the class	Average fat content analysed, %
<510	93,577	495	4.20
510-540	19,523	525	4.70
540-570	111,268	555	4.79
570-600	253,807	585	4.83
600-630	1,461,587	615	4.75
630-660	919,968	645	4.66
660-690	1,168,683	675	4.56
690-720	223,877	705	4.42
720-750	517,447	735	4.28
750-780	212,428	765	4.16
780-810	924,014	795	4.12
810-840	698,463	825	4.09
840-870	1,104,778	855	4.07
870-900	154,561	885	4.05
900-930	77,024	915	4.07
>930	26,977	945	4.13

Calculation of new factors

The results above were turned into a simple set of correction factors, dependent solely on the preceding interval. In order to do this, two assumptions were made:

1. A 24-hour recording day was assumed. This way, we can deduce the second milking interval from the one we know and mirror the fat percent for that milking.
2. Milk secretion rate was assumed to be constant around the 24-hour period. This allows us to deduce the share of the 24-hour yield produced at each milking.

These assumptions allow us to create the new correction factors by mirroring the milk yield and milk fat content in the milking whose actual data we have not got. This way, we get the following formula:

Table 4. Calculation of the mirrored milking and the correction factors.

Interval before sampling, minutes	Average fat in the sampled milking, %	Share of 24-hour milk yield in the sampled milking	Mirrored interval, minutes	Average fat in the mirrored milking, %	Calculated 24-hour average fat, %	Correction factor
<510	4.21	0.34	>930	4.14	4.16	0.989
510-540	4.77	0.36	900-930	4.08	4.33	0.907
540-570	4.82	0.39	870-900	4.05	4.35	0.903
570-600	4.84	0.41	840-870	4.07	4.38	0.906
600-630	4.76	0.43	810-840	4.09	4.37	0.919
630-660	4.66	0.45	780-810	4.12	4.36	0.936
660-690	4.56	0.47	750-780	4.16	4.35	0.953
690-720	4.43	0.49	720-750	4.29	4.36	0.984
720-750	4.29	0.51	690-720	4.43	4.36	1.016
750-780	4.16	0.53	660-690	4.56	4.35	1.046
780-810	4.12	0.55	630-660	4.66	4.36	1.059
810-840	4.09	0.57	600-630	4.76	4.37	1.070
840-870	4.07	0.59	570-600	4.84	4.38	1.076
870-900	4.05	0.61	540-570	4.82	4.35	1.073
900-930	4.08	0.64	510-540	4.77	4.33	1.062
>930	4.14	0.66	<510	4.21	4.16	1.006

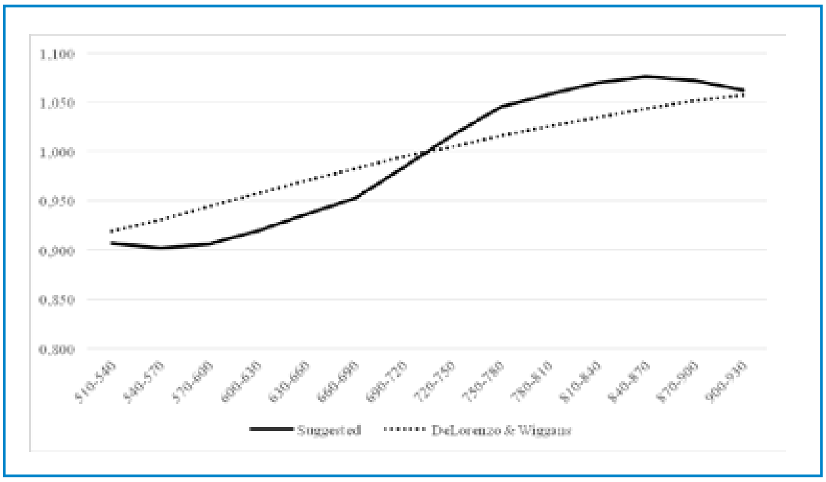

Figure 4. The obtained correction factors compared to the DeLorenzo and Wiggans (1986) factors

Table 5. Analysed and corrected fat in milk samples between September 2020 and March 2021.

Sample type	Number of samples	Analysed protein, %	Analysed fat, % (direct average)	24-h fat, % (weighted avg)
Morning samples	54,486	3.66	4.25	4.52
Evening samples	66,684	3.70	4.73	4.48
Proportional samples	22,528	3.68	4.44	4.45

*Correction factor = [(Sampled milk * sampled fat) + (Mirrored milk * mirrored fat)]/(sampled milk + mirrored milk)*

Comparing the obtained correction factors with those used in the original DeLorenzo and Wiggans method, we can see that the new factors form more of a sigmoid curve than a straight line. They will thus correct the analysed results with greater effect than the old method, especially when the preceding interval is around 10 or 14 hours.

Application of the new factors

These new factors were taken into use in the Finnish milk recording system in July 2020. Looking at the results now, we have managed to bridge the gap in 24-hour fat content between evening and morning samples. It is also easy to do a recalculation of the factors on a regular basis, and make changes if the situation has changed.

Conclusions

The earlier 24-hour calculation methods are performing on a satisfactory level. However, it seems that the historical fat correction factors needed recalculation to make the obtained estimates more accurate. The new factors were obtained from a large data set by mirroring the milking whose data did not exist. The factors have been taken into use, and are performing better than the old ones. They can easily be recalculated in the future.

List of references

Delorenzo, M.A., and G.R. Wiggans. 1986. Factors for estimating daily yield of milk, fat, and protein from a single milking for herds milked twice a day. J Dairy Sci 69; 2386.

Lazenby, D., E. Bohlsen, K. J. Hand, D. F. Kelton, F. Miglior and K. D. Lissemore. 2002. Methods to estimate 24-hour yields for milk, fat and protein in robotic milking herds. Presented at the 33rd Biennial Session of ICAR, Interlaken, Switzerland, May 26-31, 2002.

Peeters, R. and P.J.B Galesloot, 2002. Estimating Daily Fat Yield from a Single. Milking on Test Day for Herds with a Robotic Milking System. J Dairy Sci. 2002, Mar; 85(3):682-8.

Roelofs, R.M.G, G. de Jong and A.P.W. de Roos, A.P.W. 2006. Renewed estimation method for 24-hour fat percentage in AM/PM milk recording scheme. Proceedings of the 35th ICAR Session, 2006, Kuopio, Finland

Analysis of the accuracy of lactation qualification methods and use of weighting factors for genetic evaluation

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Summary

In France, the Dairy Cattle Milk Recording Guidelines allows 8 protocols, each with 6 levels of recording intervals (between 4 to 9 weeks), approved by ICAR. The percentage of cows recorded with A protocol has decreased by 42 points during the last twenty years (from 92% in 2000 to 48% in 2020). Milk Recording Organizations have indeed wished to adapt and simplify protocols in keeping with the changes in measuring equipment, new services, etc.

The aim of this study was to determine the effect of all qualification methods on prediction accuracy of 305-day production traits milk yields, fat yields, protein yields, fat%, protein% (a lactation model is used for the French genetic evaluation) and to calculate and/or update the different weighting factors applied to each method in genetic evaluation.

Two datasets have been created: one with 19047 lactations reference-305 days A4 method, another with 8250 lactations reference-305 days R4 method.

The methodology used for this study has consisted in simulating T4, Z4, C*4, Z*4 methods from reference A4 method and R*4 method from reference R4 method.

A8, T8, Z8, C*8, Z*8, R8, R*8 methods have been obtained by taking into account only one out of two test-day per lactation.

The 305-day production traits estimated for each simulated method were compared to those of the reference method. The accuracies of the other methods (5, 6, 7 and 9) have been extrapolated by means of a linear regression model. The calculation of the weighting factors for all methods took into account the coefficient of determination (R^2) and the repetability level of each trait.

The results of accuracy (R^2, bias and standard deviation of bias) underlined the fact that R^2 is lower for all traits for 8 methods compared to 4 methods.

For example, R^2 is equal to 0.945 and 0.861 for fat yields and percent for T8 method compared to 0.989 and 0.962 for T4 method. Moreover the loss of accuracy is lower for R8 method compared to A8 method.

The comparison between the old and the new weighting factors for each lactation qualification method brought out an overall underestimation for milk yields, fat yields and protein yields and an overestimation for fat%, protein% with the old factors.

For example, the new milk yields weighting factor for A8 method is equal to 0.95 compared with 0.93 previously. The weighting factors obtained for new methods are

low, mainly for fat% for which it is equal to 0.44 for C*9 method. The weighting factors will have an impact on the accuracy of the cows' index, low weighting factors leading to reduced accuracy.

Keywords: Milk recording, protocols, accuracy, weighting factors.

Introduction

In France, the percentage of dairy farms which uses A protocol is for the first time below 50% in 2020 with exactly 48% of herds. This percentage decreases each year.

Alternated one-milking recording (T) and one-milking sampling with milk weights from more than one milking (Z) represent 27% of herds, AMS protocols 13% and B protocols by farmers represent 12% of herds.

In this context, French Milk Recording Organizations (MRO) wish to answer expectations of farmers, changes in measuring equipment, new services…, and to simplify, reduce the cost of milk recording by using ICAR standard and method, with a quality level for genetic evaluation and cow management.

Many protocols and methods are proposed in France and the aim of this study is to evaluate the accuracy of these protocols and methods:

- on lactation reference-305 days from relevant data sets in comparison with A4, AR4 methods (gold standard) by calculation R2, bias, standard deviation of bias on criteria milk yields, fat%, fat yields, protein%, protein yields for each method,
- and to calculate weighting factors for genetic evaluation.

A lactation model is used in France for genetic evaluation and it was necessary to update and to calculate weighting factors from new relevant data sets.

Material and methods

Description of the datasets

Data were collected by Milk Recording Organizations, from herds in A4 scheme with use of Electronic Milk Meter Lactocorder and recording of one milk weight, one sample at each milking (pm and am) and from herds in R4 scheme with use Automatic Milking System with recording of samples for at least two milkings per test day.

Several criteria have been used to select raw data from both datasets.

For the A4 scheme dataset, recordings with missing information, with too large a difference in milk weight between milkings, before 5 and after 399 days in milk (DIM), from lactation ranks greater than 10 and with daily recorded values out of permitted range (defined in ICAR Guidelines, 2017) were excluded. 208 204 test-day records from Holstein cows were thus retained.

For the R4 scheme dataset, recordings with missing information, with daily recorded values out of permitted range (defined in ICAR Guidelines, 2017), without 24-hour reference and during a sampling period of less than 12 hours were excluded, as well as protein and fat percent from sampled milkings carried out within 4 hours of a previous sampled milking. 380 170 test-day records were thus retained, from Holstein (74%), Montbeliarde (22%), Normande, Simmental and Brown cows breed.

Estimation of the lactation accuracy for each recording method

Firstly, the data sets were used to estimate 24-hour milk yields, fat percent and fat yields, protein percent and protein yields for each recording method.

The A4 prrotocol data set was used to simulate A4 (reference method), T4 and Z4 (adjustment of milk yield and/or fat and protein yields with the ICAR approved Liu's method) and C*4 (constant one-milking recordings with adjustment of milk, fat and protein yields with the Liu's method) method. It's a new protocol used in France since 2020 (Leclerc. *et al.*, 2019).

The R4 protocol dataset was used as a reference and to simulate a R*4 method by adjusting fat percent with the ICAR approved Peeters and Galesloot's method (Peeters, R., Galesloot, P.J.B., 2002) for recordings with only one single sample during the sampling period. It's a new protocol used in France since 2019 (Minery., *et al.*, 2018).

The R*4 dataset was obtained by selecting the first sampled milking as well as the two previous non-sampled milking per cow per test day, leaving 319 076 first sampled milkings.

The A4 and R*4 data sets were split into two independent data sets: a training data set (138 222 and 212 522 records for the A4 dataset and the R*4 dataset respectively), used to estimate the Liu's and Peeters and Galesloot's regression coefficients and a validation dataset (69 982 and 106 554 records records for the A4 data set and the R*4 data set respectively) on which the regression coefficients were applied to calculate 24-hour production traits for each method.

In order to estimate 305-day production traits for each method with a 4 week-interval between recordings, each lactation had a minimum of 7 test day records separated by less than 95 days, a first record before 60 DIM and a last record after 280 DIM (and before 399 DIM for the R4 and R*4 data sets).

19 047 lactations for the A4 data set, 8 250 lactations for the R4 data set (Table 1) and R*4 data set respectively fulfilled these conditions.

For each method with a 8 week-interval between recordings, only one out of two recordings were taken into account to estimate 305-day production traits, starting from the first or the second lactation record and the same conditions were required, except for the minimum of test day records which was lowered to 4.

The statistical analysis was carried out by comparing the 305-day lactation traits thus obtained for every method to the reference 305-days lactation traits (R4 for R8, R*4 and R*8 and A4 for every other methods). The results of the accuracy (R^2, mean bias, standard deviation of bias) published for each method correspond to the lowest R^2 value and the highest mean bias values (in absolute value) obtained for each method on the validation data sets.

Table 1. Description of the initial datasets for analysis on lactation

Criteria	A4 Data set	R4 Data set
# 305-day lactations	19 047	8 250
# Cows	14 396	7 889
Average milk yields (kg)	9 172	9 495
Average fat%	3.85	3.84
Average fat yields (kg)	351	361
Average protein%	3.12	3.21
Average protein yields (kg)	285	303

The R² of the other methods (5, 6, 7 and 9) have been extrapolated from the 4 and 8 methods results by means of a linear regression model.

Weighting factors for genetic evaluation

In the French genetic evaluation of production traits, each trait is weighted according to the lactation number (1 for a first lactation and lower for future ones) and according to its coefficient of determination (R²) and its repeatability level (Rep.). The aim of the study was to estimate the latter weighting factor for each method, according to the following formula:

$$\text{Weighting factor} = 1 - \text{Rep.} / [1 - \text{Rep} + (1 - R2 / R2)]$$

with Rep= 0.5 for all yields and 0.7 for fat and protein percent.

Example: A8 method for milk yields

R²= 0.972 and Rep.= 0.5

weighting factor= 1 - 0.5 / [1 - 0.5 + (1 - 0.972 / 0.972)] = 0.95 (applied for this lactation on genetic evaluation).

Results

Analysis of the lactation accuracy for each recording method

The analysis of the coefficients of determination (R²) on 305-day lactation traits for the different methods shows (Table 2):

- a lower R² for fat yields and percent compared to the other traits;
- a lower R² for all traits for 8 methods compared to 4 methods. For example, R² is equal to 0.945 and 0.861 for fat yields and percent for T8 method compared to 0.989 and 0.962 for T4 method;
- a higher loss of accuracy for adjusted constant one-milking recording (C) compared to alternated milking. For example, R² for C*4 method is obtained with adjusted constant one-milking recording. It is equal to 0.853 for fat% compared to 0.962 for fat% for T4 method;
- a lower loss of accuracy for R8 method compared to A8 method.

The analysis of the mean bias on lactation traits for the different methods shows (Table 3) that as for R², bias are higher for 8 methods compared to 4 methods.

The different R² and bias between R and R* methods for non-adjusted traits (such as milk and protein yields and percent) are due to the fact that the records taken into account per lactation could differ between R and R* data sets, leading to different 305-days lactation traits.

Moreover, the lowest R² value and the highest mean bias values obtained for 8 methods correspond mostly to 305-day lactation traits estimated from lactation starting from the second record.

Table 2. Coefficient of determination (R^2) for each method.

Recording method	Milk yields	Fat yields	Protein yields	Fat%	Protein%
A4, R4	1	1	1	1	1
Z4	1	0.968	0.987	0.976	0.989
T4	0.997	0.989	0.997	0.962	0.997
R*4	0.997	0.954	0.997	0.878	0.997
C*4	0.966	0.940	0.971	0.853	0.955
A8	0.972	0.955	0.966	0.902	0.946
R8	0.980	0.970	0.979	0.941	0.963
Z8	0.972	0.934	0.956	0.876	0.927
T8	0.967	0.945	0.963	0.861	0.940
R*8	0.979	0.930	0.978	0.845	0.961
C*8	0.925	0.876	0.928	0.749	0.894

Table 3. Maximum mean bias (standard deviation of bias) for each method.

Recording method	Milk yields (kg)	Fat yields (kg)	Protein yields (kg)	Fat% %	Protein% %
A4, R4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Z4	0 (0)	0.10 (13.3)	0.27 (6.23)	0.00 (0.14)	0.00 (0.06)
T4	11.1 (155)	0.85 (12.2)	0.39 (4.34)	0.01 (0.10)	0.00 (0.02)
R*4	30.6 (107)	0.06 (15.7)	0.88 (3.4)	-0.01 (0.15)	0.00 (0.01)
C*4	21.3 (263)	1.11 (18.4)	0.48 (7.5)	0.01 (0.18)	0.00 (0.03)
A8	-34.0 (238)	-2.32 (15.1)	-0.14 (10.1)	-0.02 (0.15)	0.01 (0.03)
R8	-149.4 (285)	-6.11 (12.5)	-3.13 (8.6)	0.01 (0.06)	0.01 (0.04)
Z8	-34.0 (238)	-2.22 (18.3)	0.30 (10.0)	-0.03 (0.18)	0.01 (0.07)
T8	28.6 (321)	1.74 (15.5)	0.44 (9.0)	0.03 (0.14)	0.01 (0.04)
R*8	-124.4 (291)	-5.75 (19.6)	-2.38 (8.9)	0.00 (0.16)	0.01 (0.04)
C*8	46.2 (407)	1.83 (22.3)	0.55 (12.7)	0.04 (0.21)	0.01 (0.06)

Table 4. New weighting factors for each method.

Recording method	Milk yields	Fat yields	Protein yields	Fat%	Protein%
A4, R4	1	1	1	1	1
A5, R5	0.99	0.98	0.98	0.92	0.96
A6, R6	0.97	0.96	0.97	0.85	0.92
A7, R7	0.96	0.93	0.95	0.79	0.88
A8, R8	0.95	0.91	0.93	0.73	0.84
A9, R9	0.93	0.89	0.92	0.68	0.81
Z4	1	0.94	0.97	0.92	0.96
Z5	0.99	0.92	0.96	0.85	0.92
Z6	0.97	0.91	0.95	0.79	0.87
Z7	0.96	0.89	0.93	0.73	0.83
Z8	0.95	0.88	0.92	0.68	0.79
Z9	0.93	0.86	0.90	0.63	0.76
T4	0.99	0.98	0.99	0.88	0.99
T5	0.98	0.96	0.98	0.82	0.95
T6	0.96	0.94	0.96	0.76	0.90
T7	0.95	0.92	0.95	0.70	0.86
T8	0.94	0.90	0.93	0.65	0.83
T9	0.92	0.88	0.91	0.6	0.79
R*4	1	0.91	0.99	0.68	0.99
R*5	0.99	0.90	0.98	0.67	0.96
R*6	0.97	0.89	0.98	0.65	0.93
R*7	0.96	0.88	0.97	0.64	0.91
R*8	0.95	0.87	0.96	0.62	0.88
R*9	0.93	0.86	0.95	0.61	0.86
C*4	0.93	0.89	0.94	0.64	0.86
C*5	0.92	0.86	0.92	0.59	0.82
C*6	0.90	0.83	0.90	0.55	0.79
C*7	0.88	0.81	0.89	0.51	0.75
C*8	0.86	0.78	0.87	0.47	0.72
C*9	0.84	0.75	0.85	0.44	0.69

Weighting factors for genetic evaluation

The comparison between the new weighting factors (Table 4) and the previous ones (Table 5) for each method brought out an overall underestimation for milk, fat and protein yields and an overestimation for fat and protein percent with the previous factors.

For example, the new milk yield weighting factor for A8 method is equal to 0.95 compared with 0.93 previously and the new fat% weighting factor for A8 method is equal to 0.73 compared with 0.78 previously.

The weighting factors obtained for new C* protocol are low mainly for fat percent for which it is equal to 0.44 for C*9 method.

Discussion and conclusions

Regarding the context in milk recording, the willingness of France Genetics Breeding (FGE) is to propose all the protocols and methods approved by ICAR to the farmers. The wish is to simplify and to reduce the cost of Milk Recording mainly in big herds and AMS Robots.

After analyzing the results of French studies (2018 and 2019) about respectively, a new AMS protocol with only one sample (R*), a new constant one-milking recording protocol (C*), the FGE board has proposed a program of implementation of these protocols in the dairy cattle milk recording Guidelines by the end of 2019 with conditions:

- to use the Liu's method for estimating 24-hour yields with C* protocol;
- to use the Peeters and Galesloot's method for estimating 24-hour fat percent and yields with R* protocol;
- to describe Standard Operating Procedure (FGE Guidelines, 2019);

Table 5. Previous weighting factors for each method.

Recording method	Milk yields	Fat yields	Protein yields	Fat%	Protein%
A4, R4	1	1	1	1	1
A5, R5	0.99	0.98	0.99	0.96	0.98
A6, R6	0.98	0.96	0.98	0.92	0.96
A7, R7	0.96	0.93	0.95	0.85	0.93
A8, R8	0.93	0.87	0.91	0.78	0.89
A9, R9	0.90	0.81	0.87	0.71	0.85
Z4	1	0.96	0.99	0.94	0.99
Z5	0.99	0.94	0.98	0.90	0.97
Z6	0.98	0.91	0.96	0.85	0.95
Z7	0.96	0.85	0.94	0.76	0.92
Z8	0.92	0.81	0.90	0.71	0.88
Z9					
T4	0.99	0.96	0.99	0.94	0.99
T5	0.98	0.94	0.98	0.90	0.97
T6	0.97	0.91	0.96	0.85	0.95
T7	0.96	0.85	0.94	0.76	0.92
T8	0.92	0.81	0.90	0.71	0.88
T9					

- to define weighting factors (for milk yields, fat percent, fat yields, protein percent, protein yields) among the level of individual lactation qualification model use by FGE and applied for genetic evaluation.

It was necessary to update the old weighting factors from new relevant datasets and to calculate factors for all protocols and methods.

The new weighting factors will have an impact on the accuracy of the cows' index, low weighting factors leading to reduced accuracy, the main goal is to improve the quality of genetic evaluation in accordance with ICAR Guidelines.

Regarding changes and evolutions in ICAR Guidelines, another FGE study project is scheduled in 2021 about the implementation of the new Liu's method (Kuwan and Bunger, 2019).

List of references

FGE Guidelines, 2019. Dairy Cattle Milk Recording, "Standard Operating Procedure 309" about estimating 24-hour yields on alternated protocols, "SOP 310" about the description of Peeters and Galesloot method with AMS

ICAR Guidelines, 2017. Overview Section 2 - Cattle Milk Recording

ICAR Guidelines., 2017. Procedure 1 of Section 2 - Cattle Milk Recording - Computing 24-hour yields - Estimating 24-hour milk, fat and protein yields (Method of Liu *et al.*, 2000)

Kuwan, K., Bunger, A, 2019, Prague, ICAR Conference. Alternated milk recording, recalculation, results and conclusion for future test planning of new Liu's method.

Minery,S., Bourrigan, X., Saunier, D., Augier, G. 2018, Auckland, ICAR Conference. Analysis of the accuracy of a method for estimating 24-hour fat (percentage and yields) with robots protocols and one single sample

Leclerc, H., Bourrigan, X., Augier, G. 2019, Prague, ICAR Conference. Analysis of the accuracy of C method for estimating 24-hour (yields) with alternated protocols.

Peeters, R., Galesloot, P.J.B. 2002. Estimating Daily Fat Yield from a Single Milking on Test Day for Herds with a Robotic Milking System. Journal of Dairy Science, 85:682-688.

Practical application of ketosis and energy deficit milk MIR spectral predictions

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Abstract

During last 15 years different researchers were developing new calibration models linked to milk main components such as fatty acids or minerals, milk biomarkers such as ketone bodies in milk or inflammation indicators or complex components such as energy deficit, ketosis, mastitis, CH_4 or pregnancy with the help of mid-infrared (MIR) spectrometry spectral data. A major provider of MIR services is European Milk Recording (EMR), an umbrella organisation created by former OptiMIR milk recording organisations (MROs), which offer MIR standardisation and predictions. EMR's members are continuously supporting the creation and maintenance of MIR models by collaborating and participating in research projects. MROs having access to milk MIR spectra and prediction models have been increasingly integrating these predictions into services for dairy farmers. Ketosis is a metabolic disorder in ruminants caused by extremely negative energy balance (EB) in early lactation. It may induce an increase in milk fat percentages and ketone bodies as well as a rapid decrease in milk yield, body weight and feed intake. It is also known for causing secondary diseases and fertility problems. Ketosis risk and negative EB can be determined on cow level by using ketone test kits, blood analysis or by milk MIR predictions such as BHB or acetone.

The new idea of KetoMIR was the modelling of ketosis risk based on ketosis diagnosis from veterinaries as reference and milk MIR spectra absorptions as input in order to provide a better indicator in the milk recording service. The first implementation was KetoMIR1 developed by LKV Baden-Württemberg (LKV B.W.). It was based on milk components predicted from standardised milk MIR spectra and is routinely applied by the MROs LKV B.W. and LKV Austria since 2015 respective 2017.

It has to be underlined that until then in literature no information could be found of direct prediction of ketosis risk based on routine MRO spectral and diagnosis data. Since 2018 KetoMIR2 is developed in both MROs within the D4Dairy project. It is a logistic regression model based on standardised milk MIR spectra, sampling moment, lactation number and breed as input and veterinary diagnosis as ketosis reference. The spectra have been pre-processed following the OptiMIR/EMR procedure and corrected for days in milk by Legendre polynomials.

The optimal selection of input parameter was done by using the glmnet R package with lasso method and 10 fold CV. Applied to an external validation set of 11 representative farms the model showed good specificity (0.84) and medium sensitivity (0.72). The KetoMIR2 risk probability shows high correlation with energy deficit, blood BHB and milk yield. KetoMIR2 provides three classes of ketosis warning such as not, moderately and severely endangered and can also be used in herd management to detect general feeding deficiencies. Currently KetoMIR2 is evaluated by feeding advisors of LKV B.W. in a monthly EB report called EMIR. Here it is contrasted with other EB MIR predictions like KetoMIR-1, EB-NEL (as developed by LKV B.W., DLQ and optiKuh) and fatty acid groups. At LKV Austria KetoMIR-2 is being evaluated additionally with blood ketotest kits sampled in 2020.

Keywords: KetoMIR, ketosis risk, ketosis detection, energy balance, NEL, early lactation, MIR milk spectra, dairy cow, dairy farming, optiKuh, D4Dairy, EMR.

Introduction

Ketosis is the number one problem in early lactation. In this phase, up to 6 weeks, a greatly increased energy requirement for milk production dominates. The effect is additionally reinforced by breeding for higher performance. The energy deficit is covered by mobilizing body fat. This leads to an increase in long-chain, mainly unsaturated fatty acids, which are summarized under the term “Preformed Fatty Acids”, and unesterified fatty acids (NEFA). In addition, the production of short-chain and medium-chain fatty acids, the de-novo fatty acids, is falling. In extreme cases, this leads to an overload of the liver, there is an accumulation of ketone bodies in the blood as well as in urine and milk. These are mainly acetone, beta-hydroxybutyrate (BHB) and citrate (Grelet *et al*., 2016). The classic detection methods are an increased fat-protein quotient above 1.5 in dairy breeds and 1.4 in Simmental cattle. A laboratory test of the BHB concentration in the blood is used as a reference finding; here the limit values are 1.2 to 1.4 for subclinical cases and 3 mmol for clinical cases.

BHB can also be measured in milk and urine; the limit values here are 0.2 and 0.5 in milk and 4 mmol/l in urine. In the meantime, inexpensive rapid tests for blood, milk and urine are also available here. Acetone is also an indicator, the greatly increased concentration in breath, milk and urine leads to the typical fruity-sour smell. There are test methods for milk, the limit values are 0.25 or 2 mmol/l. Acetone is highly volatile, so the remaining concentration of the milk sample in the laboratory is often at the detection limit. Even severe weight loss can be used as an indicator if measured regularly. The new concept of KetoMIR is to offer a ketosis risk via milk recording samples that is more precise than e.g. the fat-protein quotient. With each milk recording sample, the farmer receives a herd screening which he can supplement with the above-mentioned methods. Participation in the OptiMIR project resulted for LKV B.W. and later also for LKV Austria in new possibilities of using MIR spectral data. IT processing and standardization was introduced in 2012.

Algorithms for milk MIR components were further or newly developed, e.g. for fatty acids, minerals, BHB, acetone and citrate and are now available. The French tool CetoMIR, based on BHB and acetone, also showed the possibility of ketosis prediction based on MIR spectra. From around 2011, the voluntary collection of veterinary diagnoses, including ketosis diagnoses, has since been started on approx. 1,200 companies based on an 86-part, simplified key based on the model of the Austrian project. The KetoMIR concept consists of the combination of MIR spectra and veterinary diagnoses to predict the risk of ketosis and was the first known work in this direction at the time. Several parameters and concentrations of the above-mentioned MIR components were used. The development began at LKV B.W. 2014, built on 3 years of data availability and went into production at the end of 2015 (Werner *et al*., 2019). The milk analysis with the help of MID infrared spectra (MIR) is a fast and inexpensive way to examine milk recording samples and tank milk samples on a large scale for the main component fat, protein, lactose and urea.

The milk sample is screened by an infrared beam in so-called FTIR analysers and the respective concentration of the substances is calculated using the absorption spectrum obtained and algorithms. The principle is based on the specific interaction of the different wavelengths with the atomic bonds in the milk molecules. With this method, models for fine components such as fatty acids, minerals, lactoferrin (Soyeurt *et al*., 2009), BHB, acetone, citrate (Grelet *et al*., 2016), etc. have also been developed over the past 15 years. In addition, models for complex characteristics such as energy deficit (Dale *et al*., 2019), ketosis (Werner *et al*., 2019), mastitis, methane emissions and pregnancy could be created. In contrast to the main components, however, a spectrum with long-term stability is required for the latter features. MIR spectra are not uniform and not long-term stable.

The reasons for the variability lie in different implementations of the manufacturers, tolerances of the components even with the same models as well as drift due to wear and tear, climatic fluctuations etc. This requires a correction by a standardization procedure (Grelet *et al*., 2015). In the standardization process, a distinction is made between manufacturer-specific processes such as those offered in the form of the Foss equalizer and Bentley stabilizer. In the OptiMIR project, a manufacturer-independent process was developed which has been offered by the OptiMIR successor consortium European Milk Recording (EMR) since 2015. In the milk recording samples and milk quality area, the bias slope correction method is used, which determines correction factors using regular pilot samples and reference measurements. Various studies have also shown that EMR standardization can significantly increase the accuracy of the MIR models.

Material and methods

The binomial KetoMIR-1 model for the first 120 days of lactation used two reference classes: “healthy” for milk recording samples from Rind GMON BW farms for which no diagnoses and health-related losses are available. Spectra were classified as “ketotic” or “sick” with a ketosis diagnosis no more than 6 days after the milk recording samples. MIR milk components such as fatty acids, minerals, BHB, acetone, citrate, classic milk recording samples ingredients and fixed effects such as breed, lactation number, lactation week and milking time were used as input parameters. The GLMNET method in R was used to model a logistic regression. A new version of KetoMIR, KetoMIR-2 is currently being developed in the D4Dairy project and made ready for production. The following innovations are used compared to KetoMIR-1. Instead of milk MIR components, the MIR spectral data are used directly, using the pre-processing established in OptiMIR (standardization, 1st derivative, 212 wavelengths).

The integration of the lactation days takes place with the help of Legendre polynomials on the MIR spectra. The basics for KetoMIR-2 were developed in the course of modelling experiments at LKV B.W. already laid at the beginning of 2017. Through the establishment of spectral data processing and standardization at LKV Austria and Zuchtdata as well as EMR membership in 2017, the MIR spectral data and ketosis diagnoses available there were also usable. The collaboration offered the opportunity to create a more robust, cross-population and cross-manufacturer MIR model. For the KetoMIR-2 calibration, data from the period 2012 to 2017 from 10,079 farms with ketosis diagnoses were used. For the milk recording samples with the classification “ketosis”, 1,638 data sets were used with a spectrum of ± 14 days around the day of diagnosis. For the milk recording samples with the classification “healthy”, 112,545 data sets were used without diagnosis within 60 days of the sample date and without health-related losses. For external test purposes, 11 farms with a high prevalence of ketosis were removed, 4 from LKV AT and 7 from LKV B.W. As with KetoMIR-1, fixed effects such as breed, lactation number and the trial period were included and a logistic regression was modelled using the GLMNET/Lazzo method with 10-fold cross-validation.

Results and discussions

The ketosis probability was calculated for the calibration and test data and the accuracy via sensitivity and specificity was determined using the 0.5 limit values. The model achieved a sensitivity of 0.72 and a specificity of 0.84 in the test. The two-class model was converted for use in a traffic light system with the classes healthy (green), subclinical/endangered (yellow) and clinical/highly endangered (red). Assuming a 20% share of subclinical cases and a 5% share of clinical cases in the first 6 weeks, the new class limits were set at 0.5 and 0.75 using a GMON database for one year. The KetoMIR1 classes were applied to the 2016 GMON annual inventory for evaluation. The representation of the class proportions for the first weeks of lactation shows the expected greatly increased proportion of endangered and highly endangered classifications. One can also see that the dairy breeds are more affected than the dual-purpose Simmental cattle. The representation of the class proportions via the lactation numbers shows that the heifers and cows from the 3rd lactation are more severely affected than the cows in the 2nd lactation. The evaluation of the mean 305-day performance over the KetoMIR classes and lactations gives the following picture. In general, the 305-day performance in the classes “at risk” and “highly endangered” is lower than in the “healthy” class. So, there is already a general, expected depression in performance.

The course of the production mean values in the respective classes over the lactation numbers follows the general course of production that is to be expected for the life of the cows with a lower production in Brown Swiss and Simmental cattle. In the KetoMIR population evaluation, the prevalence of other diseases in the same lactation was calculated based on the KetoMIR classification of the first milk recording result in the classes “at risk” and “highly endangered” relative to the class “healthy”. The value for the diagnosis of cycle disturbance is only slightly higher at 1.2. First calculations and evaluations of genetic correlations of the KetoMIR-1 index showed the potential for use in breeding selection (Hamann *et al.*, 2017).

The final KetoMIR-2 calibration model achieved a mean sensitivity and specificity of 0.76 and 0.84 in the calibration and 0.72 and 0.83 in the external test. A differentiated analysis revealed a sensitivity and specificity for the population groups LKV-AT of 0.72 and 0.81 and LKV-BW of 0.72 and 0.84, respectively. There were also differences for the racial groups. Here an accuracy of 0.76 and 0.83 was obtained for Holstein. For Brown Swiss it was 0.72 and 0.81 respectively, while for Simmental cattle a lower sensitivity of 0.58 compared to a specificity of 0.88 was determined. This is probably due to the lower prevalence of ketosis in Simmental cattle.

In contrast to KetoMIR-1, the cumulative probability curves of KetoMIR-2 showed a very different shape over the lactation weeks. The procedure for defining the threshold values for the traffic light classes therefore had to be adapted. An extensive Pearson correlation analysis of the KetoMIR-2 probability against classic MLP results and the concentration of new MIR ingredients was carried out. Pearson Correlation to the FEQ was 0.46, the MIR predictions with a closer relationship to energy deficit and ketosis resulted in significantly higher values: e.g. for energy balance NEL -0.79; Blood BHB 0.6; Blood NEFA 0.79; Blood glucose -0.76; Acetone in milk 0.65; C18_1CIS9 as the monounsaturated fatty acids 0.73; C12 -0.44, sodium 0.35. This fits well with the known physiological processes and speaks in favour of KetoMIR-2 as an improved alternative to KetoMIR-1. A need for optimization is still seen in the definition of the class limit values. The KetoMIR1-Index has been integrated in the online herd manager in section "Metabolism" at LKV B.W. since 2015 as well as at Zuchtdata/LKV-Austria since 2017.

In the "Overview" mask, the historical course of the class shares at herd level as well as the classification at animal level is displayed for the last 12 milk recording results. Flock shares in the class "endangered" over 20% are coloured yellow, shares in the class "highly endangered" over 5% are coloured red. Shares in the class "healthy" over 80% are highlighted as green. For each testday the KetoMIR-1 index is calculated and displayed to our breeders, advisors and farmers. In the table "Overview of the control year", the percentage class shares for the test year are shown and highlighted in colour according to the scheme described above. If you follow the link to the trial date, the KetoMIR classes are displayed in the traffic light system for each animal together with the milk recording results.

The KetoMIR classification can be checked against diagnoses, observation, calving, occupancy, the milk recording results, etc. via the link with the animals to the event list. In the table "Overview of the control day" you can find the known KetoMIR herd proportions with a traffic light. After more than 4 years of routine use, LKV B.W. the following conclusion can be drawn. KetoMIR was well received by the companies and consultants, late or missing results are usually asked for immediately. KetoMIR is used by the feeding consultants to assess and adjust the feeding situation. However, KetoMIR does not replace the veterinarian and the direct employment of the farmer with the individual animal and the herd. There was developed at LKV B.W. an additional milk recording report called E-MIR that focuses on the presentation of the energy balance in the herd. E-MIR integrates several MIR parameters: Energy balance EB-NEL (GfE, 2001) from the cooperation of DLQ, optiKUH and LKV B.W., the fatty acids from the EMR/OptiMIR-RobustMilk cooperation grouped into De-Novo and Preformed (FA), the energy detect classification (ED), developed by CLASEL as part of the OptiMIR project, as well as the KetoMIR classes and probability values. The KetoMIR-2 predictions are currently evaluated and fine-tuned.

The report is created in two editions, an edition for the farmer with initially less information, limited to EB-NEL and KetoMIR-1 class, as well as an extended version for LKV advisors and consultants with additional EB-NEL, KetoMIR-1and KetoMIR-2, FA and ED. The farmer receives an animal list with milk recording results supplemented by the energy balance NEL and the KetoMIR-1 classes, sorted in ascending order according to lactation days. Cows are grouped according to lactation period as cow status (primipar F and multipar K). For the KetoMIR classes, the percentage group shares are shown again and highlighted in colour according to the traffic light system. The energy balance is shown against the number of lactation days using a point graphic with a distinction between primipar and multipar. The points within the first 120 days are also stored with the KetoMIR traffic light colour.

To make it easier to classify the farm, population means are grouped according to breed, lactation week, cow status, probationary period and probation month and based on this, a green estimation curve is calculated and integrated into the graph with green colour, separated according to primipar and multipar. It can be clearly seen the typical course from negative EB values at the beginning of lactation to positive values in the middle and before the dry cattle phase. The advisor's list of animals also contains the percentage of the de-novo and preformed fatty acid groups. The De Novo group is calculated as the sum of short-chain and medium-chain fatty acids, C6 - C14. The preformed group is formed as the sum of the long-chain fatty acids, C17 + C18. In addition, the ED class is output as the energy balance parameter, which is a classification algorithm which marks animals with an extreme undersupply (-) and oversupply (+) relative to the farm average at herd level.

For the KetoMIR values, the KetoMIR-2 classification is also output as well as the respective KetoMIR probabilities as a value between 0 and 1 in order to be able to better recognize class crossers. The E-MIR table with herd averages for the LKV advisors has been expanded to include mean values for fatty acid groups and KetoMIR-2 class proportions. The graphics in the LKV consultant view have been expanded to include a separate representation of the fatty acid groups De-Novo and Preformed, based on the principle of the EB graphics. At the beginning of lactation, long-chain fatty acids from body fat mobilization dominate, the proportion from neogenesis is reduced. In the middle of lactation, things turn around. Towards the end of lactation, the preformed proportions increase slightly due to the reduced feeding and the neogenesis proportions slightly decrease.

Conclusions

Planning for 2021 foresees a strong expansion of the range at LKV B.W. in the area of MIR parameters. MIR technology continues to be viewed as a key technology. The information base is continually being expanded to further optimize herd management, notably through new reports such as Energy-MIR (E-MIR) and udder health-MIR (MastiMIR). It is also planned to integrate other MIR parameters in the herd manager, for example energy balance, fatty acids, ketone bodies, methane, MastiMIR, etc. Regarding the E-MIR report, an evaluation by LKV consultants and continuous correction has started in 2020. The following questions must be answered: Do the results correspond to the situation found? What is the influence of the composition of the food (eg fatty acid additives, protected fatty acids)? Should the presentation and limit values be optimized? Can KetoMIR-1 be replaced with KetoMIR-2? In addition, interpretation and action instructions should be developed for optimal use of the E-MIR. The usability of KetoMIR's results in breeding will also be explored in more detail.

Acknowledgments

KetoMIR2 has been developed in the international big data project D4Dairy – P2.2 Disease Detection with Milk Spectral Data (*https://d4dairy.com/en*, 2018 - 2022). Within this project the model will be further evaluated and optimized for use in routine herd management and breeding.

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Dale L.M., A. Werner, H. Spiekers, P. Hertel-Böhnke, E. Stamer, F. Gollé-Leidreiter, M. Au, F. Onken. 2019. Prediction of evaluated energy balance (NEL and ME) in dairy cows by milk mid-infrared (MIR) spectra. ICAR Technical Series 2019: 137-141

GfE. 2001. Empfehlungen zur Energie- und Naehrstoffversorgung der Milchkuehe und Aufzuchtrinder. Heft 8, DLG-Verlag Frankfurt a.M.

Grelet, C., J.A. Fernández Pierna, P. Dardenne, V. Baeten, F. Dehareng. 2015. Standardization of milk mid-infrared spectra from a European dairy network. J. Dairy Sci., 98(4): 2150-2160.

Grelet, C., C. Bastin, M. Gelé, J.B. Davière, M. Johan, A. Werner, R. Reding, , J.A. Fernandez-Pierna, F.G. Colinet, P. Dardenne, and N. Gengler. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, beta-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. J. Dairy Sci., 99(6): 4816-4825.

Hamann, H., Werner, A., Dale, L., Herold, P. 2017. Genetic analyses of ketosis and a newly developed risk indicator in Fleckvieh, Braunvieh and German Holstein. ICAR Technical Series no. 22, 105-108.

Soyeurt, H., D. Bruwier, J.-M. Romnee, N. Gengler, C. Bertozzi, D. Veselko, P. Dardenne. 2009. Potential estimation of major mineral contents in cow milk using mid-infrared spectrometry. J. Dairy Sci., 92(6): 2444–2454.

Werner A., F. Gollé-Leidreiter, K. Droessler, F. J. Auer, M. Mayerhofer, A. Köck, C. Egger-Danner, L.M. Dale. 2019. "KetoMIR2" - modelling of ketosis risk using vets diagnosis and MIR spectra for dairy cows in early l

Recording individual real yield in mozzarella cheese in the Italian Mediterranean buffalo population

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Abstract

The aim of this study was to develop a new protocol for recording real data on individual mozzarella yield to be included in breeding programs to increase mozzarella production in the buffalo population. Almost all the milk from the Italian Mediterranean buffalo is transformed into mozzarella cheese. Therefore, increasing yield in mozzarella cheese is the main goal of the breeding programs. Traditionally, yield in mozzarella cheese is estimated using milk, fat and protein by the well-known PKM formula. However, the yield in mozzarella not only depends on protein in the milk, but also on the type and on the proportion of the different protein variants. Moreover, the PKM is highly related to milk production. Therefore, selecting for the PKM might increase in the population the number of buffalo that produce more milk, and not necessarily more mozzarella cheese. The most accurate, and not expensive trait to be recorded for estimating the individual cheese yield is the Dry Matter Yield in Curd (DMYC). A total of 499 milk samples of 1 kg from 89 buffalo distributed in 8 farms from 2010 to 2015, were collected 3 to 6 times per lactation, at intervals of 40-45 days from DIM 30 to 270.

To obtain DMYC from each milk sample, the Real Yield in Curd (RYC) was first calculated with the micro-cheese technique according to the following protocol: 1 kg of milk sample was heated to 37 °C after adding 1 ml Kg^{-1} of liquid rennet, with a concentration of 160 IMCU mL^{-1}. The obtained curd was put in a plastic tray container, refrigerated at 4°C and weighted after 24 hours to getting the RYC after 24 hours (RYC24). From the RYC24, 50 g of curd were used to determine the percentage of dry matter in the curd by drying the RYC24 at 103°C. DMYC was then obtained by multiplying the weight of the curd from 1 kg of milk sample by the DM (%).

To evaluate the effectiveness of using the DMYC as estimator of individual RYC, correlation analysis of DMYC with the RYC24, PKM and APKM was performed. In addition, correlations of the average DMYC overall DIM intervals and DMYC for each DIM interval were evaluated to detect the possibility of using only one sample from a DIM interval per lactation. DMYC resulted largely correlated to RYC24; while it was lower correlated to PKM and APKM. Thus, DMYC might be used as estimator of the individual RYC. Moreover, DMYC estimated at DIM interval of 181-210 was highly correlated to DMYC overall DIM intervals.

Thus, collecting only one milk sample at DIM interval 181-210, might allow to estimate accurately the individual DMYC in the whole lactation. DMYC at DIM 180-210 is a novel, simple and not expenses trait to be recorded for the genetic selection of buffalo to increasing in the population the number of high yielding buffalo that will produce more mozzarella cheese than more milk.

Keywords: Buffalo mozzarella cheese, yield in curd.

Introduction

The increasing economic relevance of the Italian buffalo breed is due to the high market demand for the buffalo mozzarella cheese, resulting in a price for buffalo's milk that is more than three times higher than the price of cow's milk. The higher price, however, does not depend only on the higher yield of buffalo milk but also on the better flavour and, therefore, on the greater satisfaction of the consumer for the better tasty of the product. Due to its high dry matter content, buffalo milk is very suitable for processing, with a yield in cheese higher than that of cow's milk (Zicarelli, L., 2004). To produce 1 kg of mozzarella cheese are required 8 kg of cow's milk, but only 4 kg of buffalo's milk (Zicarelli, L., 2004). Therefore, in Italy, buffalo milk is primarily paid by its yield in mozzarella cheese and by its unique flavour. Moreover, the breeding schemes are aimed to achieve genetic improvement of yield in mozzarella cheese. Traditionally, the production of mozzarella cheese (PKM, kg) is estimated from the observation of milk, fats and proteins, from the formula of Altiero *et al.* (1989) (Rosati A. and D.L. Van Vleck, 2002).

This formula takes into account the amount of milk production and the percentage of protein and fat. However, the yield of mozzarella cheese does not only depend on the percentage of protein in the milk, but also on the type and on the proportion of the different protein variants (Zicarelli L. *et al.*, 2020). However, selection of the percentage of proteins in the milk does not guarantee the selection for a higher yield in mozzarella cheese.

Therefore, by selecting through the PKM, one risk is to increase the number of animals that will produce more milk, and not more mozzarella cheese. In a recent work, Parlato E., and Zicarelli L. (2015) proposed an adjusted PKM (APKM), obtained by multiplying the PKM by the ratio of its estimated yield and the mean estimated yield of the year that the lactation refers to. The APKM is lower correlated to the milk yield than the PKM and tended to increase ranking of the sire with positive EBVs for protein and fat percentage (Parlato E., and Zicarelli L., 2015).

The objective of this study was to set up a new protocol to recording individual yield in curd in the buffalo population to be used in selection program for increasing the number of high-yielding buffalo that will produce more mozzarella cheese than more milk.

Material and methods

Data

A total of 499 milk samples of 1 kg, from 89 buffalo distributed in 8 Italian farms, from year 2005 to year 2010, were collected. Buffalo were fed on a diet with the same chemical composition (15,5% of CP, 0,9 MFU/kg of DM., 130g of Ca; 76 g of P, >32% Fiber/ kg of DM., <240 gr / kg of DM). Milk samples were collected from 3 to 6 times during a single lactation, at intervals of about 40- 45 days from 30 to 270 days in milk (DIM). The day of calving was set equal to 0. From the same samples an addition of 500 ml of milk was collected for the analysis of Percentage of Crude Protein (PP) and Percentage of Fat (FP) by CombiFoss™ 7 using the dilution method.

Protocol to obtain individual Dry Matter Yield in Curd

To obtain the Dry Matter Yield in Curd (DMYC) from each milk sample, the Real Yield in Curd (RYC) was first calculated by the micro-cheese making technique according to the following protocol (Zicarelli *et al.,* 2001; Zicarelli *et al.* 2020): Liquid rennet (80% Chymosin) with a concentration of 160 IMCU mL^{-1} (International Milk-Clotting Units, mL^{-1}) was added to the milk sample (1 mL Kg^{1}) and heated to 37 °C. Since coagulation was performed with the maximum amount of rennet, the curdling and the curd formation were faster than that observed in the cheese factories. To facilitate the complete syneresis, the coagulum was cut first into a large piece then into small pieces, and then pressed to remove the residual whey. The curd was put in plastic tray containers, refrigerated (4°C) and weighted after 24 hours. The measured weight represented the yield of the curd per kg of milk after 24 hours (RYC24). After 24 hours at 4°C, 50 g of curd were used to determine the percentage of dry matter by drying the yield in curd at 103°C; Dry Matter Yield in Curd (DMYC) was obtained by multiplying the weight of the coagulum from 1 kg of milk sample by the DM.

$$DMYC = RYC24 * DM*0.01$$

PKM and adjusted PKM (APKM) were calculated for each individual milk sample by the following formula (Parlato E. and L. Zicarelli, 2015).

$$APKM = \text{milk yield, kg} * [3.5*(PP)+1.23*(FP)-0.88] / 100$$

where: C = EY / MEY; EY = [3.5*(PP)+1.23*(FP)-0.88] and MEY is the average milk yield of the year the lactation refers to.

Statistical analysis

To evaluate the effectiveness of using the DMYC as estimator of the individual RYC, correlation analysis among DMYC, RYC24, PKM, and APKM was performed by PROC CORR procedure of SAS (2005). Moreover, to detect the possibility of using only one sample per animal, as representative of the whole lactation, correlation analysis of the average DMYC per lactation with the DMYC per each DIM interval was also evaluated.

Results and discussion

Means and standard deviations of DMYC, RYC24, PKM and APKM are shown in Table 1. DMYC (113.75 g) showed the smaller mean weight compared to RYC24 (260.19 g), PKM (263.98 g), and APKM (264.33 g). The coefficients of correlation for DMYC, RYC24, PKM, and APKM are shown in Table 2. Correlation coefficients ranged between 0.52 (PKM *vs* DMYC) and 0.90 (PKM *vs* APKM). All the correlations were highly statistically significant. PKM *vs* APKM showed a large correlation because APKM is derived from the PKM. Also, DMYC *vs* RYC24 had a large correlation because DMYC is derived from the RYC24. Whereas correlations of PKM and APKM with RYC24 and DMYC were low. These results showed that PKM and APKM might not be good estimators of the RYC24 and consequently of the DMYC. The weight of the dry matter (DMYC) of the coagulum would give results more accurate than the RYC24, avoiding any bias due to the process by which the RYC24 is obtained.

Coefficient of correlations of the overall average DMYC per lactation, and the DMYC at each DIM intervals, are shown in Table 3. Correlations were all highly statistically significant. The larger (0.79) correlation was found at the DIM interval of 181-210 days. At this stage of the lactation, buffalo have reached the energetic balance, therefore, they can express completely their mammary synthesis capability. Whereas, the smaller (0.59) correlation was found at the DIM interval of 151-180 days. Since there was a large correlation between the overall average DMYC and the DMYC at DIM interval of 181-210 days, DMYC might be evaluated per animal, per lactation, only one time at DIM interval of 181-210 days.

Conclusion

DMYC at DIM 180-210 is a novel, simple and not expenses trait to be recorded for the genetic selection of buffalo to increasing in the population the number of high yielding buffalo that will produce more mozzarella cheese than more milk. Moreover, selection for DMYC will help to keep the characteristic flavour of the buffalo milk. Animals with larger yield in mozzarella cheese but less milk production are desirable to avoid flavour dilution in the milk (Zicarelli, 2020). Yield in mozzarella cheese and flavour are the most important traits for improving the profit of the buffalo farm.

Table 1. Overall unadjusted means and standard deviations of weights for Real Yield in Curd after 24 hours (RYC24), PKM; adjusted PKM (APKM) and Dry Matter Yield in Curd (DMYC), in grams, of 499 individual milk samples at different DIM of 89 animals.

Trait	Mean	Std.Dv
DMYC	113.75	14.85
RYC24	260.19	29.32
PKM	263.98	22.15
APKM	264.33	32.33

Table 2. Coefficients of correlation (above and below the diagonal) among DMYC, RYC24, PKM and APKM.*

	DMYC	RYC24	PKM	APKM
DMYC	1.00	0.85	0.52	0.65
RYC24	0.85	1.00	0.56	0.58
PKM	0.52	0.59	1.00	0.90
APKM	0.65	0.56	0.90	1.00

*: $P \leq 0.0001$

Table 3. Coefficients of correlation, r for the unadjusted mean of DMYC overall DIM intervals and DMYC at each DIM interval of 89 buffalo.*

DIM	r
30 -60	0.71
61-90	0.68
91-120	0.70
121-150	0.65
151-180	0.59
181-210	0.79
211-270	0.72

*: $P \leq 0.0001$

List of references

Altiero, V., Moio, L., and Addeo, F. 1989. Previsione della resa in mozzarella sulla base del contenuto in grasso e proteine del latte di bufala. Scienza Tecnica Lattiero-Casearia, 40(6), 425–433.

FAOSTAT. 2021. Statistical database of the food and agriculture organization of the United Nations. http://www.fao.org/faostat/en/#data/PI .

Parlato E, Zicarelli L. 2015. Genetic Parameters and Sire Ranking for the Adjusted Estimate of Mozzarella Cheese Production in Italian Mediterranean Buffalo. J Adv. Dairy Res. 3: 148. doi:10.4172/2329-888X.1000148

Rosati, A., and Van Vleck, L.D. 2002. Estimation of genetic parameters for milk, fat, protein and mozzarella cheese production for the Italian river buffalo *Bubalus Bubalis* population. Livestock Production Science, 74(2), 185–190.

SAS/STAT User's Guide, Version 9.2. 2005. Cary, NC: SAS Institute.

Zicarelli, L., Potena, A., De Filippo, C., Di Palo, R., and Campanile, G. 2001. Confronto tra bufale con differente resa alla caseificazione. Atti I Congresso Nazionale sull'allevamento del bufalo, 3–5 ottobre, Eboli. 241–246.

Zicarelli L. 2004. Buffalo milk: its properties, dairy yield and mozzarella production. Vet. Res. Com. 28: 127-135.

Zicarelli L., Di Palo R., Napolano R., Tonhati U., De Carlo E., Gagliardi R., Di Luccia A., La Gatta B. Influence of alphas1-CN and kappa CN polymorphism on the curd yield of the Italian Mediterranean buffalo (*Bubalus bubalis*) milk. 2020. Int. Dairy J. 100.

Developing a new selection index for the Italian Mediterranean Buffalo (*Bubalus bubalis*)

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Abstract

The breeding of the Mediteranean buffalo is a long-standing Italian tradition, being internationally recognized thanks to its iconic *Mozzarella di Bufala Campana*, a Protected Designation of Origin (PDO) certified mozzarella cheese. Over a population of more than 419.000 extant animals, the Italian National Association of Buffalo Breeders (ANASB) involved 121.403 animals from 380 farms mainly widespread in central and southern areas of the country. In 2019, official milk recording by certified recorders belonging to the Associazione Italiana Allevatori (AIA) and following the ICAR guidelines included 49.932 buffalo cows in 215 herds. In 1997 a first selection scheme was implemented. It was based on a traditional progeny testing and a BLUP genetic evaluation of milk productive traits. A first selection index, namely the PKM, was also developed. The breeding objective of the PKM was the mozzarella cheese yield using as selection criteria milk yield, fat and protein. Although a positive selection on milk yields was observed from 1.608 Kg in 1977 to 2.169 Kg in 2018, PKM had an unfavorable effect on the genetic trend for fat and protein.

In order to recover the unfavorable trend of milk components as well as to include morphology, a new selection index named IBMI has been developed and applied since 2019. The IBMI breeding objectives are milk production and mozzarella yield. IBMI includes five selection criteria: feet and legs, udder morphology, milk kilograms, fat and protein percentage whose relative emphasis was 24, 20, 21, 15 e 20, respectively. The expected genetic progress by generation using IBMI is +58 Kg for milk, +0.10 and 0.05 % respectively for fat and protein percentage. The next steps in the selective activity will be: a) the development of a test day model for productive traits and b) the implementation of a single step genomic EBV evaluation.

Keywords: Buffalo, genetic response, selection index.

Introduction

The Water Buffalo (*Bubalus bubalis*) is a large bovid mainly distributed in the Asian continent where the 97% of its world population is concentrated (FAO, 2020). The name 'water buffalo' is due to its adaptation to flooded or swampy areas, where it partially submerges and walks on the bottom mud without difficulty. The Mediterranean area, where the rest of the world population is raised (3%), historically have been characterized by these optimal rearing conditions. In the European continent can be found only the 0.2% of its world population and about 93% of these animals can be found in south-central Italy (Neglia *et al*, 2020). Total census in Italy increased considerably, making it one of the most important dairy species in the country. In 2019, 34,990 lactating buffaloes have been registered to the herd book. Moreover, 666,960 controlled lactations and 9,953 type traits evaluations are available and officially recorded (ANASB, 2020). Thanks to the physical-chemical properties of its milk - high concentration in protein and fat (FC ~ 8 %) and favorable coagulation (Costa *et al*, 2020) the main zootechnical interest of the Italian Mediterranean Buffalo (IMB) is to produce iconic traditional dairy products like the *Mozzarella di Bufala Campana* (Boselli *et al*, 2020), that has a great economic impact on the Italian food industry (ISMEA, 2020). Costa *et al*. (2020a, 2020b) refers to the impressive increase in heads that the IMB has experienced in the last 15 years, with an increase in terms of kilos of cheese produced, a larger herd size, a constant increase in registered herds, as well as the rise in milk price. Therefore, the economic interest in this specie makes it necessary to develop new innovative tool to improve breeding process. In 1997 a first national selection scheme was implemented. It was based on a traditional progeny testing and a BLUP genetic evaluation of milk productive traits. A first selection index, namely the PKM, was also developed. The breeding objective of the PKM was the mozzarella cheese yield using as selection criteria milk yield, fat and protein (Rosati and van Vleck, 2002). Although a positive selection on milk yields was observed– from 1.608 Kg in 1977 to 2.169 Kg in 2018, PKM had an unfavorable effect on the genetic trend for fat and protein. Moreover, no emphasis was given to health related or functional traits. An effective breeding objective should be defined for the genetic improvement of any population such that the future individuals, e.g., buffalo cows, will produce the desired products more efficiently under expected future economic production environment (Lopez-Villalobos and Garrick, 2005). Attending to mozzarella cheese manufacturers and farmers community, who both asked for a more balanced breeding objective which included also health related traits, annual genetic response for milk yield (MY), milk components (MC), composite feet and legs (FL) and mammary system (MS) and their correlated response with mozzarella cheese production were estimated in the IMB using selection index theory to eventually develop a new and more up-to-date aggregate selection index.

Material and methods

Different selection indices (I) and breeding objectives (H) were constructed using selection index theory (Hazel,1943).

The selection indices investigated in this study considered the following traits: MY, fat content (F%), protein content (P%), mozzarella cheese production (MCY) estimated as 116.615 + 2.015 * (P% * F%) + 2.929 x $(P\%)^2$, and two composite traits FL and MS. The indices were of the form

$$I=b_1\, x_1+b_2x_2+....+ b_m\, x_m = \mathbf{b'x}$$

where x_i is an observation on the *ith* trait and b_i is the selection index coefficient (or weight) for that trait. In vector notation, $b' = [b_1, b_2, \dots , b_m]$ and $x' = [x_1, x_2, \dots, x_m]$.

Three alternative breeding objectives were formulated according to relative weights given to MY, MCY, FL and MS. The different breeding objectives and relative weights considered in this work are shown in Table 1

The three scenarios were formulated in order to give an increasing relative emphasis on milk yield, but keeping the emphasis to MCY above 30%. The breeding objectives were of the form

$H = w_1\, g_1 + w_2\, g_1 + \dots + w_m\, g_1 = w'\, g$

where g_i is the additive genetic value of the *ith* trait and x_i is the relative economic emphasis of the trait. In vector notation, $w'=[w_1, w_2, \dots ,w_m]$ and $g'=[g_1, g_1, \dots ,g_m]$. The vector **b** was derived from the equation $\mathbf{b = P^{-1}Ga}$

where **P** is the *n x n* phenotypic variance–covariance matrix of the traits (*n*) used as selection index, and **G** is the n *x m* genetic covariance matrix between traits in selection index (*n*) and traits in the aggregate genotype (*m*).

Matrices **P** and **G** were estimated using data from 7199 buffalo cows and a pedigree including 19574 buffalos. A multi-trait animal model was fitted using a Bayesian implementation via Gibbs sampling.

Genetic response (**GR**) to selection for each trait considered in the selection indices was calculated with a deterministic procedure (Cameron, 1997) applying the equation

$$GR_j = \frac{b'G_j}{\sqrt{b^i P b}}$$

where $\mathbf{GR}_j$ is the **GR** for trait *j* and $\mathbf{G}_j$ is the *jth* column of matrix G.

Results and discussions

The genetic correlation among the breeding goals and the selection criteria used to estimate the response to selection for the three different breeding scenarios are in Table 2

Mozzarella cheese production had an unfavourable genetic correlation with MY (- 0.54), a null correlation with MS (-0.01), and a favourable correlation with FL (0.25), F% (0.87) and P% (0.96). correlation. Those values had an impact on the selection response for all traits considered in the three scenarios which are shown in Table 3.

The breeding objective S1 showed large and positive genetic response in terms of mozzarella cheese production, fat and protein %. However, MY was strongly penalized, with a negative genetic response. The breeding objective S2 had the best results for health related traits, namely feet and legs and mammary system. In this scenario, 35% of the relative emphasis in the breeding objective was given to health related traits. The last scenario - S3 - showed the best overall results with a positive genetic response for all traits included in the breeding objective ranging from 0.02 (MYC) to 0.21 (P%). The scenario S3 was eventually chosen as the official new selection index for the IMB, called IBMI.

Selection index combines different sources of information that can be used to predict an animals' breeding value. It combines this information by weighting it with its relative importance based on the relationship with the breeding goal. In the IMB the breeding goals were identified as: Cheese Production, Milk Yield and Longevity. The need for a change was mainly based on a frankly discussion among ANASB technicians, farmers and cheese makers. The observed negative genetic trend for fat and protein content suggested the need for a substantial change. From one side there was the need to keep increasing the kg of milk but at the same time there was the urgency of recovering the fat and protein content negative genetic trend. Moreover, the farmers asked for including some linear traits, especially the ones related to longevity and health (e.g., udder and feet and legs). Indeed, the new breeding goal gives more emphasis to the cheese production and at the same time increases milk yield, looking for the first time at the functional morphology.

Table 1. Relative emphasis (%) on traits included in alternative breeding scenario for the IMB.

	Traits in breeding objective^s			
Breeding scenario	**MY**	**MCY**	**FL**	**MS**
S1	5	45	30	20
S2	30	35	15	20
S3	45	45	5	5

Table 2. Estimates of genetic correlations among the breeding goals and the selection criteria in the IMB.

Traits	FL^a	MS^a	MY^a	F%	P%	MCY^a
FL	1.00	0.19	0.00	0.15	0.28	0.25
MS	0.19	1.00	0.29	- 0.08	0.03	-0.01
MY	0.00	0.29	1.00	-0.55	-0.47	-0.54
F%	0.15	-0.08	-0.55	1.00	0.69	0.87
P%	0.28	0.03	-0.47	0.69	1.00	0.96
MCY	0.25	-0.01	-0.54	0.87	0.96	1.00

[a] Traits in the breeding objectives.

Table 3. Genetic response (expressed in genetic standard deviations) for the three breeding objective.

	Traits^b					
Breeding scenario^a	**MY**	**MYC**	**P%**	**F%**	**FL**	**MS**
S1	-0.24	0.05	0.42	0.32	0.14	0.13
S2	0.03	0.03	0.25	0.15	0.16	0.22
S3	0.11	0.02	0.21	0.12	0.14	0.20

[a] relative weight given to MY, cheese production, FL and MS: 5:45:30:20 (S1), 30:35:15:20 (S2) and 45:45:5:5 (S3)

[b] MY = Milk yield (kg/270d), MYC = mozzarella cheese production (MCY) estimated as 116.615 + 2.015 * (P% * F%) + 2.929 x $(P\%)^2$, P% = protein %, F% = fat 5, FL = composite feet and legs, MS = composite Mammary System

Conclusions

The new selection index for the Italian Mediterranean buffalo (IBMI) is extremely different from the former selection index (PKM). Introducing it as a new selection tool has actually been a cornerstone of buffalo breeding in Italy, affecting bulls and dam rankings. Indeed, the best individuals are no longer those with the highest milk EBV, but those that best match the need for a more balanced breeding objective (production, quality and functionality). Moreover, changing the breeder's attitude to look at only one trait, milk production, is not *as easy as pie*. It takes time and needs the right piece of information.

The IBMI is the very first attempt to use selection index theory to set up an aggregate index in the Italian Mediterranean buffalo. However, it can be adjusted especially as concerns the phenotypes used to improve the quality of milk for mozzarella cheese production. In the present research, fat and protein percentage were used as proxies of milk quality but several studies have already showed that milk coagulation properties like rennet coagulation time or curd firmness 30 min after coagulant addition are important traits for enhancing the efficiency of the dairy industry.

Those new phenotypes coupled with the use of genomic selection are the on-going and very next ANASB breeding objectives.

List of references

ANASB, 2020, (Associazione Nazionale Allevatori Specie Bufalina). *http://www.anasb.it/statistiche/* [Accessed May 11, 2020].

Boselli C, De Marchi M, Costa A, Borghese A. 2020 Study of Milkability and Its Relation With Milk Yield and Somatic Cell in Mediterranean Italian Water Buffalo. Front Vet Sci 7(432):432. doi: 10.3389/fvets.2020.00432.

Cameron, N.D., 1997. Selection Indices and Prediction of Genetic Merit in Animal Breeding. CAB International, Wallingford, UK.

Costa A, Neglia G, Campanile G, De Marchi M. 2020b Milk somatic cell count and its relationship with milk yield and quality traits in Italian water buffaloes. J Dairy Sci 103(6):5485-94. doi: 10.3168/jds.2019-18009.

Costa A, Negrini R, De Marchi M, Campanile G, Neglia G. 2020a Phenotypic Characterization of Milk Yield and Quality Traits in a Large Population of Water Buffaloes. Animals 10(2):327. doi: 10.3390/ani10020327.

Food and Agriculture Organization (FAO). 2020. Live Animals. Available online at: http://www.fao.org/faostat/en/#data/QA [Accessed September 28, 2020).

Hazel, L.N., 1943. The genetic basis for constructing selection indexes. Genetics 28, 476–490.

ISMEA. 2020. *http://www.ismeamercati.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/5186* [Accessed Sept 15, 2020].

Lopez-Villalobos, N.,Garrick,D.J., 2005.Methodology for the design and enhancement of genetic improvement programs illustrated in the context of the New Zealand dairy industry. Agrociencia IX, 553–568.

Neglia G, de Nicola D, Esposito L, Salzano A, D'Occhio MJ, Fatone G. 2020. Reproductive management in buffalo by artificial insemination. Theriogenology 150:166-72. doi: 10.1016/j.theriogenology.2020.01.016.

Rosati A and Van Vleck L.D. 2002 Estimation of genetic parameters for milk. fat. protein and mozzarella cheese production in Italian river buffalo population. Livestock Prod Sci 74:185-190.

Data ownership, privacy, use, sharing and stewardship - The U.S. experience

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Abstract

The Council on Dairy Cattle Breeding (CDCB) is a non-profit collaboration between four sectors of the U.S. dairy industry: dairy records providers, dairy records processing centers, Purebred Dairy Cattle Association (PDCA) and National Association of Animal Breeders (NAAB). The purpose of CDCB is to host the national cooperator database (NCD) on behalf of the dairy community and use data analytics to provide value back to dairy producers through genetic evaluations and management information. The NCD is composed of three basic data types: animal relationships (pedigrees), management and performance records (phenotypes) and single nucleotide polymorphisms (SNP) markers (genotypes).

Phenotypes and pedigrees have been collected over a century by dairy herd improvement (DHI) services contracted by dairy producers to enhance decision-making process at the herd. Dairy producers own and control the use of data generated from their herds, and access to data is regulated by agreements signed with the DHI service providers. Genomic nominators provide tissue sampling and data collection services and serve as conduits between farmers, genotyping laboratories and CDCB for genomic predictions. Control over the use of genomic data is also exerted by animal owners and regulated by commercial agreements between service providers and users. The NCD receives data from a multitude of sources, and CDCB has material license agreements with each one establishing data access and use limits. A material transfer research data exchange agreement between the Agricultural Research Service (ARS) of U.S. Department of Agriculture (USDA) and CDCB allows ARS researchers access to the NCD for research purposes only. Other research organizations can only access data from the NCD if formally authorized by the data controllers. International data sharing initiatives mainly involve genotypes and are negotiated by data controllers and executed by CDCB accordingly.

CDCB is also investing in novel phenotypes data generation projects such as feed efficiency, which require a new business model to secure sustainable data flow. Dairy herds data from sensors is growing rapidly in the industry but in a disordered process that lacks standards, quality assurance, and means to be properly integrated to the existing data systems. Dairy herd data belong to dairy producers who invest in technology and pay for all services. Therefore, dairy data must serve primarily to improve the decision-making process at the herd level and the role of farmer-based organizations such as CDCB is to facilitate an effective integration of the existing and emerging data streams.

Keywords: Data stewardship, CDCB, U.S. dairy.

Introduction

Data ownership has become a common discussion in the dairy industry since dairy herds started the transition from small-medium technology-empirical management to larger-hi-tech-professional management kind of operations. Nowadays most herds need to be managed as an enterprise that optimizes economic outputs by controlling costs and using the right technologies. This optimal management can only be achieved by using reliable data to monitor all aspects of the operation and therefore have a sound decision making process in place. The sector has accompanied the trends of precision agriculture and an abundance of new technologies to generate data at the farm have been incorporated into the system.

Besides the traditional milk recording data collected periodically (milk yield, components, somatic cell counts, reproductive events, culling and dry off events), now on farm sensors generate data 24 hours a day, a large proportion of calves are genotyped right after birth and genomic predictions are used as management tools, and a myriad of other service providers offer specialized data to be added to the farm hard disks (vets, nutritionists, artificial insemination planners, feedstuff suppliers, crop specialists, milk buyers, etc.). One can easily assume that dairy farmers are overwhelmed with data and integration of all these pieces of information is still far from adequate. The question to be addressed here is who owns these data, who has access to it and what can be done with it. This paper will focus on the specific situation of the U.S. dairy industry and how data ownership, privacy, use, sharing and stewardship are currently managed.

Dairy data in the U.S.

Dairy data in the U.S. has a long history and usually it is traced back to 1908, when the dairy herd improvement associations (DHIA) started collecting regular milk recording data. From the start, the U.S. Department of Agriculture (USDA) provided logistic support and research that evolved into the modern services supporting decision making and genetic evaluations. For most of this history, the Agricultural Research Service (ARS) maintained the dairy national cooperator database (NCD) and provided genetic evaluation services. In 1999 the CDCB was formed as a nonprofit corporation with the purposes of providing a forum in which to share information and coordinate activities that improve dairy cattle genetics and maintaining the integrity of data used in the genetic evaluation of dairy cattle. CDCB served as a communication channel between ARS, the dairy data providers and the organizations using and promoting U.S. dairy genetics. The three sectors represented in the CDCB board were the DHIA sector, the breed associations and the artificial insemination (AI) industry.

When the genomics era initiated in 2008, demand for data management and genetic evaluation services increased dramatically and both ARS and the dairy industry stakeholders realized that a new business model was necessary to fulfil the industry needs and continue at the edge of the scientific knowledge. A significant part of the research that allowed the genomic technologies to be adopted in dairy cattle was carried out by ARS researchers using data from the NCD. It became evident that it was time for the industry to take responsibility over the services and allow ARS to focus on the research. As a consequence, the NCD stewardship and the genetic evaluation services started to migrate from ARS to CDCB in 2013 and the process was completed in December 2015.

A material transfer research data exchange agreement between the two organizations establishes that ARS continues to have full access to the NCD for research purposes only and CDCB, besides receiving ARS legacy programs and expertise, continues to receive scientific support from ARS researchers and is committed to maintain quality certification programs to ensure the NCD data quality.

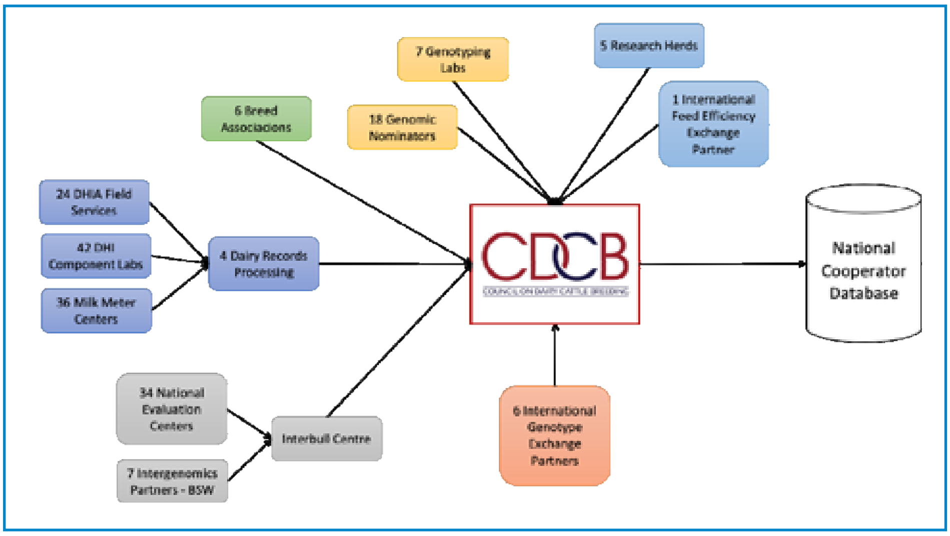

Figure 1 - Number of organizations providing data to the National Cooperator Database hosted by the Council on Dairy Cattle Breeding (April 2021).

Dairy data management

Figure 1 shows the number of organizations providing data to the NCD. Data types flowing into the NCD can be described as animal identification, genealogy, performance, management, health, conformation appraisals, dry matter intake, breeding values and genomic markers. Quality certification for animal identification, performance, health and management data is provided by Quality Certification Services (QCS), for international genealogy and breeding values by the Interbull Centre and for genomic and genealogy data by CDCB. Quality control for novel data types (e.g., dry matter intake) is overseen by CDCB.

Data ownership, access and use

The CDCB members position is that dairy data belongs to the owner or controller of the animal from which data was generated. Dairy producers pay for all services and technologies that generate dairy data. All the other agents included in Figure 1 are either data processors, stewards or users. This is a fundamental principle irrespectively of the data type. As a consequence, only dairy producers or animal controllers can authorize access and use of data stored in the NCD. CDCB is the NCD steward and follows the access and use policies established by data providers.

DHI data access and use is regulated by agreements between dairy producers with the DHI service providers. Genomic nominators provide tissue sampling and data collection services and serve as conduits between farmers, genotyping laboratories and CDCB for genomic predictions. Control over the use of genomic data is also exerted by animal owners and regulated by commercial agreements between service providers and users. Since the NCD receives data from a multitude of sources (Figure 1), CDCB has material license agreements with each data provider establishing data access and use limits. Research organizations can only access data from the NCD if formally authorized by the data controllers. International data sharing initiatives mainly involve genotypes and are negotiated by data controllers and executed by CDCB accordingly. CDCB is also investing in novel phenotypes data generation projects such as feed efficiency, which require a new business model to secure sustainable data flow. Dairy herds data from sensors is growing rapidly in the industry but in a disordered process that lacks standards, quality assurance, and means to be properly integrated to the existing data systems.

Take home message

Dairy herd data belong to dairy producers who invest in technology and pay for all services. Therefore, dairy data must serve primarily to improve the decision-making process at the herd level and the role of farmer-based organizations such as CDCB is to facilitate an effective integration of the existing and emerging data streams and follow the access and use options of dairy producers as the national data steward.

iDDEN: An international perspective

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Abstract

Dairy industries worldwide are facing many of the same challenges: fewer and larger dairy farms, more investment in on-farm technology and significant industry demand for the efficient collection, exchange and integration of this data. The on-farm technology choices traditionally available from a few brand name equipment manufacturers is now being crowded with a growing number of start up sensor and technology companies and increasing market penetration of herd management software companies. The ability to effectively move and manage data with a growing number of suppliers and lack of industry standardization has created an increasingly inefficient process. As well, equipment manufacturers are continually pressured to meet the data interface demands of multiple (national) organizations and this detracts from their primary objectives of developing innovative milking and herd management technologies.

To address this increasingly costly issue, seven farmer owned organizations have collaborated in the establishment of a unified international dairy data exchange network partnership (iDDEN GmbH). Together the organizations represent about 20 million dairy cows on 200,000 dairy farms in 13 countries on three continents. The objective of the partnership is a critical mass of organizations that will coordinate unified data exchange and integration with dairy equipment manufacturers and other entities involved in herd management. Given our intent to integrate ICAR-ADE (Animal Data Exchange) standards wherever possible, we have started work with three initial manufacturers given their intent to switch to the ADE standards. Once completed, we will continue with the addition of manufacturers and then expand the geographic service regions. The end goal is to provide global access for other organizations while retaining leadership by a farmer- led organization.

iDDEN purchased the former NCDX data exchange system used in the Nordic countries and is currently adding a connection to cloud-based data repositories. In principle the newer data exchange technologies (JSON-REST) using ICAR-ADE standards will be implemented where possible and available. The objective is to create a standard process for parties involved in data exchange being milk recording, genetic evaluation, or other groups.

As it relates to data governance, the data will be real-time transfer and no data would be stored or retained by iDDEN. As well, appropriate authentication will be required by the farm and the end user to ensure that only approved parties will have access to farm data – either direct or via cloud connections where available. Other than data transfer security, the data governance issues of privacy and ownership will remain the responsibility of the organizations and farms using the exchange service.

Keywords: Dairy cattle data, data exchange, international cooperation, dairy cattle data.

The company and motivation

iDDEN GmbH was founded in May 2020 and brought together a group of organizations interested in data exchange that were seeking the opportunity to do so with less effort and reduced cost due to standardization and reuse of existing solutions.

The founding members are listed in Table 1 below to show the worldwide collaboration. However, more than these founding members benefit from using the iDDEN system as a platform for data exchange, all strategic partners and other customers will also be able to exchange data more easily with more potential partners in future.

The number of farms, herds and cows managed by these founding members are shown in Figure 1 “Potential of iDDEN” below. These high numbers of potential resources for data exchange will drive a lot of momentum to manufacturers of milking equipment and other on-farm systems to take the opportunity to be involved in the iDDEN data exchange.

Vision and design goals

The vision of iDDEN is to be the worldwide accepted standard platform for data exchange of dairy related data. To achieve this, iDDEN acquired the NCDX system of the Nordic countries developed by Mtech, Finland. Based on this foundation iDDEN will evolve the capabilities of the system and implement the standards developed by the ICAR-ADE working group.

Table 1. Founding members of iDDEN GmbH.

Shareholder	Designated area responsibilities
CRV Holding B.V.	The Netherlands and Belgium
DataGene Ltd.	Australia
Lactanet Canada	Canada
NDHIA Inc.	USA
NCDX ApS	Denmark, Iceland, Finland, Norway, Sweden
RDV GmbH	Austria, Germany
VIT w.V.	Germany, Luxemburg

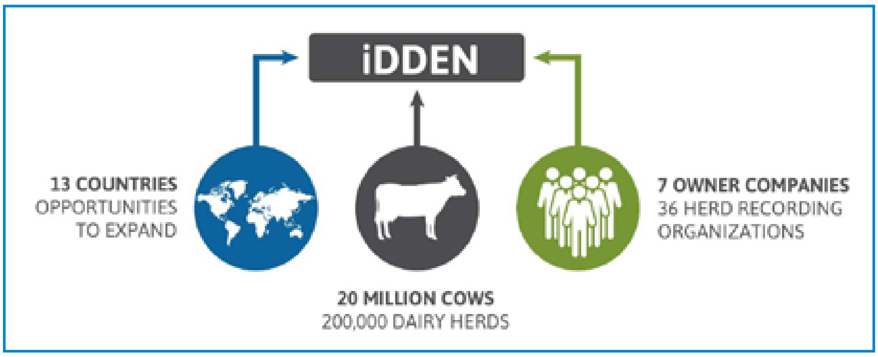

Figure 1. Potential of iDDEN.

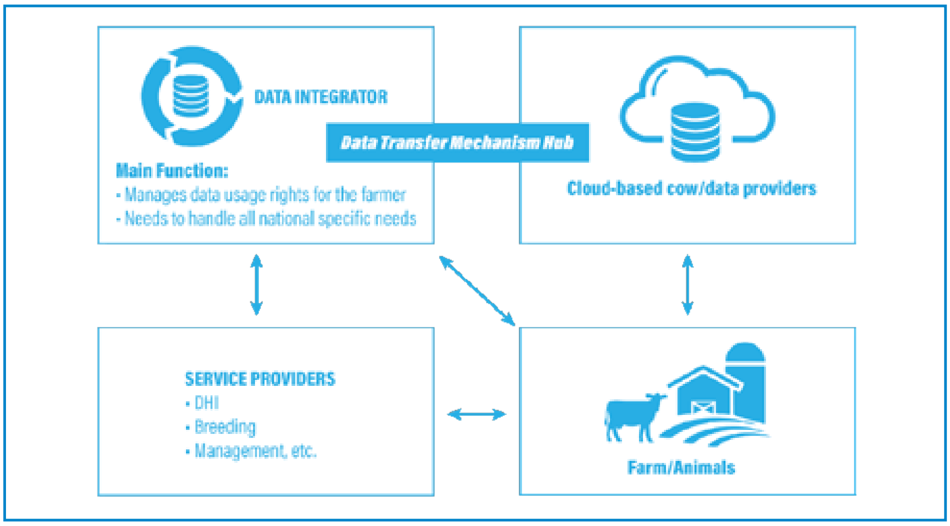

Figure 2. Schematic of data flow between on-farm data and other (central) data.

The future of data exchange between data integrators (like Milk Recording Organizations (MROs)), Original Equipment Manufacturers (OEMs), and other on-farm systems or service providers on behalf of farmers is shown in Figure 2.

The design goals for the iDDEN system are as follows:

- **Reuse as much as possible**

  By building the iDDEN system on the infrastructure of the already established NCDX system, it will utilize the well-tested and working functionality of the original NCDX system.

  Since all partners currently operate some kind of rights and mandates checking system, it was decided to use these services also in the iDDEN system for authentication and authorization purposes.

- **Standardize as much as possible**

  The iDDEN system will work on well-established open standards like web services using the JSON/REST approach.

  As messages it will use those defined by the ICAR-ADE working group. This is an open-source development for standardization of animal data. Therefore, it will be suitable for a wide range of partners and provide a stable set of messages for communication with all potential partners in the future.

- **Integrate only once**

  Using a Hub architecture, all partners will only need to implement “one” interface communicating directly with the iDDEN system. All routing and transforming (if needed) will be handled inside the Hub. It will be possible to enhance the system ith local specialties, but these should be avoided wherever possible.

Roles and architecture

Within the iDDEN system there are four different roles defined for systems involved in data exchange. These roles are listed and described in Table 2 below.

All three external roles can act as active or passive partners in the data exchange via the iDDEN Hub. Active means that the partner is initiating the conversation against the Hub. It will either request data from another partner using a ‘GET’ request or it could also send data using a ‘POST’ request. From a client/server architectural point of view this will be the client part. The passive partner will listen for requests and either send the requested data or accept the data sent from the active partner and store it in their database system. This will be the server part in data exchange.

Table 2. Roles defined in iDDEN.

Role	Description
FMS	Farm Management System - This can be any software running on the farm which is involved in data exchange. It could be a herd management software or a milking system (robot, parlour, etc.) system or any analytic software.
Cloud	Cloud provider – This is the central cloud system of software running on the farm. It gets some data directly from the FMS.
Data Integrator	Data Integrator – This is the role of the iDDEN participant parties. It can be an MRO (Milk Recording Organization) or any other data integration system.
Hub	The iDDEN system responsible for routing and transformation of data.

Security considerations

By using the established authentication and authorization services from each partner providing data, the partners have to ensure the right of the requesting partner to access the data in question. Therefore, they need the consent of the farm to share the data with other partners.

Each partner system will be identified by an iDDEN-ID. These are assigned by iDDEN during the registration of each organization to the iDDEN program. Alongside with this iDDEN-ID also an iDDEN-API-Key will be provided. These credentials will be used to authenticate against the Hub system before it will forward the request to the target partner.

The same iDDEN-ID will be used to authenticate against any authentication service from any other data delivering partner. For obvious security reasons this login must be done outside the Hub before any communication via the Hub. The token delivered in this step could be used in a full session of data exchange with this partner over a serial of requests.

In the case of Farm Management Software (FMS), it will use the credentials of the farm where the software is running to authenticate against the data delivering service. This is because the same FMS system is running on several farm locations and therefore the partner providing data needs to know which instance is requesting data to ensure the confidentiality of the data.

Examples of data exchange

To show the steps involved in data exchange via the iDDEN Hub two scenarios are shown below. The first one (Figure 3) is a ‘GET’ request from an FMS system to acquire data from an MRO for a specific farm.

The second example (Figure 4) shows how an MRO sends data to an OEM cloud via a ‘POST’ request.

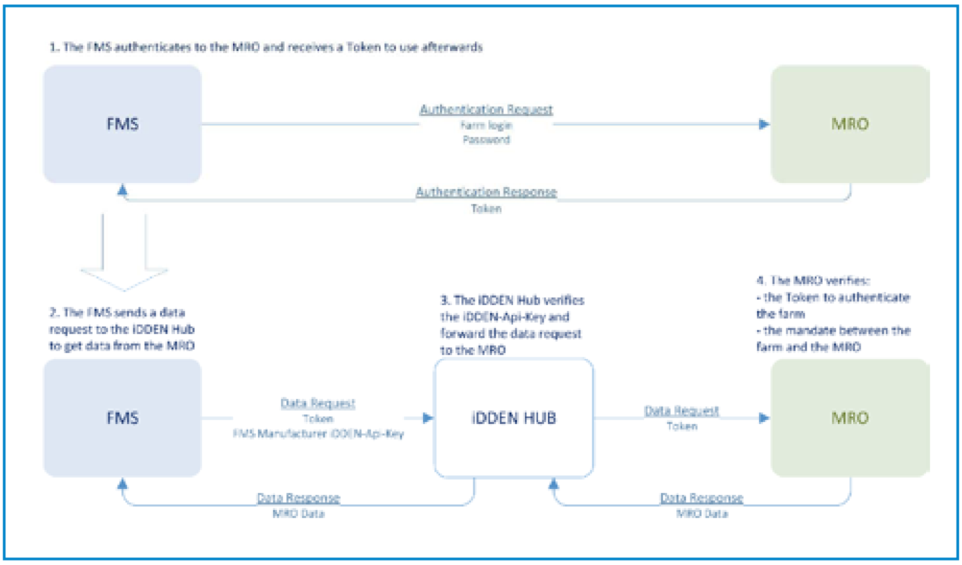

Figure 3. Get data from MRO to FMS.

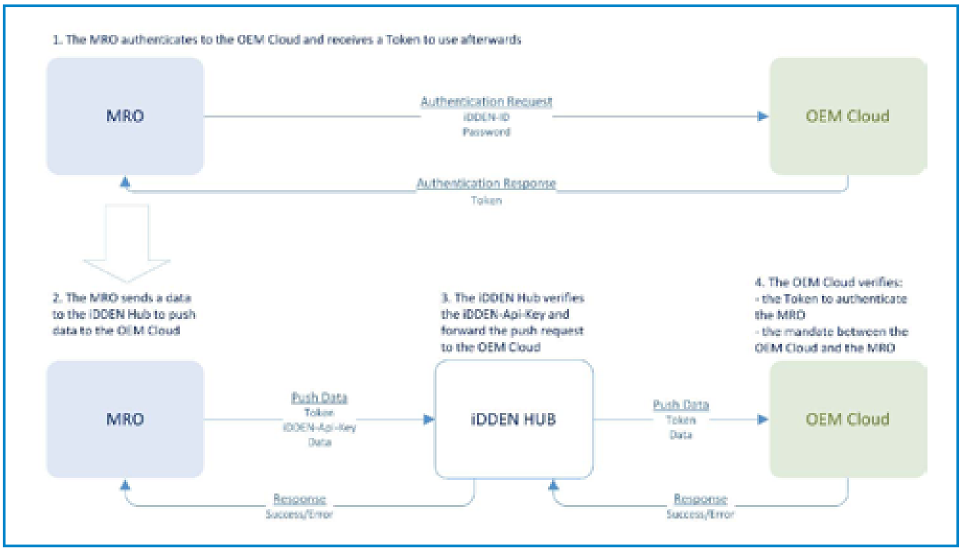

Figure 4. Send data from MRO to OEM Cloud.

List of references

ICAR-ADE project on GitHub: *https://github.com/adewg/ICAR*

iDDEN Homepage: *https://www.idden.org/*

Collecting, integrating, harmonizing and connecting data from dairy farms: the US Dairy Brain project experience

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Abstract

Modern dairy farms in the US generate vast amounts of data, and with constantly emerging new technologies and implementations, the frequency, diversity, and sheer quantity of these data are increasing. While each source of data can be valuable on its own, the integration of data from different on-farm sources offers a worthwhile opportunity to add significant value to the processes of farm management and decision-making. While this diversity in data collection platforms can be beneficial to the farmer, and to the adoption of automation procedures in general, this diversity also complicates the integration of data from all of these different systems. There is currently no standardization of data organization from a single source (e.g., milking parlor data), meaning that successful integration of data at a large scale requires that each individual source x vendor combination has a unique standardization process to make the data interchangeable and generically available to analysis algorithms.

As part of the University of Wisconsin Dairy Brain project, we are developing an Agricultural Data Hub (AgDH) to do just that. The AgDH is a system which obtains data from on-farm sources and implements a variety of parsing scripts, each one designed to handle the translation of data from one source x vendor combination into a source-specific (but vendor-generic) format. This standardized data structure is then stored and organized in a way that reflects the relationships and interdependencies between different on-farm data sources, facilitating the integration of on-farm data sources, and making the data available for further analysis.

The AgDH is being implemented using an extensible Apache Airflow system of Directed Acyclic Graphs defining a library of workflows or sequence of instructions that orchestrate container-based standardization algorithms and Structured Query Language based data storage along with a data-serving Application Programming Interface endpoint that makes it available to the analytical services further down the value chain.

Keywords: Dairy brain, agricultural data hub, data integration.

Introduction

Dairy farms are data rich but analysis poor because their data streams reside in isolated silos. Insights from data integrated deployed to dairy farms can make large strides in farm efficiency and profitability (Cabrera *et al.*, 2020). Although there are some promising emerging technologies such as Connecterra (2019), JoinData (2020), Idden (2020), the University of Wisconsin-Madison Dairy Brain project (Ferris *et al.*, 2020), among others; the dairy production sector, in general, has been slow to adopt data integrative technologies.

Analytical tools that utilize these integrated data can improve profitability, sustainability, and resilience of farms (Lovarelli *et al.*, 2020). Analyses from integrated data can bring novel insights that are not realized when using only one source of data. These become increasingly important as we move up in the tools' hierarchical level from simple descriptive dashboards to involved predictive simulations to highly sophisticated prescriptive models. Integrated data analysis, also, allows us to envision what would be the unintended consequences of a management change in one area of management to another distant management area, something that is not normally in the radar of the decision makers.

Therefore, as part of the Dairy Brain project, we are developing a framework to connect dairy farm data from various sources and make the data interchangeable and integratable in preparation for downstream analysis, the Agricultural Data Hub (AgDH; Ferris *et al.*, 2020).

The Dairy Brain Agricultural Data Hub (AgDH)

The AgDH collects, cleans, and integrates dairy farm data into a centralized data hub, and makes them accessible to the Dairy Brain analytical modules. More specifically, it involves 5 critical steps: (1) Accessing, (2) Decoding, (3) Cleaning, (4) Homogenization, and (5) Integration (Figure 1).

In brief, data from different sources and of different types (e.g., feed, milk, and health) are collected on-site from standard outputs from different farm software packages and uploaded periodically into the AgDH using minimal software installation on a farm computer (1). Data are then parsed and loaded into a database in a relatively intact native format (2). Next, data are cleaned by verifying validity and duplication (3). Following, same data types from different software are transformed and homogenized to a common format (4). Homogeneous data are then integrated into a data warehouse (5) where they can be accessed via a secure and authenticated web Application Programming Interface (API).

Accessing

The most advanced software components could be cloud-based and offer access to data via API's, but this is still rare in dairy production systems. More often, the data is collected by different systems produced by different companies, and is only available by accessing files stored on a local computer located at the farm. A client process installed in a computer at the farm detects new data files from the systems and triggers the transfer of those files via the internet into a centralized set of servers that host the reminder of the AgDH functionality.

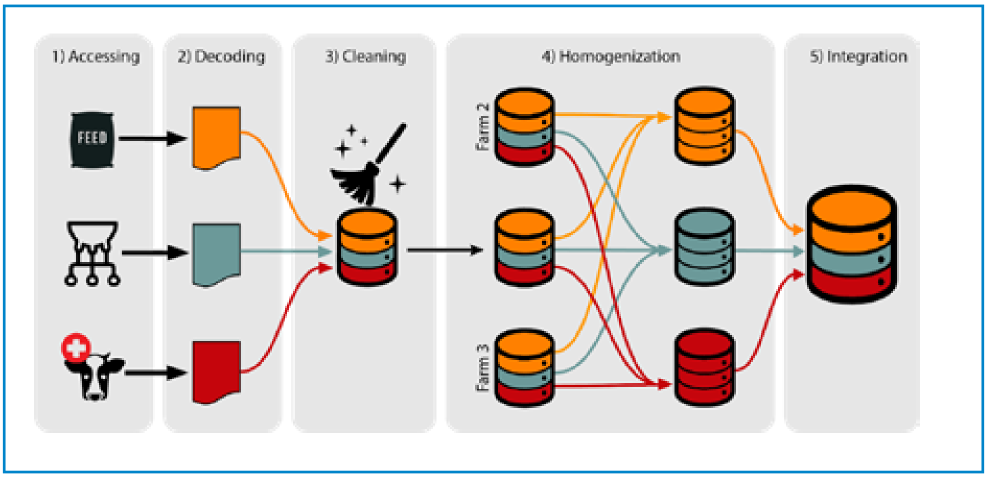

Figure 1. The Agricultural Data Hub (AgDH) framework to integrate dairy farm data.

Decoding

Accessible data are extracted from its raw source and transferred to a more readily available format for easier processing, i.e., ingested. On-farm data sources tend to vary greatly on their level of machine readability due to the fact that existing farm software can output data in many diverse formats depending on vendors, software, and farms, creating a large matrix of data readability type combinations that need to be handled by the ingestion process. Thus, unique routines need to be developed for each data source to handle its peculiarities, which is labor-intense and time consuming. These onerous tasks are alleviated by a modular framework with a flexible approach for parsing scripts that extract and ingest the data. Although there is still a need for custom language for each new software, our framework allows scripts to be combined to assemble a custom ingest pipeline for each farm.

Cleaning

Data used by the system have to meet certain quality standards. Quality ensures the integrity and tractability of the data. Integrity signifies the validity of the data. The first step to guarantee integrity is enforcing data type on the data input replacing data that do not conform with the predefined data type with a null value. Then, exception handling is used to harmonize different null representations that might exist in different systems. Then, a process checks validity of the values by, first, comparing the values against a list of valid entries and then, by applying logic filters such as hard boundary checks, reasonable physical bounds, or mathematical reasoning to identify inconsistent observations. Data deemed invalid are handled with care and transparency following a moldable logic decision tree to determine the level of invalidation (observation, row, or dataset) and documenting the process for human review.

Tractability ensures that any data element and derivative data products can be traced back to its original source and recreated. This is accomplished by capturing every step detail in the data transformation pathway by following a data lineage or data provenance approach and recurring to the use of metadata documents that describe the original source and form of the data. The data lineage begins identifying the farm, the software details, and the format of the data source. Each iteration of the ingestion process receives a unique identifier, which is attached to each entry made or updated from that iteration together with any logs or messaging from the ingestion iteration. These allow to link each data element to a single instance configuration and therefore recall the full ingest process to its original source.

Homogenization

Data follows a horizontal integration that involves identifying and extracting data commonalities among different software that record the same type of data. Hence, it requires standardization of units, terminology, types of measures, intervals, and other relevant details among input data. This process stores and serves standardized data for each farm regardless of the farm specific data collection system. Homogenization is challenging and complex as dairy data is highly heterogeneous and is collected by a number of dissimilar systems.

Integration

Data from different sources are connected following a process of record linkage that involves developing a pipeline to map records from one data source with those from other sources. Since each record from each system does not usually have a global unique key that links the record to a single organism, data connection among systems is complex. Hence, connecting data relies on a combination of linking variables.

With large differences by countries, regions, and even farms, dairy animals are issued a unique identifiable number. In some places, this number could comply with an official government issued number that could or could not be used by the software recording systems of the farm. Most normally, individual animal identifiers in a farm are multiple and likely inconsistently used among data collection systems. Therefore entity match is a critical step for data connections.

Another issue arises from the fact that data on dairy farms resides at different levels of aggregation. Dairy farms have data collected at the individual animal level such as daily milking, data at the group level such as pen feed consumption, and at the herd level such as milk bulk tank composition. Thus, data relationships need to be inferred using yet other data sources. For example, the details of a feeding event from the feeding recording software can be linked to a pen of animals from the event tracking software by using unique animal identifiers and pen allocation. This variation in data available forces aggregated analyses such as averages reducing the tractability of individual animal information and subsequent precision.

Automation and Deployment of the AgDH

We rely on a workflow automation system called Apache Airflow (Airflow, 2020), in which we define a library of workflows or sequence of instructions defined as Directed Acyclic Graphs (DAG) that orchestrate container-based standardization algorithms and Structured Query Language (SQL) based data storage. These DAGs are well suited for diverse data, as the ones encountered on dairy farms, as they can be flexible, dynamic, and modular. All previously defined steps of the AgDH workflow are controlled by DAGs. The automated system retries failed actions and generates alerts and messages if issues persist. The vision is to make the integrated data accessible through API endpoints hosted by the AgDH service. This service will be accessed via secure https connections, which will retrieve JavaScript Object Notation (JSON), Comma Separated Values (CSV), or eXtensible Markup Language (XLM) outputs. The first and main data consumer will be the analytical services of the Dairy Brain, but the system will be prepared and open to serve other research, industry, or consultant groups.

Integrated Decision Support Systems

The vision for the Dairy Brain is a real-time analytical engine capable of performing longitudinal historical analyses and forecasting the future from past information in a continuous loop grounded on data provided by the AgDH. We categorize our models as those descriptive like summary dashboards that show the current situation and might include some simple calculations. A number of our tools will include predictive or forecasting capabilities and will be updated continuously. The most advanced models are conceptualized as prescriptive tools that will provide suggestions, mostly from optimizations, of the best course of action.

Acknowledgement

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List of references

Airflow, 2020. The Apache Software Foundation. *http://airflow.apache.org/* (accessed 5 November 2020).

Cabrera, V.E., J.A. Barrientos, H. Delgado and L. Fadul-Pacheco. 2020. Real-time continuous decision making using big data on dairy farms. Journal of Dairy Science 103:3856–3866.

Connecterra, 2019. Connecterra. *https://www.connecterra.io/* (accessed 14 September 2020).

Ferris, M.C., Christensen, A., Wangen, S.R., 2020. Symposium review: Dairy Brain—Informing decisions on dairy farms using data analytics. J. Dairy Sci. 103, 3874–3881.

Idden, 2020. International Dairy Data Exchange Network. https://www.idden.org (accessed 4 November 2020).

JoinData, 2020. Join Data. *https://join-data.nl/en/* (accessed 4 November 2020).

Lovarelli, D., Bacenetti, J., Guarino, M., 2020. A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production? J. Clean. Prod. 262, 121409.

An approach to coordinating and encouraging investment in phenotypes

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Abstract

Performance records have always been the critical "raw material" for genetic improvement. This is even more the case in the genomics era, when genomic relationships enable leveraging of phenotypic data across large numbers of animals that may not be recorded at all. In extensive industries such as beef cattle and sheep, performance recording has traditionally been conducted by individual seedstock enterprises, with the volume and quality (in terms of trait coverage and identification of fixed effects) of data varying widely within (and between breeds).

Genomics enables leveraging of the recording effort, but if that effort is not rewarded, there is a risk that breeders will withdraw that effort, leading to decline in accuracy of breeding values. Addressing this coordination problem requires a mechanism(s) for cost-sharing, which needs to address diversity in quality of data (which is linked to contribution to accuracy) and cost of recording. In addition, recording effort may be augmented by government or industry contributions, particularly for hard-to-measure or "future" traits.

A simple model is presented that equitably and efficiently shares costs and rewards of recording across multiple players. The model accounts for diversity in recording cost and data quality, and provides a framework for optimising pooled investment in novel traits, or traits for which market signals are imperfect or absent.

Keywords: Genomic reference, recording, costs, coordination.

Introduction

The reference population is the critical core or foundation for implementation of genomic selection (Goddard and Hayes, 2009). This can be considered as an extension of the fundamental requirement for performance records for genetic evaluation and subsequent selection, but with the added dimension that a genomic reference population can be leveraged to a greater extent than performance records can be under simple BLUP evaluation.

By leverage, we mean the ability to use information contained in the reference in evaluation of other animals, in particular those without their own performance records. Within a single breeding unit or organisation such leveraging is effectively an extension of the standard principle of focussing recording effort in a nucleus, from which genetic improvement is disseminated throughout the tiers of multiplication and commercial production (Bichard, 1971). In such cases the transition from BLUP-based to genomic evaluation may not necessitate any significant organisational or investment modification: relevant traits will be recorded in a small component of the overall population, and genotyping may be used primarily to achieve increases in accuracy of estimated breeding values and to extract more value from recording hard-to-measure traits.

In the situation typical of beef cattle and sheep breeding and production, and especially so in countries with larger populations of these species, where there are large numbers of breeding units and (typically) larger numbers of commercial production enterprises, this relative simplicity may not apply. Here, the recording efforts of what may be a small number of enterprises can be “harvested” by many others, both breeders (enterprises selling bulls or rams) and commercial producers. If this harvesting erodes competitive advantage for the enterprises that do record, incentive to continue recording will likely decline, leading to loss of accuracy in genomic prediction. Such harvesting is a form of “free-riding”, and the risk it poses suggests that some mechanism(s) for sharing the cost of the reference should be developed.

Cost and accuracy of the reference

The simplest model of a reference population would be a single herd (or flock) where recording is practiced for the objective traits, and costs can be clearly defined, and some number of enterprises “draw on” the reference, by genotyping. Here, the accuracy generated and costs incurred would be simple to determine, and the costs could be shared simply by a levy on genotyping, equal to the reference cost divided by the known or estimated number of genotypes sampled outside the reference.

A more typical situation would be where a number of herds (flocks) collect performance data and genotypes, potentially including specific resource herds (flocks) – who could be termed contributors. Almost invariably, there would be variation in both the accuracy generated reflecting different combinations of traits recorded by different contributors and different costs, reflecting variation in herd (flock) size, business arrangements etc.

The contributor herds (flocks) could include industry- or government-funded or assisted units, likely particularly for hard-to-measure traits. Costs for farmer-owned units may need to be estimated by survey, or in more sophisticated models by some form of tender system.

This variation in accuracy generated and cost incurred across some number of contributors makes no difference to how costs of the reference can be shared across genotyping activity, but adds a dimension of complexity in allocating any levy collected amongst those contributors to the reference.

In this situation, we have:

- A range of contributors, varying in the acuracy their records generate when genotyping is added, and the cost of the accuracy they contribute, and
- Some number of “drawers”, who will also vary in the accuracy they obtain via genotyping, but likely little or no variation in genotyping cost

For each agent (contributor or drawer) we can in principle estimate (ex ante) or determine (ex post) the combination accuracy obtained and cost incurred, and hence for each case, the return on investment, as accuracy/cost. This can form the basis of determining equitable levies (charged to drawers) and rebates (distributed to contributors).

Equalising return on investment via levies and rebates

Costs can be equitably shared across contributors and drawers provided the following are known:

- Cost of reference ie amount to be collected [$cost_{ref}$]
- Accuracy of genomic breeding values for reference animals [acc_{ref}]
- Accuracy of genomic breeding values for genotype-only animals [acc_{go}]
- Costs of genotyping at each level (ie can be different) [$cost_{ref}$ & $cost_{go}$]
- Expected numbers of animals genotyped in the two categories [n_{ref} & n_{go}]

The levy to be imposed on genotypes is calculated as:

$$\text{Levy} = [(acc_{go} \times cost_{ref}) - (acc_{ref} \times cost_{go})] / [acc_{ref} + ((n_{go}/n_{ref}) \times acc_{go})] \qquad (1)$$

and the rebate applied to reference data contributions is calculated as:

$$\text{Rebate} = \text{Levy} \times (n_{go}/n_{ref}) \qquad (2)$$

The application of this simple approach can be illustrated with an example. This is based on beef cattle in Australia, and assuming three categories of contributors and one of drawers (Table 1). The 3 categories of contributors reflect "research-level" recording – which might involve some industry or government assistance to record a trait such as individual feed intake, farms where all traits that can be readily recorded are, and farms recording only minimal records (such as weaning weight).

Table 1: A simple example of the cost-sharing approach

Parameter	Contributors			Drawers
	A	B	C	
Number of animals	150	450	500	12,500
Accuracy generated or obtained	0.55	0.38	0.20	0.25
Cost of phenotyping	$143	$55	$20	
Cost of genotyping	$30	$30	$30	$30
Levy				$3.36
Rebate	$99.60	$34.29	$28	
Final cost after levy and rebate	$73.40	$42	$29.69	$33.36
Return on investment*	0.75	0.75	0.75	0.75

*: calculated as Accuracy x 100/final cost.

Challenges for implementation

The simple example shows how costs of the reference can be shared equitably, and that under realistic conditions, the required levy can be modest. This is desirable, as the core of the approach is to share the reference costs over large numbers of animals that are only genotyped, but in practice, that number cannot be known in advance. This points to challenges for practical implementation.

The first is the number of animals that will be genotyped can either be estimated allowing the levy to be estimated for an upcoming year or other time period, or observed over a period and the retrospective number used to calculate the levy. Either way, there is likely to be some error, generating some difference in return on investment for contributors and drawers, and/or potentially some loss (or benefit) for whatever organisation is managing the levy and rebating system.

The second challenge is that the calculations involved depend on a single basis for calculating accuracy. This implies that the individuals comprising contributors and drawers can agree on some common breeding objective – such that accuracy is the accuracy for the objective. This may not be readily achieved where individuals vary widely in their goals for the breed.

The third challenge is simply to establish some coordinating mechanism: some group or process that determines the levy and rebates, communicates those, and actually collects the levy and distributes the rebates. This might be a breed association, or in situations where there is industry and/or government involvement, a genetic evaluation unit or service. No matter the situation, communication must be clear and effective – the mechanism needs to be understood and accepted.

The fourth challenge is around inclusion of traits for which there is no current market value. An example is methane output – in some countries, absence of any market price means that consideration of inclusion of methane emission in the objective involves more uncertainty than inclusion of a trait like sale weight. This uncertainty, or more precisely the resulting under-investment in recording, may be alleviated by some form of industry and/or government investment. If this is quantified, the approach used here can be extended to include such traits, with their cost of recording adjusted to reflect the investment, but the decision on the economic weight to be applied to such traits must be made in such a way that it is accepted by the contributors and drawers.

Two final practical challenges for implementation can be identified. The first is that the value of performance records and genotypes collected on reference animals varies through time: basically, there are diminishing returns to additional records (Goddard and Hayes, 2009). Strictly, this should lead to rebates declining as more reference animals are recorded – this can be handled mathematically in real-time, but in practice, the rebating mechanism may need to be simplified such that the first n records receive rebate x, the next n records some fraction of x, the next n records some smaller fraction of x, and so on. This would require very clear communication. The second is that the mechanism requires that the coordinating group – for example, a breed association – acts as a bank, particularly in making payments to the contributors. It may be simpler to avoid such payments by adjusting all levies and rebates to only addressing the recording costs not considered as “normal costs of business” – for example, focussing on the recording of hard to measure traits, but this does not address variation in the level of recording of traits that can be normally done on farm without expensive equipment. It is hard to avoid concluding that all costs, and hence all contributors, need to be eligible for rebates.

Implications

The underlying message of this paper is that phenotyping, and the accuracy that it generates, is the fundamental resource of genomics (“phenotyping is king!”). Whereas in the BLUP era, the costs and benefits of phenotyping were largely respectively borne and captured by the invidual or business investing in the recording, with genomics recording generates a shared or public good (strictly, a club good). Such goods typically require some coordinating or governance mechanism, at least more than simply leaving decisions about investment in recording to the market (Ostrom, 1990). Such mechanisms can be effective over long periods and across a wide range of shared and/or co-created resources (Ostrom, 1990), but require active engagement of the participants. For breed associations, or industry- or government-supported organisations, this implies effective and ongoing communication – primarily around the breeding objective(s) and how recording generates accuracy, and around understanding the shared or common pool resource that is being managed. Organisations that cannot deliver this will almost inevitably suffer from sub-optimal investment in recording, and resulting loss of competitiveness.

List of references

Bichard, M. 1971. Dissemination of genetic improvement through a livestock industry. Animal Production 13: 401-411

Goddard, M.E., and B.J. Hayes, 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature Reviews Genetics 10: 381-391

Ostrom, E., 1990. Governing the Commons: The Evolution of Institutions for Collective Action. Cambridge, UK: Cambridge University Press.

The US Dairy Brain Project: Data integration and data applications for improved farm decision-making

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Abstract

Data pervades the dairy farming industry. However, specific data streams are most often ad-hoc and poorly linked to each other and to decision making processes. Dairy farms have embraced large and diverse technological innovations such as sensors and robotic systems, and can now stream vast amounts of data, but they have not been able to integrate all these data effectively to improve whole-farm decision making. Consequently, the benefits of the new smart dairy are not being fully realized. In order to address this, it is imperative to develop a system that can collect, integrate, manage, and analyze on- and off-farm data in real-time for practical and relevant analyses that can be used to improve on-farm decision making. Hence, we are developing a real-time, data-integrated, data-driven, continuous decision-making engine: The Dairy Brain by applying Precision Farming, Big Data analytics, and the Internet of Things. This is a trans-disciplinary research and extension project that engages multi-disciplinary scientists, dairy farmers, and industry professionals. We are using the state-of-the-art database management system from the University of Wisconsin-Madison Center for High Throughput Computing to develop our Agricultural Data Hub that connects and analyzes cow and herd data on a permanent basis. This involves cleaning and normalizing the data as well as allowing data retrieval on demand. We have a four-part strategy: (1) Create a Coordinated Innovation Network (CIN) to shape data service development; (2) Create a prototype Agricultural Data Hub (AgDH) to gather/disseminate multiple data streams relevant to dairy operations; (3) Build the Dairy Brain – a suite of analytical modules that leverages the AgDH to provide insight to the management of dairy operations and serve as an exemplar of an ecosystem of connected services; and (4) Design and execute an innovative Extension program. We illustrate our Dairy Brain concept with a practical application that predicts CM onset. The application uses machine learning algorithms to identify cows at higher risk of contracting CM seven milkings before the onset. The application integrates data from management software and data from the milking parlor. Our preliminary results indicate that our predictions are 72% accurate. Integration of more data streams and incorporating larger historical datasets will improve accuracy even further. We demonstrate that it is possible to develop integrated continuous decision-support tools. Tomorrow's dairy industry will be built on the effective capture and integration of more data streams, not fewer. This is a critical moment to develop the structures that can move the industry towards modernized data exchange. This is an ongoing innovative project that is anticipated to transform how dairy farms operate.

Keywords: Data integration, decision support tools, dairy brain.

Introduction

Dairy farms generate large amounts of data. With the adoption of new technologies these amounts of data have increased. These data are an important tool to help improve farm decision making in animal welfare, performance, and long-term sustainability of the farms (Lovarelli *et al*., 2020). Nevertheless, using data in efficient way is a complex task as it is highly heterogenous, different data sources are normally independent from each other (Wolfert *et al*., 2017) and the analysis of big data is a challenging task (Morota *et al*., 2018).

As a result, daily management is a difficult task since data integration of different data sources (i.e., production, feeding, health, etc.) is lacking (Koltes *et al*., 2019; Cockburn, 2020; Cabrera and Fadul-Pacheco, 2021). It has been recognized that collection, aggregation and analysis of data will transform the way decision making is done, making farms realize large gains in productivity, efficiency and profitability (Bronson and Knezevic, 2016; Newton *et al*., 2020) and providing farmers a better understanding of the past, present and future of the farm (Lioutas *et al*., 2019).

Data integration is crucial to improve data quality and algorithm performance (Hogeveen, *et al*., 2010; Menéndez González *et al*., 2010) and it has been identified to be an important component to the decision-making process on dairy farms (Eastwood *et al*., 2017; Dairy Brain, 2020a). In addition, automatized data integration is recognized as a tool to give holistic advice on management practices (Gengler, 2019).

Knowing the potential benefits of data integration, it is imperative to develop a system that can collect, integrate, manage, and analyze on- and off-farm data in real-time for practical and relevant analyses that can be used to improve on-farm decision making. Hence, we are developing a real-time, data-integrated, data-driven, continuous decision-making engine: The Dairy Brain by applying Precision Farming, Big Data analytics, and the Internet of Things. This is a trans-disciplinary research and extension project that engages multi-disciplinary scientists, dairy farmers, and industry professionals.

The Dairy Brain

The Dairy Brain project as a continuous decision-making engine as described on Figure 1, first data is collected at the farm, then farm data from multiple sources is transferred to a central location, where data is transformed and homogenized to then apply analytics to it and finally access to the developed analytics farmer via web interface. It consist of a four-part strategy: 1) Create a Coordinated Innovation Network (CIN) to shape data service development; 2) Create a prototype Agricultural Data Hub (*AgDH*) to gather/disseminate multiple data streams relevant to dairy operations; 3) Build the *Dairy Brain* – a suite of analytical modules that leverages the aggregation service and available data to provide insight to the management of dairy operations and serve as an exemplar of an ecosystem of connected services and 4) DataMoney: an innovative Extension program.

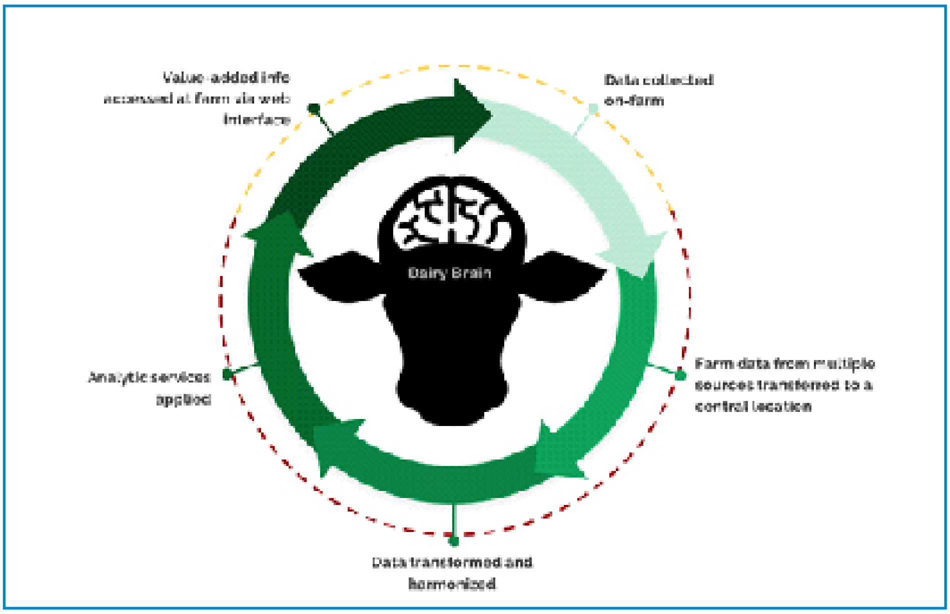

Figure 1. Dairy Brain project phases.

Create a Coordinated Innovation Network (CIN)

A Coordinated Innovation Network (CIN) is, in general, "a large community of stakeholders that addresses bottlenecks in critical areas by bringing together experts from different disciplines and domains to identify innovative and synergistic solutions" (USDA, 2021). In the context of the Dairy Brain project, the *bottlenecks* make reference to the data management challenges in the dairy industry. Therefore, the CIN of the dairy brain project is a network of stakeholders from the dairy industry (i.e., farmers, industry, researchers and extension professionals) shaping the structure of the Dairy Brain project itself and serving as a basis for broader implementation of data services and standards.

The main roles of the CIN are to raise awareness, facilitate the exchange of opinions and generate discussion and crate guidelines about data management in the dairy industry. To start the discussion process, the CIN published five opinion articles that covered some data management challenges that were identified by the CIN members and the Dairy Brain team.

The first was an introductory article to the Dairy Brain project and the roles of the CIN (Dairy Brain, 2020a). This was followed by a discussion about data security and data privacy (Dairy Brain, 2020b). The next article was about data collection and data standardization (Dairy Brain, 2020c). Then, another article was the adoption of decision support tools (Dairy Brain, 2020d), and the last opinion article was related to the value added of data to help improve management practices at the farm (Dairy Brain, 2020e). These opinion articles are the foundation for more technical and scientific articles: the CIN design documents, which are under development. To learn more about the perception of a number of key topics related to data challenges in the dairy industry, we are currently conducting a survey at DairyBrainSurvey (*https://uwmadison.co1.qualtrics.com/jfe/form/SV_0HDBzawvvRygQVE*).

Create an agricultural data hub (AgDH)

Farms have different data sources that are normally not connected to each other, to address this issue, the main objective of the agricultural data hub (AgDH) is to collect, integrate and homogenize the dairy farm data so that all these data can be used by the DairyBrain to develop decision support tools. Data integration through the AgDH consist in five main steps: 1) Assessing; 2) Decoding; 3) Cleaning; 4) Homogenization and 5) Integration. The AgDH is built in a modular fashion under the consideration that these five steps will be automatized with minimal or no human supervision. Once the data is fully processed, these integrated data will be accessible through a set of application programming interfaces or API's endpoints hosted by the AgDH service. It is important to mention that these endpoints will be secure so that only individuals authorized by the farm will have access to them. Also, the API's will make the data available to the analytical module offered though the DairyBrain portion of our project.

Build the DairyBrain

The actual Dairy Brain portion of the project is a data-driven engine of decision making to advance analytics and dairy farm sustainability. We categorize our models as descriptive, predictive and prescriptive. The descriptive models are normally for short-term decision making, and they are mainly visualizations (i.e., dashboards). They might also include some simple calculations, as for example, feed efficiency. Even though feed efficiency is based on simple algorithms, it requires data from different data sources which means it needs data integration.

Predictive models include performance projections to the future. One example of this type of models is selection of genetic traits to reduce the incidence of clinical mastitis (Fadul-Pacheco *et al.*, 2021). And, finally, the most advanced, the prescriptive models are those that provide suggestions, mostly from optimization, of the best course of action. Among these models we can find the continuous nutritional accuracy (Barrientos-Blanco *et al.*, 2020) that can help provide accurate diets to cows as an effective strategy to control cost, increase revenue, enhance feed efficiency and reduce environmental impact. It is important to keep in mind that all these models need to be adjusted with continuous data. Data integration from multiple data sources though the AgDH and the application of advance analytics is an example of added value to the raw and disparate data.

DataMoney: An innovative extension program

The objective of the extension program of the Dairy Brain project is to increase farmers, advisors, and county educators' awareness on data use and management at the farm level. More precisely, demonstrate the benefit of data integration and the appropriate use of decision support tools. We have developed a program called "DataMoney." The program consists in working individually with farm teams in particular farms. The first step is to perform an assessment of the data usage at the farm and, according to the farm priorities, needs assessment, and farm data availability, work collaborative developing farm-specific decision support tools. This process will spark farmer and farm workers interest in the use and application of farm data for better decision making.

List of references

Barrientos-Blanco, J.A., H. White, R.D. Shaver, and V.E. Cabrera. 2020. Improving nutritional accuracy and economics through a multiple ration-grouping strategy. Journal of Dairy Science 103:3774–3785. doi: 10.3168/jds.2019-17608.

Bronson, K., and I. Knezevic. 2016. Big Data in food and agriculture:. Big Data and Society. doi:10.1177/2053951716648174.

Cabrera, V.E., and L. Fadul-Pacheco. 2021. Future of dairy farming from the Dairy Brain perspective: Data integration, analytics, and applications. International Dairy Journal 105069. doi:10.1016/j.idairyj.2021.105069.

Cockburn, M. 2020. Review: Application and Prospective Discussion of Machine Learning for the Management of Dairy Farms. Animals 10:1690. doi:10.3390/ani10091690.

Dairy Brain. 2020a. Help Us Help You Make Better Use of Dairy Data. Accessed September 24, 2020. *https://hoards.com/article-27981-help-us-help-you-make-better-use-of-dairy-data.html*.

Dairy Brain. 2020b. Farming out Data-Driven Decisions. Accessed September 24, 2020. *https://hoards.com/article-27982-farming-out-data-driven-decisions.html*.

Dairy Brain. 2020c. Data: Think Big, but Start Small. Accessed September 24, 2020. *https://hoards.com/article-27983-data-think-big-but-start-small.html*.

Dairy Brain. 2020d. Making Data Work on the Farm. Accessed September 24, 2020. *https://hoards.com/article-27984-making-data-work-on—the-farm.html*.

Dairy Brain. 2020e. Creating Value from Data. Accessed September 24, 2020. *https://hoards.com/article-27985-creating-value-from-data.html*.

Eastwood, C.R., B.T. Dela Rue, and D.I. Gray. 2017. Using a ‘network of practice’ approach to match grazing decision-support system design with farmer practice. Anim. Prod. Sci. 57:1536. doi:10.1071/AN16465.

Fadul-Pacheco, L., H. Delgado, and V.E. Cabrera. 2021. Exploring machine learning algorithms for early prediction of clinical mastitis. International Dairy Journal 119:105051. doi:10.1016/j.idairyj.2021.105051.

Gengler, N. 2019. Symposium review: Challenges and opportunities for evaluating and using the genetic potential of dairy cattle in the new era of sensor data from automation. Journal of Dairy Science 102:5756–5763. doi:10.3168/jds.2018-15711.

Hogeveen, C., C. Kamphuis, W. Steeneveld, and H. Mollenhorst,. 2010. Sensors and Clinical Mastitis—The Quest for the Perfect Alert. Sensors 10:7991–8009.

Koltes, J.E., J.B. Cole, R. Clemmens, R.N. Dilger, L.M. Kramer, J.K. Lunney, M.E. McCue, S.D. McKay, R.G. Mateescu, B.M. Murdoch, R. Reuter, C.E. Rexroad, G.J.M. Rosa, N.V.L. Serão, S.N. White, M.J. Woodward-Greene, M. Worku, H. Zhang, and J.M. Reecy. 2019. A Vision for Development and Utilization of High-Throughput Phenotyping and Big Data Analytics in Livestock. Front. Genet. 10. doi:10.3389/fgene.2019.01197.

Lioutas, E.D., C. Charatsari, G. La Rocca, and M. De Rosa. 2019. Key questions on the use of big data in farming: An activity theory approach. NJAS - Wageningen Journal of Life Sciences 90–91:100297. doi:10.1016/j.njas.2019.04.003.

Lovarelli, D., J. Bacenetti, and M. Guarino. 2020. A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production?. Journal of Cleaner Production 262:121409. doi:10.1016/j.jclepro.2020.121409.

Menéndez González, S., A. Steiner, B. Gassner, and G. Regula. 2010. Antimicrobial use in Swiss dairy farms: Quantification and evaluation of data quality. Preventive Veterinary Medicine 95:50–63. doi:10.1016/j.prevetmed.2010.03.004.

Morota, G., R.V. Ventura, F.F. Silva, M. Koyama, and S.C. Fernando. 2018. Big data analytics and precision animal agriculture symposium: Machine learning and data mining advance predictive big data analysis in precision animal agriculture. J. Anim. Sci. 96:1540–1550. doi:10.1093/jas/sky014.

Newton, J.E., R. Nettle, and J.E. Pryce. 2020. Farming smarter with big data: Insights from the case of Australia's national dairy herd milk recording scheme. Agricultural Systems 181:102811. doi:10.1016/j.agsy.2020.102811.

USDA. 2021. Data Science for Food and Agricultural Systems. Accessed April 14, 2021. https://nifa.usda.gov/resource/dsfas-faq#_Toc61252312.

Wolfert, S., L. Ge, C. Verdouw, and M.-J. Bogaardt. 2017. Big Data in Smart Farming – A review. Agricultural Systems 153:69–80. doi:10.1016/j.agsy.2017.01.023.

Blockchain for the Dutch dairy industry

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Introduction

For most people, blockchain technology is associated with bitcoins and other cryptocurrencies: An intriguing, wild-west, fast-money, hard to understand, virtual economy, that has nothing to do with real tangible processes like the production of milk.

This is a misconception as blockchain technology brings great opportunities for the real world too. Large companies like IBM and Microsoft have become convinced about this and invested heavily in so called “Third Generation Blockchains”. These are without all the obscure “bitcoin like” features and designed specially for supply-chains.

Qlip has embraced this new technology to create a platform for the Dutch dairy Industry because it brings two big advantages for the future:

- **It helps to improve cooperation:** The technology used in a blockchain makes that you can be absolutely sure that shared data is only visible for that specific “other” stakeholder and for no-one else. In a normal central database there is always an “administrator” who can access all stored information. This unwanted feature makes dairy companies reluctant sharing data on a central platform (and thus prevents cooperation and efficiency).
- **It is a great opportunity for tracking & tracing and food provenance.** There is absolutely no possibility to tamper with data in a blockchain, without anyone (or the blockchain network itself) noticing it. This makes it the ultimate technology for food supply chains where tracking and tracing and food provenance are of the utmost importance.

Trust is essential

The only way you really can be sure that the above-mentioned advantages really exist, is by investing in knowledge. That is the only way to be able to be certain that you can trust blockchain technology. This advice is especially relevant for business people. That does not mean that business people need to learn how to program a blockchain, but you need to understand what technical concepts are implemented so you don’t have to believe that blockchain brings value, bat that you know that blockchain brings value and even more important: you understand how it brings value.

Bring value to the Dutch dairy industry

in this trust for ourselves, Qlip started to build a blockchain for raw milk. Three different dairy companies participated. Every milk collection of the participating dairy companies is stored in a blockchain. You are only able to see a milk collection if you are the delivering party or the receiving party. We experienced that this new technique brings great opportunities for the future.

Plans for the future

Our ambition is to create a new digital infrastructure for all the raw milk that is produced in Holland. With this you can make al information about milk collections available to whoever receives the milk.

This dairy blockchain could be used for several things.

- Reduction of administrative or inspection costs because all data is tamper free stored and can be provided to a inspector if you choose so.
- If a dairy producer chooses to share his production data (form the manufacturing plant) on the blockchain to, you could print a QR code on the package and so give the end consumer insight in what milk was used for his end product (i.e., infant nutrition for export markets)
- When milk is exchanged it is much easier to be sure that it is Dutch milk or foreign milk to see what type of milk it is, what the lab results are etc.

How to run milk recording effectively- case Finland

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Abstract

In Finland there is one milk recording organisation called ProAgria. There are currently 4,009 recorded herds (71% of all herds) and 211,369 cows (83% of all cows). Milk recording is mostly done by farmers themselves, distribution between methods are A= 2 %, B= 93 % and C= 5 %. There is effective network around milk recording. ProAgria is leading and organizing it, analysing of samples is done by the dairies and calculation and reporting to customers is done by Mtech Ltd.

Keywords: Milk recording, network.

Introduction

ProAgria organises the milk recording in Finland. Association of ProAgria Centres is coordinating and supporting a network consisting of ProAgria area centres, Mtech, dairy laboratories and customer service. Figure 1 illustrates the network.

Running milk recording in practise

In practice running milk recording in a network means that there must be someone who keeps the strings in their hands. This person works in the Association of ProAgria Centres. She leads the development, and the customer service and takes care that everybody in the network is aware of new practices and tools in milk recording.

Test milking

As stated, most test milkings (93%) are done by farmers. There are guidelines and instructions for them how test milking should be done. If there are challenges with test milking, technical expert can consult the farmer. In the future, standard operation procedures (SOPs) will be created for test milking and they are delivered to customers.

Yield calculation and reporting

There are two major sources to develop calculation and reporting: feedback from customers and ICAR guidelines. Feedback is collected via customer service, chat, email and experts from ProAgria Centres. We also have good connections to ICAR, because experts from Mtech and ProAgria have members of ICAR working groups and sub-committees. Every third week, there is a meeting with ProAgria and Mtech where all the feedback and expert comments are reviewed and decisions are made on the

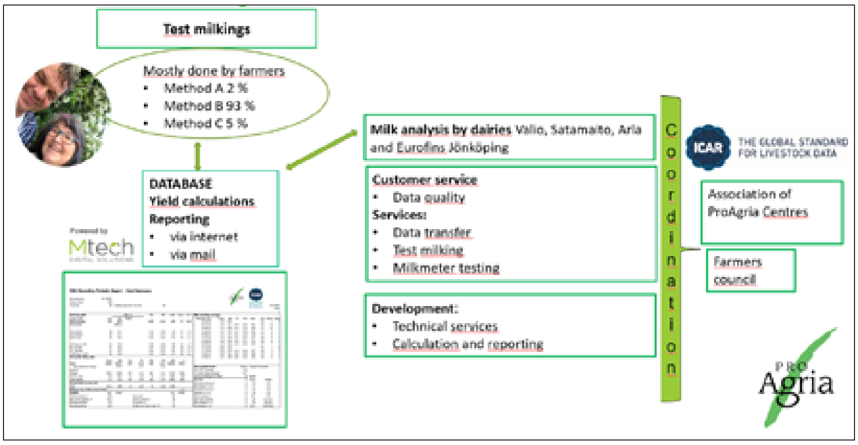

Figure 1. Milk recording network in Finland.

further handling of the cases. Yield calculation, reporting and the related development takes five person-years from Mtech and 0,2 person-years from ProAgria.

Analyzing milk samples

Milk analysis is handled by dairies. There are three laboratories in Finland and about 1,2 million samples analyzed per year. There is one meeting per year between ProAgria and every laboratory, and additional meetings whenever necessary. If there are quality problems in samples, that information goes from the laboratory to the customer service and customer service contacts the customer and tells him what the problem is and what should be done better next sampling. If the laboratory needs development, then ProAgria and laboratory staff will have a discussion.

Customer service

Customer service assists the farmers, stakeholders and ProAgria experts. Service is available via phone, email, and chat. The Customer service develops data quality together with area centres and contacts customers that have low data quality points. The Customer service can also for example take remote access to an AMS and help with data transfer when needed. There is three person-years working in the customer service.

Services for farmers

Test milking

Farmer can buy service for test milking so that they can outsource it partly or completely to a ProAgria technician. These services are organized by ProAgria centres and the Association of ProAgria Centres is responsible for the education of experts and service development. 197 customers purchased this service in 2020 and 1.4 person-years were used.

Data transfer and recording

Farmers can buy a service to record their data to the database. An expert will then take the data directly from the milking system or the farmer can send data by mail, email or via WhatsApp. This service is organised by three area centres and there have been 352 customers in 2020, with 1 person-year used on this.

Milk meter testing

All portable milk meters are tested in one testing place, in Riihimäki. Meters are transported by mail to the testing place and back to customer. In 2020 there have been 730 customers and two person-years have been used. Stationary AMS and parlour meters are mostly tested by manufacturer servicemen.

Conclusions

The ProAgria milk recording in Finland is running effectively. We spend 13,2 person-years running the system, also including its development. The system is working satisfactorily, but there is need for make it more linear from the customer point of view. The number of recorded herds will decrease in the coming years and there will be lot of new tools that are offered to the farmers from other operators. In the future, we must have more time to develop better services for milk recording.

Performance recording and genetic evaluation of *Equus genus*

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Abstract

The ICAR Board based on interest of Breeding Organizations (BO) agreed to include performance recording and genetic evaluation of *Equus genus* into its programs. The ICAR Equid Ad Hoc Advisory Committee (Equid AC) was proposed by ICAR with the objectives to evaluate the benefits that ICAR can provide in the fields of performance recording and genetic evaluation to current ICAR members and other equids' organisations. The priorities for the Equid AC are to establish a list of worldwide contacts involved in Equid recording and genetic evaluation and to liaise with ICAR governing and technical bodies to identify the range of activities that ICAR could potentially offer to support recording and genetic evaluation of equids. In order to achieve these objectives, a Survey to equid stakeholders have been proposed to determine the scope of current and potential future activities that could be supported by ICAR in the future. The Survey has been carried out by the means of a questionnaire including basic information and specific questions. The emphasis has been placed on future activities, namely parentage verification based on SNP markers. Members of Equid BO have been invited to fulfil the questionnaire through online application developed for this purpose available at the following link: https://rodica.bf.uni-lj.si/EquidAC. The Survey response is in ongoing phase and so far 33 breeding organizations have responded. A total of 149,334 animals were included in the breeding work of these organisations, comprising approximately 58,997 broodmares and 5,472 stallions. The results are encouraging and show the great interest in harmonization under ICAR umbrella for almost all traits. To evaluate feedback from Equid interests, a comprehensive report with recommendations will be prepared for consideration by the ICAR Board.

Keywords: Equus genus, Equid AC, Breeding organisations, Survey, Online application.

Introduction

Performance recording in horses and donkeys is not yet standardised worldwide. Therefore the ICAR Board, recognizing the interest of Breeding Organisations (BO), agreed to include performance recording and genetic evaluation of *Equus genus* into its programs. The first step was to establish the ICAR Equid Ad Hoc Advisory Committee (Equid AC) following Terms of Reference (ToR) with the objectives to evaluate the benefits that ICAR could provide in the fields of performance recording and genetic evaluation to the current members of ICAR and other organisations interested in the *Equus genus*. The following priorities of the Equid AC have been pointed:

1. Establishing a comprehensive list of worldwide contacts with an interest in Equid recording and genetic evaluation.
2. identifying the range of activities that ICAR could potentially provide to support equid stakeholders.
3. Conducting a Survey of Equid interests.
4. Evaluation of feedback from equid stakeholders by preparing a comprehensive report with recommendations for consideration by the ICAR.

In order to achieve these objectives and get feedback from the worldwide BO, the Equid AC developed online data collection through Survey. The questionnaire concern interest in performance recording, parentage verification, and genetic evaluation of Equids. The main breeds of interest are local (indigenous) horse and donkey breeds which lost economic value over the last century.

Material and methods

The Survey was developed with the assistance of all Equid AC members. The questionnaire included basic information (BO contact data, country, city, address, e-mail, contact details of organization representative person) and specific questions including information about the breed (breed and breed type, number of breeders, number of equines, number of breeding animals - broodmares and stallions, and number of foals), main and optional use of the breed, information about parentage verification and parentage verification method, selection criteria (main and additional traits according to breeding programme), and future plans and interests (parentage verification, collaboration with ICAR, potential traits of interest and future activities). The Survey has been hosted at the University of Ljubljana, Biotechnical Faculty, Department of Animal Science and accessed via link *https://rodica.bf.uni-lj.si/EquidAC*. A link to an online questionnaire was distributed via e-mail lists from BO. The Survey was launched in September 2019 and is in ongoing phase. At the beginning, the response was lower than expected because of several reasons such as English language (many people are not familiar with foreign language), lack of knowledge, lack of relevant contacts for each breeding organization – often volunteer / not professionals in breeding organisations). For the last, the European Regional Focal Point for Animal Genetic Resources i.e. National Coordinators were involved to obtain relevant BO contacts. The feedback was positive and questionnaire was sent to many BO. Since the most of the contacted persons need to be reminded several times before responding, the automatic reminder was generated under the online application.

Statistical package SAS (SAS Inst. Inc., 2009) was used to analyse data and to perform descriptive statistics and graphs.

So far 33 BO have responded to the online Survey (Figure 1). Most of them bred warm-blood horse breeds (around 50%). The rest includes cold-blood breeds (25%) followed by pony and small horses (13%).

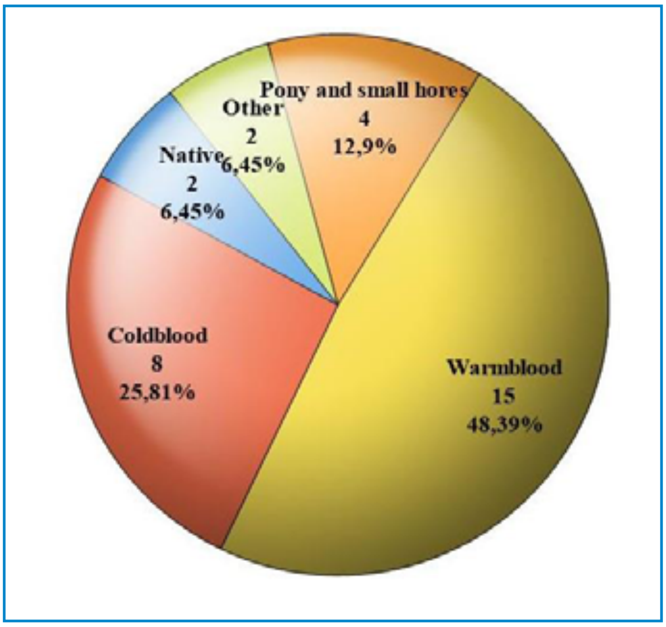

Figure 1. Distribution of breed type by breeding organisations

Results and discussion

All together around 31K breeders have participated in the work of BO. A total of 150K animals are included in these organisations, comprising about 59K broodmares and 5,500 stallions (Table 1).

Main use of the breed refers to the most important traits according to the economic use and breeding objectives (riding school, leisure, sport driving, agricultural work, milk production, etc.). For majority of BO, sport riding (33%) is the most important trait, followed by leisure, leisure riding, agriculture work, meat production, and leisure driving (Figure 2a). The questionnaire also provides possibility to select optional use of breed. The most important optional use of the breed is leisure driving (around 30%), followed by leisure riding, sport riding, meat production, and tourism (Figure 2b).

Most BO perform parentage verification for all registered animals (Table 2), however in some no parentage verification is used. Parentage verification method is based on the ISAG standard microsatellite (MS) test (62%) and own MS test (38%).

Table 1. Number of breeders, horses, broodmares, stallions, and foals.

Item	Mean	Std	Min	Max	Sum
Members/ breeders	1068.0	1,761.3	1.0	9,000.0	30,972.0
Horses	5,333.4	13,294.3	100.0	70,000.0	149,334.0
Broodmares	1,843.7	4,034.3	20.00	22,738.0	58,997.0
Stallions	171.0	247.6	4.0	1000.0	5,472.0
Foals per year	895.2	2,477.3	15.0	13,652.0	26,857.0

Table 2. Group of animals used for parentage verification by Breeding Organisation (BO).

Parentage verification	N1	%
Not in use	5	16.1
Breeding stallions	4	12.9
Breeding animals	2	6.5
All registered animals	20	64.5

[1] N= number of BO.

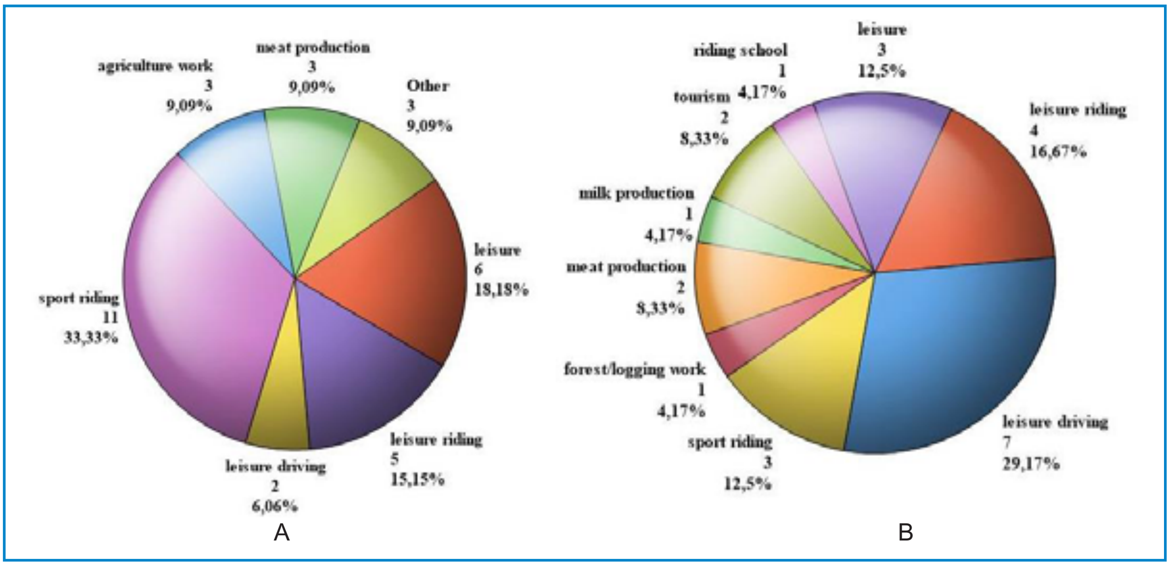

Figure 2a and 2b. Frequency distribution of traits according to main and optional use of the breed.

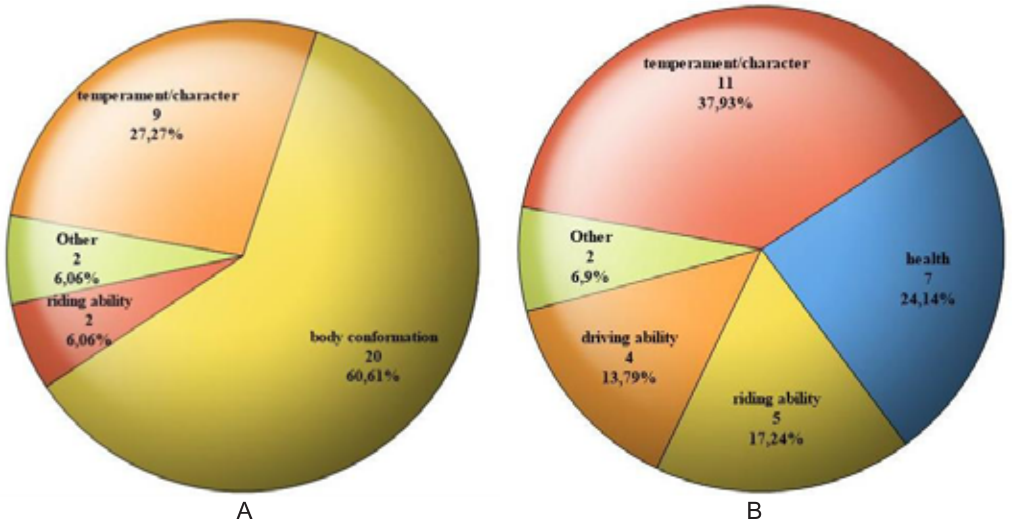

Figure 3. Frequency distribution of the most important traits (a) and additional used traits (b) from selection point of view.

Figure 3a and 3b. Frequency distribution of the most important traits (left) and additional used traits (right) from selection point of view

Table 3. Part of the population under performance recording.

Criteria	N[1]	%
Whole population	13	43.3
Animals selected for breeding	12	40.0
Part of population	3	10.0
Breeding females	1	3.3
Stallions	1	3.3

[1] N= number of BO.

Table 4. Future parentage verification method.

Future parentage verification method	N	%
ISAG standard MS test[1]	18	58.1
Breed specific MS test[2]	1	3.2
SNP based test[3]	12	38.7

[1] ISAG – International Society for Animal Genetics.
[2] MS – Microsatellites.
[3] SNP – Single Nucleotide Polymorphism.

From the selection point of view, body conformation (60%) is the most important selection trait (Figure 3a) followed by temperament/character (27%) and riding ability (6%). The additional traits important for selection purposes (Figure 3b) are temperament/character (38%), followed by health traits (24%), riding (17%), and driving ability (14%).

For selected traits, recording is performed mostly for the entire population (Table 3), followed by animals selected for breeding. In small proportion, it is used for only breeding females and stallions. Unfortunately, phenotype is still the most important criterion for selection (90%). Only 10% of BO performs genetic evaluation.

Questions regarding future plans or interests were related to parentage verification method (Table 4). It seems that genomics becomes more important in horse breeding since BO started to implement parentage verification using SNP based testing for all registered animals. Furthermore, the BOs are interested to include additional traits (body conformation and temperament/character) in the breeding program or in recording and selection in the future.

Conclusions

The results of the Survey, although not conclusive, are encouraging and show the great interest in harmonization under the CAR umbrella for almost all investigated traits. Feedback from the Survey will enable the Equid AC to determine current activities and potential future activities that might be facilitated by ICAR.

List of References

SAS Inst. Inc. 2009. SAS/STAT® 9.2 User's Guide, Cary, NC: SAS Institute Inc

Methane, energy balance and feed efficiency MIR predictions evaluation on dairy cow's population

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Abstract

Methane (CH4) is one of the major greenhouse gas (GHG) emissions. Along with other sources it is naturally produced during the rumen fermentation process of ruminants. Different factors could have an impact on CH4 quantity emitted i.e. feed, feeding system, herd management or genetic selection for efficient animals. The study aim was to compare milk MIR CH4 predictions relevance obtained with different equations based on same reference dataset but including or not fix effects (1,203 data). The CH4 models were built on SF6 tracer gas method and respiratory chambers measurements with PLS, CPPLS and GLMNET methods and considered the stage of lactation. The model performances have been statistical validated in yearly dairy data production. Pearson correlation analysis has been made with milk MIR indicators. For energy efficiency were found positive correlation with GLMNET model. Negative correlations were found between all CH4 models and blood NEFA, C18-1Cis9, Energy-Detect, KetoMIR, INSAT and positive correlation showed SCFA and MCFA and SAT. The GLMNET models showed positive correlation with EB NEL and ME. The correlations fit well with the usual metabolic effects of extreme body fat mobilization e.g. an increased concentration of NEFA, ketone bodies, long chained unsaturated fatty acids, a decreased concentration of MCFA and an extreme negative EB. The effect can be explained by reduced feed intake and rumen activity in these situations. The limits of applicability must still be defined in order to ensure the relevance of the predictions obtained, therefore in ReMissionDairy project the GLMNET model predictions are used in a production report for field consultants and advisors in order to reduce the CH4 emission and the CH4 per kg milk. To better understand the CH4 results team work with filed experts and also climatic environmental experts is necessary.

Keywords: MIR spectra, ECM, CH4, energy balance, feed efficiency, energy efficiency

Introduction

Mid infrared spectroscopy (MIR) is using the infrared light from the electromagnetic spectrum which shows specific absorption patterns when sent through a milk sample caused by frequency dependent interactions with the chemical bonds of the chemical milk components. With the help of milk MIR spectra, a wealth of information can be obtained by establishing relationships with reference methods. Since 2012 researchers in the MIR spectroscopy are working on creating predictions models based on milk MIR spectral data and dairy cow phenotypes like standard milk components, new milk MIR components like ketone bodies (Grelet C. *et al*., 2016), fatty acids (Grelet C. *et al*., 2014), minerals (Soyeurt H. *et al*., 2009) and MIR based blood components (BHB, NEFA, Glucose, IBF1, Insuline, Calcium) (Dale L. *et al*. 2019 and traits like energy balance (NEL and ME) (Dale L. *et al*., 2019). The current research focus is the detection of indirect quantities, such as: methane (CH4) emissions (Dehareng *et al*., 2012), furthermore Vanlierde (2019) present a set of CH4 models based on spectral data and SF6 and climatic chamber measurements. CH4 is one of the major greenhouse gas (GHG) emissions. Along with other sources it is naturally produced during the rumen fermentation process of ruminants. Different factors could have an impact on CH4 quantity emitted i.e. feed, feeding system, herd management or genetic selection for efficient animals. The eMissionCow project focused on the prediction of emission and efficiency related phenotypes and their potential applications, whereas the ReMissionDairy project is testing the estimated values for feed efficiency, energy balance and methane emissions on different pilot farms in 4 federal states of Germany. The ReMissionDairy projects aim is to develop feeding strategies to increase efficiency and to decrease emissions of CH4. The study aim was to compare milk MIR CH4 predictions relevance obtained with different equations and compare energy balance, energy efficiency and different CH4 equations based on MIR spectra predictions. The objective of this study was to evaluate different CH4 equation based on SF6 tracer gas method and respiratory chambers in order to improve the quality of the predictions obtained and choose the best model.

Material and methods

From the work at the Walloon Agricultural Center and University of Liege, Gembloux Agro Bio-Tech, in cooperation with OptiMIR/EMR partners, the Methagene Group and the European project GplusE, a CH4 equation with SF6 tracer gas method and respiratory chambers measurements was created. The spectral data set was first standardized by applying the OptiMIR/EMR method (Grelet *et al*., 2015) and pre-processed by Savitzky-Golay first derivative to remove the offset differences between samples for baseline correction, before performing Legendre polynomial modelling. To identify the main variables that were positively or negatively associated with CH4 emission, the data was submitted to polynomial regression in combination with lasso parameter optimization as implemented in the “glmnet” R package.

The Legendre polynomial data based on DIM for the 212 OptiMIR wavenumbers of spectral data were used as input variables. The global spectrometric equations for energy balance calculated by the two evaluation systems net energy lactation (NEL) (GfE, 2001) and metabolizable energy (ME) (Susenbeth, 2018) is the result of a collaboration between the German optiKuh project consortium and the “German Association for Quality and Performance Testing e.V.” (DLQ). optiKuh is a collaborative project, of 12 research farms from different German states such as Baden Württemberg, North Rhine-Westphalia, Bavaria, Schleswig-Holstein, Rhineland-Palatinate, Lower Saxony and Mecklenburg-Western Pomerania, funded by the German Federal Ministry of Food and Agriculture. Between 2014 and 2017 a dairy cow feeding experiment with weekly collection of milk samples and daily individual feeding data provided reference values of both energy balances (NEL, ME). The local MROs and associated milk laboratories, organized in DLQ, provided milk recording results and standardised as

Table 1. Milk MIR models for CH4 emission and energy status.

Milk Biomarker	Unit	#LV	ϕ	SD	SEC	R^2c	SECV	R^2cv	RPD	Use
CH4 Emission	[g/d]	12	1266	97.00	50.00	0.73	54.00	0.69	1.80	-[1]
Energie Balance – NEL*	[MJ/d]	12	2.47	17.29	8.27	0.75	8.27	0.75	2.00	0[2]
Standardized							7.53	0.84	2.50	
Not Standardized							8.08	0.76	2.00	
Energie Balance – ME**	[MJ/d]	12	0.08	23.54	8.99	0.85	8.94	0.85	2.58	0
Standardized							8.42	0.89	3.05	
Not Standardized							9.06	0.84	2.48	
Energie Efficiency – NEL	[MJ/kg]	8	2.47	17.29	0.27	0.89	0.27	0.89	3.00	+[3]
Standardized							0.28	0.90	3.10	
Not Standardized							0.29	0.88	2.93	
Energie Efficiency - ME	[MJ/kg]	8	7.54	1.48	0.42	0.91	0.42	0.91	3.26	+
Standardized							0.42	0.91	3.30	
Not Standardized							0.46	0.89	3.05	
Feed Efficiency	[ECM/kg]	10	1.54	0.27	0.09	0.90	0.08	0.89	3.03	0
Standardized							0.08	0.90	3.13	
Not Standardized							0.08	0.88	2.97	

[1] Use "-" means RPD between 0-2 a very poor model class with application as allowing to compare groups of cows and distinguish between high or low values.
[2] Use "0" means RPD between 2-3 a poor model class with application as rough screening.
[3] Use "+" means RPD between 3-5 a fair model class with application as screening.
*NEL balance (GfE 2001); ** ME balance (Susenbeth, 2018)

well as non-standardised MIR spectral data from FOSS and Bentley FTIR analysers. With financial support from DLQ the weekly milking and MIR spectral data were combined with energy balance data to establish the MIR calibration equation of the two energy balances. Following the same Methodology and using the same optiKuh feeding experiment data source energy efficiency reference values and MIR spectra were combined within the eMissionCow project. The energy efficiency is defined here as the quotient of the energy intake and the amount of ECM per cow and day. Global models for energy efficiency NEL and ME and feed efficiency with a cross-validation trial from all research farms with around 1,511 animals were then created. In order to evaluate the effect of non-optimized feeding regimes on animal health, veterinary diagnosis and treatment data were linked from the LKV BW dairy database. This type of data is routinely recorded at LKV BW starting with the GMON project in 2011 for around 1,000 farms. Combined with milk recording data each milk sample from these farms can be associated with a health status.

Results and discussions

The CH4 calibration model was performed with 10-fold cross validation on a subset of 1,203 samples. This model is now also available to DLQ through the eMissionCow project as a new consortium member. The CH4 model is expanded to include measurements on Simmental cows. This increased the variability of the data and the robustness of the equation (Table 1). With a RPDcv of 1.8 it can be used for distinction of high and low values. Nearly 26,000 energy balances on NEL basis and nearly 29,000 energy balances on ME basis of the 12 experimental farms have been used for the new equations. It can be emphasized that there is a difference of 0.5 in RPD (Table 1.) between standardized and non-standardized devices. But for a better variability and a better robustness of the models, we have combined the standardized and non-standardized spectra and this model will be used for validation in commercial herds. It has to be pointed out that for the non-standardized spectral data only a maximum of 1 % relative deviation between the official fat content provided by the laboratories and the fat content derived from the RobustMilk MIR equation was accepted.

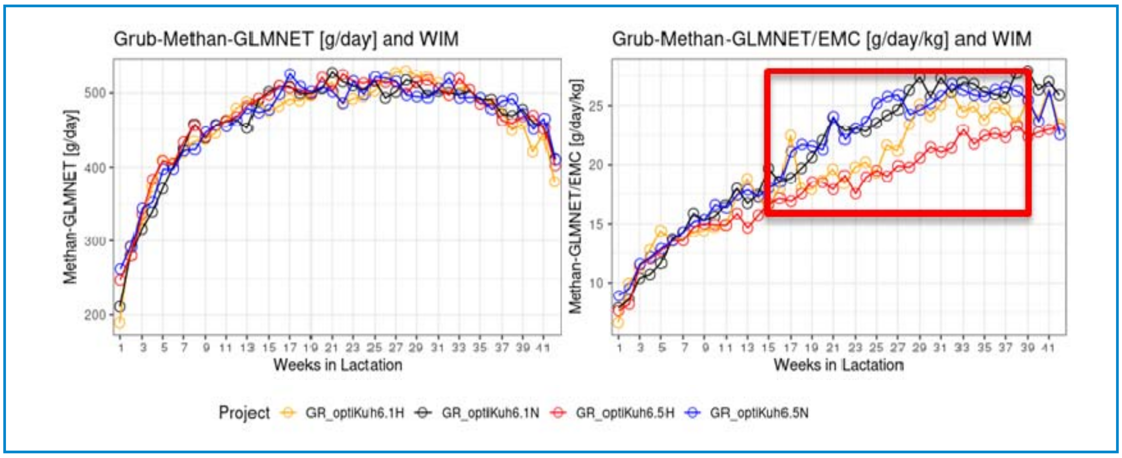

Figure 1. Absolute and relative (per kg ECM) calculated CH4 emission over weeks in lactation in different feeding regimes

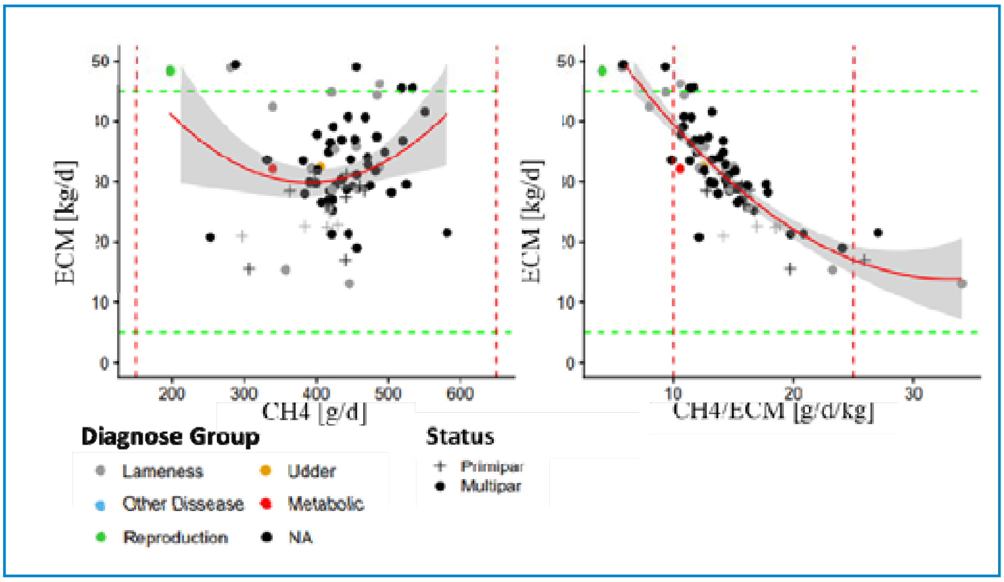

Figure 2. Absolute and relative CH4 and ECM according detected health

Therefore, the equation quality is better and the RPD is higher as 2. In the case of the feed efficiency the RPD is between 3 and 5 (Table 1.), which means: the equation is satisfactory and can be used for fine grading. With the same data was modelled the feed efficiency in kg ECM/kg dry matter intake. Here the RPD is more then 3 (Table 1.). Figure 1. shows the predictions of the CH4 equation with spectral data from the optiKuh project for each lactation week. It can be seen that the coloured data series are different. This is due to the four different feeding regimes, based on rations with 6.1 and 6.5 MJ NEL per kg forage DM and each with lower (N) or higher amounts of concentrates (H). The graph on the left shows the CH4 emissions in grams per day, the graph on the right shows the CH4 emissions relative to milk, in grams per kg ECM. The difference is much clearer visible in the relative emission. In the range from 100 to 270 lactation days, the values of the different feeding regimes differ most clearly. The differences between the rations with lower or higher amounts of concentrates also appear very clearly here. It is very easy to see that feeding regimes with lower energy density of forage and low amounts of concentrates and a higher proportion of forage, have more CH4 per day and kg of milk than feeding regimes with higher energy. In cooperation with eMissionCow and ReMissionDairy a report draft has been prepared for LKVBW test farms in order to evaluate the relevance and usability of the new predictions for herd management. The reports were designed for farmers and consultants: In the “Climate Status: CH4, feed efficiency and energy status report” can be seen the milk MIR parameters that reflect the energy status of the cow or herd. What is displayed: first two main groups of fatty acids: the de-novo group which consists of short and medium-chain fatty acids, mostly formed from the feed, and the preformed group that consist on long-chain fatty acids, mostly from the metabolization of body fat. Then there is energy balance: here is the NEL energy balance, feed efficiency, energy efficiency and energy consumption, and last CH4 with absolute CH4 in grams/day and relative CH4 in grams/ECM kg. Also, in the report it can be seen a set of plots displaying the link between milk parameters and daily CH4 emission and daily CH4 emission per ECM kg (Figure 2).

In the Figure 2 it is shown the daily ECM kg over CH4 emission. Compared to the left plot one can see that the milk yield has a stronger and clearly negative correlation with CH4 emission per kg ECM showing that cows with higher ECM yields have a lower CH4 emission per kg ECM. Figure 3. shows the link between energy efficiency and CH4 emission. Again, it can be seen a better correlation with CH4 emission per kg ECM. It is underlined that cows with a lower CH4 emission per kg milk also have a better energy efficiency meaning less energy is used per kg ECM. The main message to be provided to the farmer and advisor is that the predictions should be kept well within borders for which reasonable thresholds have to be developed. Here is also shown higher degree regression curves with standard error borders. Furthermore, the veterinary diagnosis registered for the cows near the milk recording date were also plotted in different colours: e.g. the fertility or reproduction problems were plotted in green, the udder diseases are in orange, the metabolic distortion are red, etc. It can be seen that cows with health problems appear rather at the borders and it can

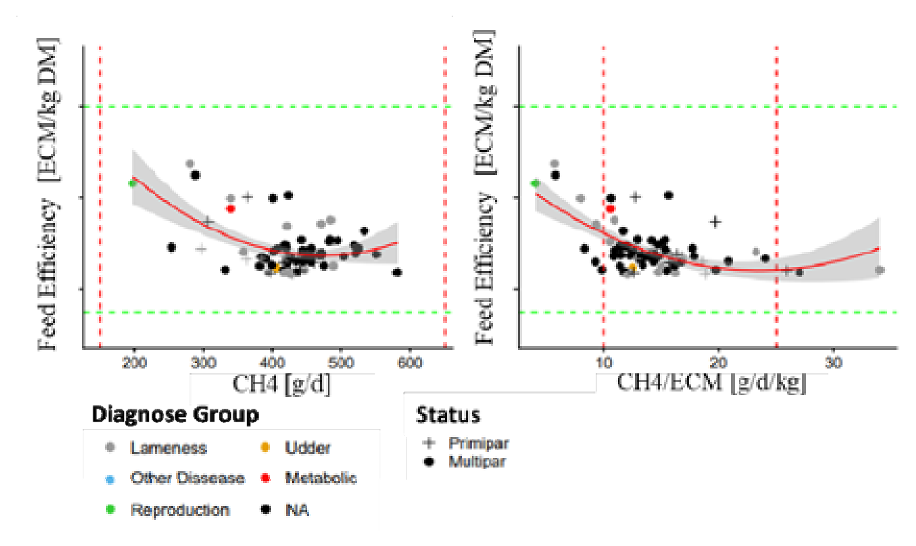

Figure 3. Absolute and relative CH4 and feed efficiency according detected health.

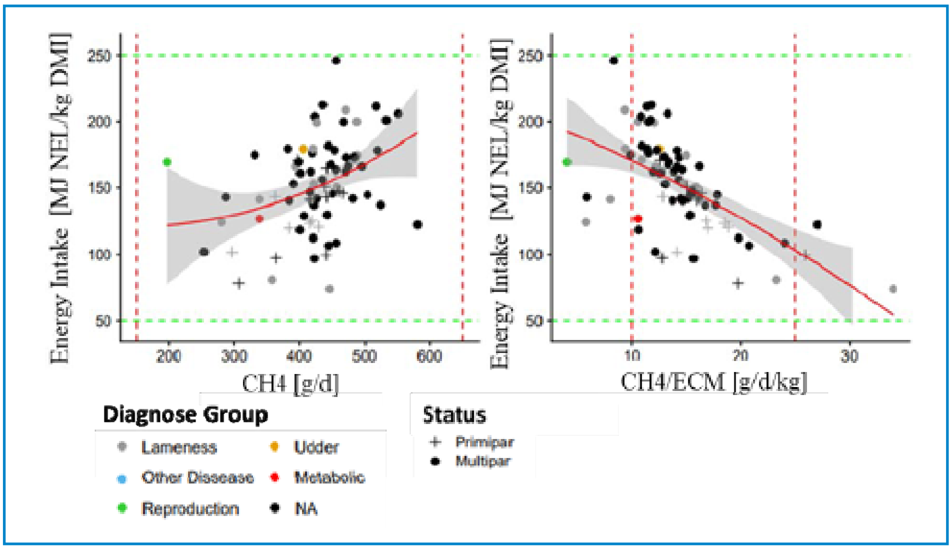

Figure 4. Absolute and relative CH4 and energy intake from feed dry matter according detected health.

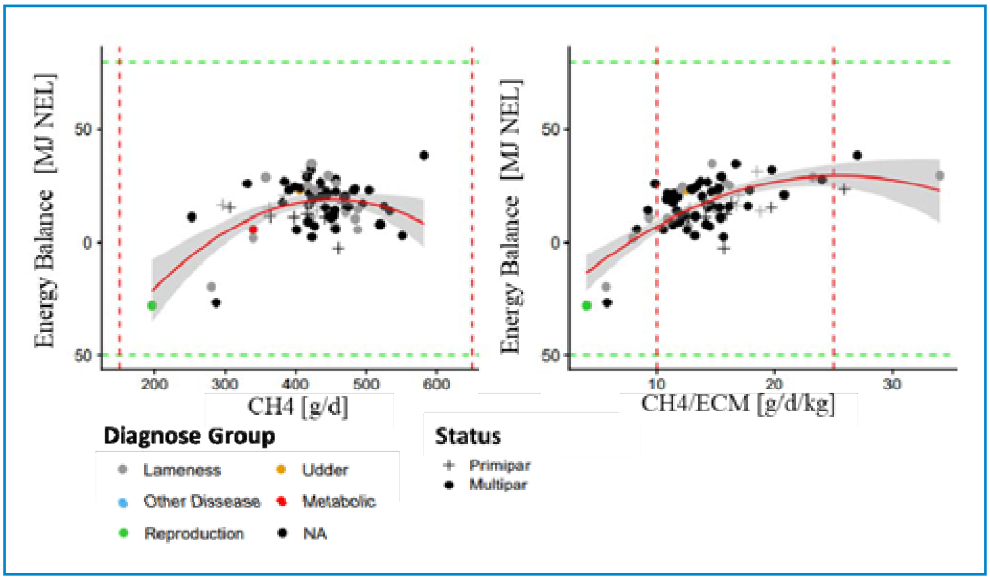

Figure 5. Absolute and relative CH4 and energy balance NEL [MJ] according detected health.

tell whether the herd management goes in the right direction with regard to feeding efficiency management. This link has to be elaborated further. In the Figure 4. it can be seen the plots over absolute and relative CH4 emission per day and energy intake from feed dry matter.

The plots show the energy intake per kg dry matter and the higher energy concentration in the feeding is linked with the lower CH4 emission per kg ECM. The plots at the bottom show the link to energy balance again on the right side with a stronger and clearly positive correlation to CH4 emission prediction per kg milk. Though negative energy balance (Fig. 5.) is linked to lower CH4 emission this should be avoided because extreme negative energy balance comes mostly with reduced feed intake. Extreme body fat mobilisation followed by health problems. This is indicated by the red versus the green bounded area. There is a correlation between diagnosis frequency in that day and the relative CH4 emission per day but also to other milk component concentrations. The diagnoses observed on that testday were mainly lameness but also udder und metabolic or reproduction diseases. It has been determined that the diagnosis frequencies had positive correlation to fat and almost all fatty acids (FA) groups but not with De Novo FA. In the same time, it can be seen that CH4 had a positive correlation to energy intake from feed dry matter as well as ECM and MY and also to DeNovo FA. Negative Pearson correlation to CH4 was found for fat, protein, feeding efficiency, as well as to lactoferrin, natrium and magnesium, also to blood components: adiponectin, insulin, IGF, Preform FA and C17 and total C18.

Conclusions

The Clima Status report for farmers and advisors emphasises energy related MIR predictions. The report is designed to show the link between these predictors in order to help them understand the effects and improve the feeding management. The equations for the determination of CH4, the energy balances (NEL, ME) and the feed efficiency (NEL, ME) are statistically highly meaningful and allow a comparison between groups of cows. The future developments that could be carried out at all levels are for example reports and applications for feed and energy efficiency, because MIR-based applications for reducing emissions and optimizing feeding are good tools for reducing emissions and optimizing feeding. Another future development could be the calculation of new breeding values based on correlations between genetics and MIR indicators.

Acknowledgments

The EE, FE and EB NEL and ME work was part of the collaborative project optiKuh, funded by the German Federal Ministry of Food and Agriculture. The KetoMIR2 model was conducted within the COMET-Project D4Dairy (Digitalisation, Data integration, Detection and Decision support in Dairying, Project number: 872039) that is supported by BMK, BMDW and the provinces of Lower Austria and Vienna in the framework of COMET-Competence Centers for Excellent Technologies. The COMET program is handled by the FFG. 10 years (2011-2021) of spectral standardization. This work was concepted by CRA-W, founded by OptiMIR project with the support of INTEREG IV B and it is under enhancement and continuous development of EMR-EEIG. Clima Status report is part of eMissionCow and also to ReMissionDairy projects, funded the German Federal Ministry of Food and Agriculture.

List of references

Dale L.M., A. Werner, H. Spiekers, P. Hertel-Böhnke, E. Stamer, F. Gollé-Leidreiter, M. Au, F. Onken. 2019. Prediction of evaluated energy balance (NEL and ME) in dairy cows by milk mid-infrared (MIR) spectra. ICAR Technical Series 2019: 137-141

Dehareng, F., C. Delfosse, E. Froidmont, H. Soyeurt, C. Martin, N. Gengler, A. Vanlierde and P. Dardenne. 2012. Potential use of milk mid-infrared spectra to predict individual methane emission of dairy cows. Animal, 6(10): 1694-1701.

GfE. 2001. Empfehlungen zur Energie- und Naehrstoffversorgung der Milchkuehe und Aufzuchtrinder. Heft 8, DLG-Verlag Frankfurt a.M.

Grelet, C., J.A. Fernández-Pierna, H. Soyeurt, F. Dehareng, N. Gengler, and P. Dardenne. 2014. Creation of universal MIR calibration by standardization of milk spectra: example of fatty acids. EAAP – 65th Annual Meeting, Copenhagen. p.108.

Grelet, C., J.A. Fernández Pierna, P. Dardenne, V. Baeten, F. Dehareng. 2015. Standardization of milk mid-infrared spectra from a European dairy network. J. Dairy Sci., 98(4): 2150-2160.

Grelet, C., C. Bastin, M. Gelé, J.B. Davière, M. Johan, A. Werner, R. Reding, , J.A. Fernandez-Pierna, F.G. Colinet, P. Dardenne, and N. Gengler. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, beta-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. J. Dairy Sci., 99(6): 4816-4825.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL *https://www.R-project.org/*.

Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres, and N. Gengler. 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. J Dairy Sci. 89(9): 3690-3695.

Susenbeth, A. 2018. Der Energiebedarf von Milchkühen heutiger Rassen. Tagungsband optiKuh, LfL-Schriftenreihe (2/2018): 40- 43

Vanlierde, A. 2019. Development of equations to predict methane eructed by lactating cows from milk mid-infrared spectra. Gembloux Agro-Bio Tech - Université de Liège.

Meta-analysis of some risk factors affecting somatic cell score in dairy cattle

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Abstract

Mastitis and milk quality in dairy cows are an ongoing concern of great relevance to animal welfare and productivity in modern dairy production. High somatic cell scores (SCS) are an indirect measure of presence of mastitis and low milk quality, they are relatively easy to record and values of heritability are higher than records of mastitis. However, published SCS values are highly variable across studies which makes it impossible to have a reliable reference value. The objectives of this study are to perform a meta-analysis (1) to estimate this reference value and the extent of its variability and (2) to identify whether and how factors of variation (year and country of publication, parity, and breed) influenced this value. Information on SCS was retrieved from 138 papers published between 1979 and 2020 in 40 countries and analyzed with the Metafor package in R software. Standard deviations were estimated from available data or imputed using a Bayesian hierarchical modelling approach. Results of the meta-analysis revealed a significant decrease of 0.04 units in mean SCS with the year of publication, an increase with the number of parities and a significant variability across countries. The reference SCS value was estimated at 3.68 (3.59 - 3.76) and total heterogeneity across studies at 1.24 (1.11-1.38). Further analyses are necessary to verify arguments provided to explain the results.

Keywords: SCS, meta-analysis, dairy cattle, risk factors, mastitis.

Introduction

Ensuring high quality of milk products is of crucial importance as they are part of the official nutritional recommendations in many countries worldwide (Rozenberg *et al*., 2016). Globally, cow milk represents 80% of total milk production in all regions (Navarro and Emery., 2015) and its consumption has spread around the world in the last forty years (Wiley, Andrea S, 2007). Milk quality depends on many factors, one of which is very important, namely the number of somatic cells (SCC) it contains. Indeed, high milk SCC are an indicator of subclinical infections (Heringstad *et al*., 2000; Pösö and Mäntysaari, 1996) which is associated with low milk production (Sert *et al*., 2016), deteriorated flavor quality and shelf life (Sobczuk-Szul *et al*., 2015), reduced milk processing yield (Najafi *et al*., 2009) and low protein content (Sharma *et al*., 2012). Besides infection, many other factors influence directly or indirectly milk SCC. They include cow characteristics (e. g., parity, season of calving, age, stage of lactation, udder conformation), geographical regions (e.g., temperature and humidity) and management factors, (e.g., transition to an automatic milking system, herd size (Barkema *et al*. 1998; Feliciano *et al*., 2020; Oleggini *et al*., 2001; Van den borne *et al*., 2021).

It is therefore not surprising that SCC values reported in the literature are variable. However, a part of this variation is also associated with random errors during the measuring process. Several measures (e.g., Q test, I^2 statistics, funnel plots) have been proposed to quantify heterogeneity of results across studies and to determine whether this heterogeneity goes beyond what would be expected by chance (Sedgwick, 2015; Melsen *et al*., 2014). If it is the case, alternatives exist to study the reasons for this heterogeneity and to generate a pooled SCC value (Cordero and Dans, 2021) such as the meta-regression models (Baker *et al*., 2009).

The goal of this study is to explore the diversity in SCC values reported in the literature and to determine the level of heterogeneity across studies.

Material and methods

The first step consisted in searching the web with a combination of keywords and subject headings for the following concepts: “Somatic Cell(s) Count(s)”, “Somatic Cell(s) Score(s)”, “Dairy Cattle”, “factors affecting somatic cell(s)”, “parity”, “season”. Globally, articles must have been published between 1970 and 2020 and include information related to a measure of SCC. Measures of SCC included test-day and lactation averages. Reports were mostly written in English, but other languages were allowed. Once an article was selected, all articles referenced within it were consulted. When available, information on breed, country, parity, and year was retrieved in each article. Six authors were contacted personally and provided us information missing in their articles (Knob *et al*., 2018; Pritchard *et al*., 2012; Heins *et al*., 2008; McParland *et al*., 2013; Koç, 2007, Koç and Kizilkaya, 2009). Finally, records were stored into a Zotero library.

The second step consisted in standardizing the reported values. Indeed, means values were reported as SCC, transformed in logarithm of SCC or in SCS. Herein, SCS were all expressed following the proposition of Wiggans and Shook (1987). When available, information of the variability around the means was retrieved and transformed into SCS standard deviation. When it was not possible to compute these standard deviations from the information available in the article, they were imputed following the procedure of Sung *et al.* (2006), assuming missing variances come from same lognormal distribution.

The last step consisted in evaluating level of SCS heterogeneity across SCS means. We implemented two random-effects models with the function “rma” of the “metafor” R package to obtain REML estimates of the effects included in both models and to create funnel plots (Röver, 2018). Besides the overall mean (fixed intercept), the first model included two random effects both assumed to be normally distributed with zero mean and with between- and within-study variances, respectively. This model allowed us to create the funnel plot and to compute the amount of heterogeneity ($\hat{\sigma}^2$) across SCS means. Tests for funnel plot asymmetry (which may be indicative of publication bias) was obtained with function “ranktest.rma”. In addition to these random effects, the second model included the fixed effects of breed and parity of the animals, country of publication and the linear effect of year of publication (covariate). This model allowed us to estimate the pseudo-R^2 value, i.e., the amount of heterogeneity that is accounted for by these fixed effects. The p-value threshold for statistical significance was set at 5%.

Results and discussion

After editing according to the inclusion criteria, we retrieved 637 SCS records from 138 peer-reviewed publications in 40 countries. The number of SCS records used to compute the mean ranged from 4 (Singh N *et al.*,2019) to 13 786 064 (Dezetter *et al.*,2015). The number of mean SCS per study varied from 1 to 22. The predominant breed was Holstein (58.64% studies), followed by Jersey (4.33 %) and Brown Swiss (4.09 %). Most studies were from Europe (46.75 %), Asia (20.60 %) and America (17.75 %).

The SCS mean over all studies is 3.68 (3.59 - 3.76) and Ä² is 1.24 (1.11-1.38). The funnel plot (Figure 1) reveals that 95% of the SCS means had standard errors less than 0.24 units which may suggest a bias in favor of studies with a large number of records. Indeed, 65.3% of the studies that reported the number of records used to compute the SCS mean signaled more than 1000 records. No significant difference in the number of studies on each side of the vertical line on the funnel plot was observed, in line with the observation that 57% of the studies reported SCS means lower than 3.68.

Effects included in the second model accounted for 60.87 % of the heterogeneity across the records. Most differences were found across years and countries of publication and across parities. The REML estimates of the effects of country and parity are given in Figures 2 and 3, respectively. In comparison with Austria, estimates were two units higher for countries such as Colombia, Egypt, New Zealand, Poland, South Africa and Turkey. Results from New Zealand may be incorrect as the method used to transform SCC in SCS was unusual (Lembeye *et al.*, 2015 and Lembeye *et al.*, 2016) and this will be corrected in a later report. For the other countries, further analyses are necessary to explain differences. Indeed, studies differed in the nature of the SCS measure (e.g., lactation or test-day, season of measure), in the individual (e.g., health status) and herd characteristics (Khaitsa *et al.*, 1998; Erdem *et al.*, 2010) or weather conditions (Carabaño *et al*, 2014). Figure 3 reveals that REML estimates increases across parities, being highest for parity higher than 3. Many possible causes could also be suggested to explain this observation. Among others, Sandrucci *et al.* (1992) observed increased amounts of epithelial cells in milk as the number of lactations rises. Breen *et al.* (2009) reported higher risk of clinical mastitis in cows with middle and high parity. A final significant effect was the linear reduction of 0.04 units per increased year.

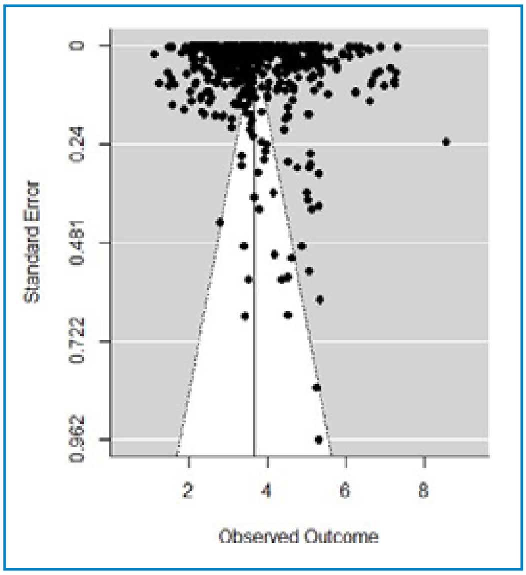

Figure 1. Funnel plot showing the SCS means (observed outcome) and corresponding standard errors for each study

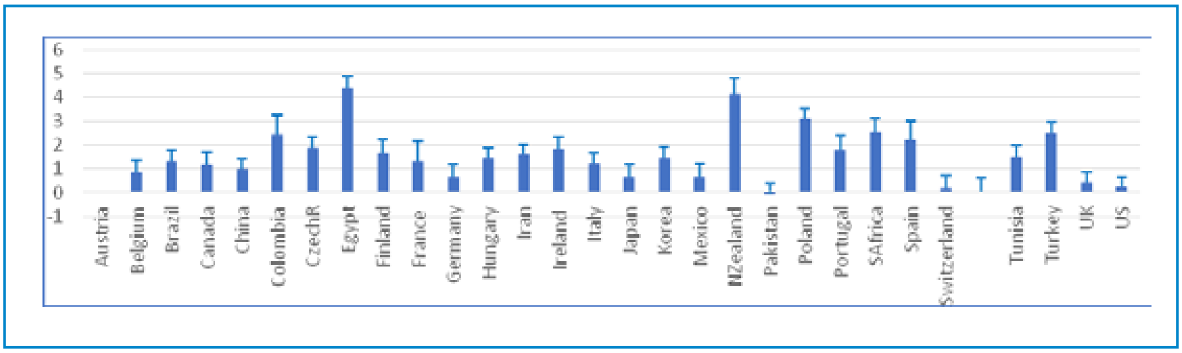

Figure 2. Estimates of the differences in SCS means for Holstein with respect to Austria, adjusted for the effects of parity and year of publication.

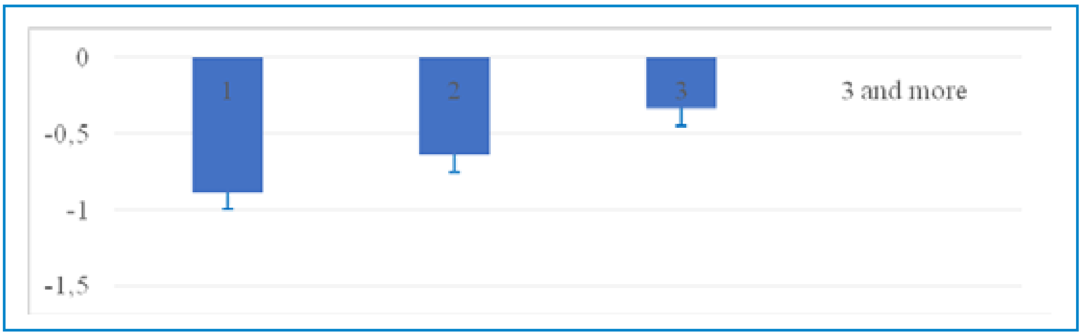

Figure 3. Estimates of the differences in SCS means for dairy cows with respect to parity higher than 3, adjusted for the effects of breed, country and year of publication.

Better management techniques (Hiitiö *et al.*, 2017), utilization of robot milking system (Frössling *et al.*, 2017; Johansson *et al.*, 2017) and genetic selection are some of the possible explanations for this trend.

Conclusion

The large number of studies from very different environments have allowed us to highlight significant differences in SCS across studies linked to parity, year, and country of publication. It remains to dissect the results to find clues towards a better understanding of these results.

List of references

Andrea S. Wiley., 2007. The Globalization of Cow's Milk Production and Consumption: Biocultural Perspectives, Ecology of Food and Nutrition, 46:3-4, 281-312.

Baker, W.L., White, C. M., Cappelleri, J.C., Kluger, J., and Coleman, C.I., 2009. Health Outcomes, Policy, and Economics (HOPE) Collaborative Group. Understanding heterogeneity in meta-analysis: the role of meta-regression. Int J Clin Pract. 63(10):1426-34.

Barkema, H. W., Schukken, Y.H., Lam, T.J.G.M., Beiboer, M.L., Wilmink, H., Benedictus, G., and Brand, A., 1998. Incidence of Clinical Mastitis in Dairy Herds Grouped in Three Categories by Bulk Milk Somatic Cell Counts. Journal of Dairy Science, 81 (2): 411-419.

Breen, J. E., Green, M.J., and Bradley, A.J., 2009. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J Dairy Sci. , 92: 2551-61.

Carabaño, M.J., Bachagha, K., Ramón, M., and Díaz, C., 2014. Modeling heat stress effect on Holstein cows under hot and dry conditions: Selection tools. Journal of Dairy Science. 97, 7889–7904.

Cordero, C. P., and Dans, A. L., 2021. Key concepts in clinical epidemiology: Detecting and dealing with heterogeneity in meta-analyses. J Clin Epidemiol., 130:149-151

Dezetter, C., Leclerc, H., Mattalia, S., Barbat, A., Boichard, D., and Ducrocq, V., 2015. Inbreeding and crossbreeding parameters for production and fertility traits in Holstein, Montbéliarde, and Normande cows. J Dairy Sci., 98(7): 4904-13.

Erdem, H., Atasever, S., and Kul, E., 2010. Determination of Milk Production Characteristics and Milk Losses Related to Somatic Cell Count in Jersey Cows Raised in the Black Sea Region of Turkey. Asian Journal of Animal and Veterinary Advances. 5: 217-222.

Feliciano, R. J., Boué, G., and Membré, J. M., 2020. Overview of the Potential Impacts of Climate Change on the Microbial Safety of the Dairy Industry. Foods, 9, 1794.

Frössling, J., Ohlson, A. and Hallén-Sandgren, C., 2017. Incidence and duration of increased somatic cell count in Swedish dairy cows and associations with milking system type. Journal of Dairy Science, 100(9), 7368-7378.

Heins, B.J., Hansen, L.B., Seykora, A.J., Johnson, D.G., Linn, J.G., Romano, J.E., and Hazel, A.R., 2008. Crossbreds of Jersey×Holstein compared to pure Holsteins for production, fertility, and body and udder measurements during first lactation. Journal of Dairy Science, 91: 1270-1278.

Heringstad, B., Klemetsdal, G., and Ruane, J. 2000. Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries. Livestock Production Science, 64 (2–3), 95-106,

Hiitiö, H., Vakkamäki, J., Simojoki, H., Autio, T., Junnila, J., Pelkonen, S., and Pyörälä, S., 2017. Prevalence of subclinical mastitis in Finnish dairy cows: changes during recent decades and impact of cow and herd factors. Acta Vet. Scand., 59, p 22.

Johansson, M., Lundh, Å., De Vries, R. and Svennersten Sjaunja, K., 2017. Composition and enzymatic activity in bulk milk from dairy farms with conventional or robotic milking systems. Journal of Dairy Research, 84(2), 54-158.

Khaitsa, M. L., K. H. Hoblet, K. L. Smith, T. Wittum, and P. Morley, 1998. Herd characteristics and management practices related to high milk production and production of high milk quality in two regions of Ohio. 244–245. Natl. Mastitis Counc. Annu. Mtg., National Mastitis Council, Madison, WI. p 244-245

Koç, A., 2007. Factors Influencing Daily Yield, Somatic Cell Count and Non-Fat Dry Matter Content of Milk. Indian Veterinary Journal. 85:630-632.

Koç, A. and Kizilkaya, K., 2009. Some factors influencing milk somatic cell count of Holstein Friesian and Brown Swiss cows under the Mediterranean climatic conditions. Arch. Anim. Breed., 52, 124–133.

Lembeye, L., Lopez-Villalobos, N., Burke, J. L., and Davis, S.R., 2015. Estimation of breed and heterosis effects for milk traits and somatic cell scores in cows milked once and twice daily in New Zealand. Proceedings of the New Zealand Society of Animal Production 2015. Vol 75:60-63

Lembeye, L., Lopez-Villalobos, N., Burke, J.L., and Davis, S.R., 2016. Estimation of genetic parameters for milk traits in cows milked once- or twice-daily in New Zealand. Livestock Science, 185, 142-147.

Melsen, W.G., Bootsma, M.C.J., Rovers, M.M., and Bonten, M.J.M., 2014. The effects of clinical and statistical heterogeneity on the predictive values of results from meta-analyses. Clinical Microbiology and Infection, 20 : 123-129.

McParland, S., O'Brien, B., and McCarthy, J., 2013. The association between herd- and cow level factors and somatic cell count of Irish dairy cows. Irish Journal of Agricultural and Food Research, 52: 151–158.

Najafi, M. N., Mortazavi. S. A., Khorami, J. and Rekik, B., 2009. Fat and protein contents, acidity and somatic cell counts in bulk milk of Holstein cows in the Khorasan Razavi Province, Iran. International Journal of Dairy Technology, 62: 19-26.

Navarro, J., and Emery, J., 2015. Annex B - Dairy Industry Background Report

Oleggini, G.H., Ely, L.O., and Smith, J.W., 2001. Effect of region and herd size on dairy herd performance parameters. Journal of Dairy Science, 84 (5): 1044-1050.

Pösö, J., and Mäntysaari, E.A., 1996. Relationships Between Clinical Mastitis, Somatic Cell Score, and Production for the First Three Lactations of Finnish Ayrshire. Journal of Dairy Science, 79 (7): 1284-1291.

Pritchard, T., Coffey, M., Mrode, R., and Wall, E., 2013. Genetic parameters for production, health, fertility and longevity traits in dairy cows. Animal, 7 (1), 34-46,

Rozenberg, S., Body, J.J., Bruyère, O., Bergmann, P., Brandi, M.L., Cooper, C., Devogelaer, J.P., Gielen, E., Goemaere, S., Kaufman, J.M., Rizzoli, R., and Reginster, J. Y., 2016. Effects of Dairy Products Consumption on Health: Benefits and Beliefs-A Commentary from the Belgian Bone Club and the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases. Calcif Tissue Int. 98(1):1-17.

Sandrucci, A., Tamburini, A., and Rapetti, L., 1992. A study on the use of a linear score for the evaluation of milk somatic cell count. Proceedings of the 27th Simposio Internazionale di Zootecnia, Societa per il Progresso della Zootecnia, Milan, Italy, p 175-190.

Sedgwick, P., 2015. Meta-analyses: what is heterogeneity? BMJ. 16;350:h1435. doi: 10.1136/bmj.h1435.

Sert, D., Mercan, E., Aydemir, S., Civelek, M., 2016. Effects of milk somatic cell counts on some physicochemical and functional characteristics of skim and whole milk powders. J Dairy Sci., 99(7): 5254-5264.

Sharma, N., Rho, G.Y., Hong, Y.H., Lee, T.Y., Hur, T.Y. and Jeong, D.K. 2012. Bovine mastitis: an Asian perspective. Asian Journal of Animal and Veterinary Advances, 7: 454–476.

Singh**, N., Sharma, S., Sharma, V., Saini, S., Joshi, R., and Karnani, M.,** 2019. Effect of non-genetic factors and body condition score on udder health and milk composition traits of Rathi cattle Indian J. Anim. Prod. Mgmt., 35 (1-2): 1-5.

Sobczuk-Szul. M, Z. Wielgosz-Groth, Nogalski, Z., Mochol, M., Rzemieniewski. A, and Pogorzelska-Przybylek, P., 2015. Changes in the fatty acid profile of cow's milk with different somatic cell counts during lactation. Veterinarija ir Zootechnika 69(91):52-57

Sung, L., Beyene, J., Hayden J, Nathan, P.C., Lange, B., and Tomlinson, G.A.A., 2006. Bayesian meta-analysis of prophylactic granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in children with cancer. Am J Epidemiol. 163:811–7

van den Borne, B. H. P., van Grinsven, N. J. M., and Hogeveen, H., 2021. Trends in somatic cell count deteriorations in Dutch dairy herds transitioning to an automatic milking system. Journal of Dairy Science, 104(5): 6039-6050.

Wiggans, G. R. and Shook, G. E., 1987. A lactation measure of somatic cell count. J Dairy Sci., 70(12): 2666-72.

Development of the first Russian genomic reference database and genomic evaluation results

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Abstract

At the moment, in the Russian Federation there is no established methodology for calculating the genomic breeding value of sires and dams. Routine selection (breeding) is carried out manually based on the dam's productivity only. But to perform effective breeding, it is important to choose the worst and best cows and rank animals with higher accuracy.

Genomic breeding values are widely used all around the world, but have not been implemented in Russia due to a lack of a local reference database. Foreign reference databases have advantages in size. However, the reliability of ranking animals according to foreign bases may be inaccurate due to different conditions of keeping animals, climate and other external factors.

In 2020, we launched a project to introduce genomic selection in the Udmurt Republic, which is the fourth largest milk producing region in Russia with many farms. The first genomic reference database in Russia was developed for the Holstein breed, the most popular dairy breed. This database contains more than 8000 animals with genotypes, including cows from frontrunning breeding farms in the region and bulls from the largest suppliers of semen in the region.

In the Russian Federation not all animals have unique IDs. So, at the first stage of our work we assigned each animal its own identifier. It is necessary to illustrate pedigree reliably. For this purpose phenotypic data was analyzed and filtered. We describe the most common mistakes in accumulated phenotypic data and how to process it.

We present our experience in the development of the first genome reference database with more than 8000 genotypes, which provides more accurate breeding values estimation and information about genetic diseases most represented in the Russian Federation.

The first genomic evaluations were calculated with the single-step genomic BLUP (ss-gBLUP) method for the following parameters: productivity, fat (absolute value and percentage), protein (absolute value and percentage) and longevity. The reliability of estimated genomic breeding values is up to 66%. We show the difference in evaluation between the pedigree-based breeding values and genomic breeding values.

We developed the web service for agricultural holdings which allows accurate breeding based on the GEBV (genomic estimated breeding values) and other results of genetic tests. Our solution allows breeders to find animals with the large and low genetic potential to increase the productivity of the next generation, select animals for crossing, and choose animals for embryo transfer, as well as to identify genetic abnormalities and economically useful traits in cows at an early stage.

Keywords: Genomic evaluation, reference database, russian holsteins, ss-gblup.

Introduction

The population of dairy cattle, represented mainly by the Holstein breed, is located in many regions of Russia, where climatic, feeding, and technological conditions vary significantly. Most of the cattle are concentrated in the Central, Volga, Southern, and North Caucasus districts. According to the Dairy Intelligence Agency, the number of dairy cattle in Russia in 2019 was 10.7 million heads with 4.6 million cows among them. Of these, the herd of the largest holding is 183 thousand head, with average productivity of 8 tons of milk per cow.

There are several types of organizations in Russian dairy cattle breeding and each has several distinctive features. There are large and medium-sized agricultural organizations, small farms and individual entrepreneurs. The main difference between them is in the dairy cattle population distribution. Thus large and medium-sized agro-holdings account for up to 69.5% of total livestock in Russia, while small farms account for 30.5%. In addition, differences are observed in farming conditions and contribution to overall dairy production. For instance, the difference in cow productivity can be observed between different organization types. The average productivity of dairy cattle in Russia was 4642 kg per cow per year of farms, while the productivity of agricultural holdings was 6290 kg, and for individual entrepreneurs 3791 kg per cow per year as of 2019, the most successful farms produce 13 000 kg per cow on average.

Agricultural organizations are an important player in the dairy cattle sector accounting for 85% of all milk production in Russia. The typical customer of the Ksitest company is an agricultural organization with a breeding status. The breeding status implies some obligations such as annual reports on livestock and productivity of animals and fixation of several indicators. Breeding farms are obliged to record inseminations, calvings, disposal reasons, evaluation of the exterior, and live weight at various stages of an animal's life. Also, they are required to conduct a control milking at least once a month, whereas they record the cow's milk yield, and send samples to the laboratory, determining the minimum fat, protein, somatic cells count, and other indicators. All these indicators are recorded in a local herd management program Selex (*https://plinor.spb.ru/index.php?l=0&p=3*). In contrast, industrial farms (non-breeding farms) are generally not restricted to anything and can only record as much or as little data as they find necessary.

The program was registered in 1997 and most of the breeders started to incorporate it in their data collection process in the last 20 years. Before that, they had preferred to use handwritten cards, which needed to be transferred to the electronic form. Some of the animal's cards go up to the 1970s.

Some farms can often use a second program for herd management: DairyComp, UniForm Agri, Afifarm, and others. Such programs can produce herd reports and reduce the human factor in filling data by using chips on animals, as well as automatic data collection from milking parlors, the results of control milkings, etc.

Data collected by breeders may have several problems. While many Russian breeders have started to introduce unique identifiers for animals, the data could still have some identification issues and, therefore, the pedigree errors. Besides, most of them use microsatellites as a kinship analysis, however, there is still data with parentage verification by immunogenetics. Therefore, the first step in reference database development is to correct all factual inaccuracies and use as much accumulated data as possible. Verification of animal records is especially important to get accurate estimated breeding values based on pedigree (EBV) and estimated breeding values based on genomic information (GEBV).

Russian dairy farms differ in environmental conditions, for instance, in climatic regime: Volga and Southern districts average July temperatures are 14,7°-24,7° and 18,0°-29,4° respectively. There are also differences in feed composition, care, management, and breeding programs. Therefore, cows from different Russian districts can not be comparable by their 305d productivity. Given this diversity, ranking animals by their genomic breeding values is the only way to compare dairy cattle in Russia by their genetic worth, and genomic selection seems particularly desirable.

The proper reference database plays a crucial role in the accurate breeding values estimation and is an essential step towards genomic selection implementation. The Holstein population in Russia is huge and spread throughout the country in different environmental and management conditions, phenotypic recordings of which have accumulated over several generations. However, local breeders still use the selection based on the dam's productivity, missing the opportunity of shorter generations and higher rates of genetic gain. The objectives of this work are to describe the steps of the first reference genomic database formation and to show the main results of the genomic evaluation of the Russian Holstein population as a beginning of genomic selection in Russia.

Stages of the genomic reference database creation

Data collection and filtration

As was mentioned before all breeders in Russia have duties on data recording, so therefore as a start we use dumps from herd management programs.

The phenotypic data gathered from these programs along with pedigree information are reported in table 1.

After uploading animal data to the Ksitest database, we perform pedigree verification. Verification helps solve problems such as (1) assigning multiple IDs to the same animal, or (2) having multiple records with the same inventory number for different animals. Problem (1) can arise, for example, when animals move between farms; problem (2) occurs due to the assignment of the same inventory number to two different animals from different farms. Errors associated with incorrect assignment of identifiers can distort

Table 1. Phenotypic data collected from Selex Dairy desktop.

Milk production	Milk, kg per day (starting from 5 days after calving) sum of milking (in case of 2 or 3 milking times) 305 days productivity and throughout lactation amount of milk fat, protein, somatic cells (laboratory checked data) daily milk yield in kg/time-consuming for milking during the day, min
Service period	The value of the service period in days
Longevity	The value of productive life in the number of lactations
Insemination	Date, bull, method
Calving	Date, result, calf weight, ease
Other information	Dry period (date, method), disposal (reason, date)

the breeding index and pedigree. To solve these problems, we assign each animal its own unique identifier. This approach allows tracking the movement of animals. For example, when the animal's birth farm and current location are different. To identify and correct data errors, we compare phenotypic data, pedigree and other information contained in records about the animal. The data is analyzed for inconsistencies in total for more than 10 indicators, including: data conflicts in dates of birth; conflicts in household data of birth; conflicts in nickname data; conflicts of data on the sex and age group of the animal; conflicts in the records of the breed of the animal. Phenotypic data derived from the Selex database is also checked for outliers. Besides, we include data that is directly confirmed by farmers as reliable.

Data genotyping

The process of biological data collection and subsequent genotyping began in the summer of 2019 and is still ongoing. There are several ways of getting genotypes:

1. customers collect biological material and we send it for genotyping at the DNA Laboratory;
2. customers provide genotypes previously obtained by them at other laboratories;
3. we obtain genotypes from international databases (CDCB, GenoEx, WWS), directly or through partners.

Depending on the way the data are accessed, the biomaterial used for genotyping differs. Hair follicles from the tail are the most used method because of the ease of obtaining and exporting. However, some customers prefer ear tissue sampling instead of follicles (<2% of samples). Blood sampling option is still under testing.

The selection of animals for genotyping is based on the goals and financial capabilities of farms. Generally, between 100 and 2,000 animals are collected from each farm. The main criteria are as follows: mother-daughter pairs or daughters with parents previously genotyped are collected; cows should not exceed 10 years of age; cows should have milk recordings for at least the first lactation; 305d lactation yield > 2,000 kg.

Genotyping is performed on a medium-density chip - the Weatherbys Scientific Bovine VersaSNP 50K. Usually it takes 1-1.5 months from the time the material is collected until the laboratory uploads the genotypes to the ftp-server. In addition to data from Bovine VersaSNP 50K, we also have genotypes from other chips (EuroG_MD, GGP_HD, ZMD, etc.) that differ from the a fore mentioned one in both density and content. An imputation procedure between chips is planned to add this data to the database in the nearest future.

The quality-check procedure is performed for collected genotypic data. Genotyped samples with call-rate < 0.9 and duplicates are removed. Duplicate identification is performed with PLINK v1.9 —genome option (Chang *et al.*, 2015). All animals meeting the following criteria Z0 <= 0.15 & Z1 <= 0.15 & Z2 >= 0.7 are considered to be duplicates and are being removed. The filtered genotypes are then used to search and verify the parentage, to identify statuses of farming traits and diseases, and calculate genomic breeding values.

Parentage verification

As the DNA Data Interpretation center accredited by ICAR (accreditation was obtained at 08.01.2021), we perform the parentage verification procedure directly according to the ICAR Guidelines for Parentage Verification and Parentage Discovery Based on SNP Genotypes.

Genomic evaluation

A single-step Genomic Best Linear Unbiased Prediction (ss-gBLUP) methodology is used to predict breeding values of both genotyped and non-genotyped animals in order to combine a pedigree and genomic information (Misztal *et al*., 2009). The fixed effects are as a combined effect year-season of first calving, agro-holding, farm, the age of heifer at first calving, mother's lactation number at the birth of the animal, and weather conditions. Estimated traits are 305d milk yield (kg), milk fat (absolute value, percentage), milk protein (absolute value, percentage), longevity (month).

The quality of data is determined by the coefficients of reliability, i.e. the proportion of explained variability by models with the use of all effects. Prediction accuracies of EBV and GEBV were expressed as square root of reliability, calculated from prediction error variance.

Total merit index

During the project, The Total Merit Index (KSI) was also developed (Miesenberger, J., and Fuerst, C., 2006). KSI is composed of estimated breeding values of the most valuable productive traits: 305d milk yield (kg), protein and fat content (%), and longevity (month). Each breeding value of a specific trait is weighted according to its economic importance under the Russian dairy production system. The economic importance is measured by the marginal profit per additional unit of the respective estimated breeding value in rubles assuming all other traits remain constant (Hazel, 1943). Economic data was provided by the Ministry of Agriculture of Udmurt Republic. Milk price is the largest contribution in revenue, such that milk with base protein and fat content equal to 25 rubles per 1 kg. The replacement cost is a main cost factor in the production system and amounts to 60 thousand rubles. In general, the profitability of milk production in the Russian system is about 10%. The final formula of KSI is following:

$$\begin{aligned} KSI = {} & 2.6\ \text{½}\ *\ BV_{\text{305-d milk yield}} + 2501\ \text{½}\ *\ BV_{\text{protein content, \%}} + \\ & + 2170\ \text{½}\ *BV_{\text{fat content, \%}} + 98.6\ \text{½}\ *BV_{\text{longevity, month}}, \end{aligned} \tag{1}$$

where BV corresponds to estimated breeding value derived from ss-gBLUP evaluation model. The Spearman correlation was calculated between KSI and the published Total Merit Indices of Iran, Israel and Czech Republic for auxiliary results verification (Sadeghi-Sefidmazgi *et al*., 2009, Krupová *et al*., 2018, Ezra and Weller, 2012).

Results and discussion

As part of the project for genomic reference database development, we collected more than 7,300 genotypes of the Udmurt Holstein dairy population in 2 month. In order to account for all available data, we also combined phenotypes and genotypes data from other regions of various previous local projects. Overall, by the end of 2020 we had 161 048 animals in the database with 17 924 milking cows and 1299 evaluated bulls from 5 regions of Russia.

During the data collection, Selex databases from dairy farms were received and processed. Mistakes, typos, duplicates, and deviations from expected values have been filtered from the data. Every animal has been provided with a unique identifier. There were 9 farms associated with the Udmurt region; overall 15.7% of the animal records were filtered. For each farm, on average 2% of animal records did not pass the data validation. For other 4 regions about 1% were filtered.

All the animal-parent pairs for which the genotypes were obtained were checked for consistency of parentage in the pedigree with parentage by genotype. In the case of a mismatch between the parents of an animal by pedigree and genotype, the search for the true parents was performed.

The obtained results are presented in table 2 and 3.

With the formed reference population of five regions, EBV and GEBV were estimated for both cows and bulls. The distribution of calculated reliability of 305d milk yield EBV and GEBV is shown in figure 1.

Table 2. The number of animals with accepted and excluded dams.

Genotypes in total	Dam is accepted	Dam is excluded	True dams found
8426	2086 (75.9%)	187 (7.9%)	114

Table 3. The number of animals with accepted and excluded sires.

Genotypes in total	Bulls genotypes	Sire is accepted	Sire is excluded	True sires found
8426	23	922 (86.5%)	92 (8.6%)	91

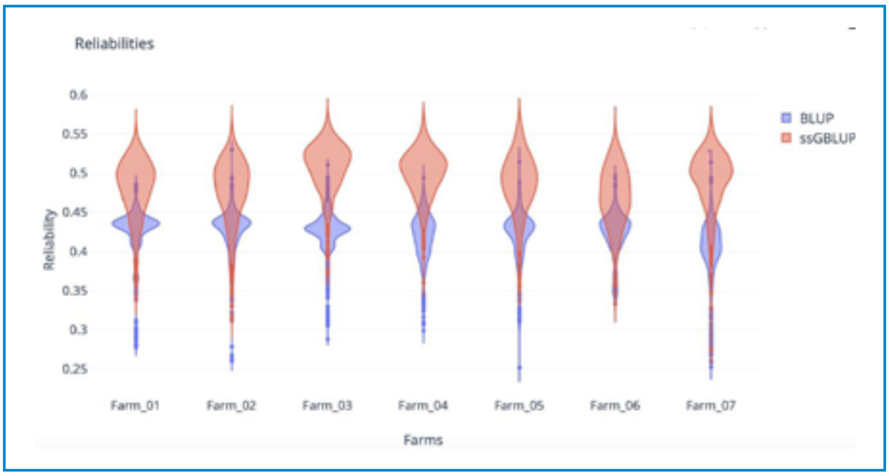

Figure 1. Reliability from BLUP and ss-gBLUP models of Udmurt region data.

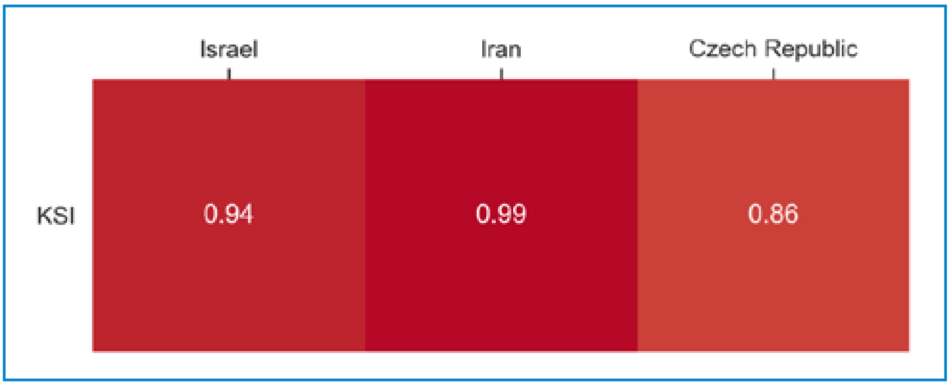

Figure 2. Spearman's correlation coefficients with foreign total merit indexes.

As expected, the result showed the larger reliability values with genomic information added. Considering the results of 305d milk yield prediction, the maximum reliability value of GEBV is 0.66 for cows and 0.93 for bulls, medium reliability value is 0.53 for cows and 0.58 for bulls. The lowest reliability performance both for GEBV and EBV is associated with low-quality phenotypic data from farm 4. Average gain in reliability values of ss-gBLUP are about 8%.

After the genomic evaluation process the total merit index, KSI, was implemented. Spearman's correlation coefficients between KSI and Israel, Iran, Czech Republic Indexes are 0.94, 0.99, 0.86, respectively (Figure 2). The 305d milk yield trait has the biggest contribution (76%) in KSI variance, since the milk price has the greatest impact on Russian agro-holding economics.

Moreover, a web service was implemented (https://app.ksitest.ru/) for local breeders. Ksitest web-service combined almost all features described above for each animal obtained database: their own information (name, farm, birth date), breeding values, genomic passports, total merit index, parentage verification, pedigree information. Genotyped animals also have results from genetic tests, such as monogenic disease (e.g. Bovine Leukocyte Adhesion Deficiency) or selection trait (e.g. polledness) statuses. As a result, web-service allows breeders:

- to rank animals by their genetic potential;
- to explore analytics (e.g. mean milk yield on the farm, dynamics of EBVs and GEBVs in years);
- to chose animals for embryo transfer or sale;
- analyze bull's performance (e.g. descendants production)
- receive documents (e.g. genomic passports and genotyping results in Illumina Final Report format).

In the next year 200 bull's genotypes and 22 500 cow's genotypes will be produced. The reference database will be expanded to 3 more Russian regions. Furthermore, to make a more efficient KSI total merit index for the dairy farmers, calving interval, fertility and conformation traits will be implemented and more economic parameters will be obtained by the end of 2021.

Conclusion

We aimed to create the first genomic reference database in Russia and perform genomic evaluation collaboratively with the Ministry of Agriculture of Udmurt Republic. We collected and validated more than 160 thousand animals data to the ksitest database. Besides, as of July 2021, the database contains records from 18 farms of 8 Russian regions. Currently we have data on 234,582 animals in total with 23,811 milking cows and 1561 evaluated bulls with 8974 genotypes and 328,917 phenotypic records. Genomic evaluation of obtained data outperformed the pedigree based evaluation. In addition, we incorporated all developments to our web service. Our results imply that genotyping information tends to reach higher reliability of prediction and higher selection accuracy. Genomic selection could replace currently common selection by dam's productivity in Russia and can improve accuracy for young animals without phenotypic information. Evaluations of new traits and more genotypes are expected in 2021. We expect that the accuracy of genomic evaluation will continue to improve with more data and these results pave the way for genomic selection implementation in Russia.

List of references

Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M. and Lee, J.J., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience, 4(1), pp 13742-015.

Ezra, E., and J. I. Weller. 2012. The Israeli Selection index. Page 28 in The Dairy Industry in Israel 2012. D. Hojman, Y. Malul, and T. Avrech, ed. Israel Dairy Board and Israel Cattle Breeders Association, Caesaria Industrial Park, Israel.

Hazel, L.N., 1943. The genetic basis for constructing selection indexes. Genetics, 28(6), pp.476-490.

Krupová, Z., Wolfová, M., Krupa, E., Zavadilová, L. and Pøibyl, J., Economic weights of traits in the breeding objective for Czech Holstein cattle.

Manichaikul, A., Mychaleckyj, J.C., Rich, S.S., Daly, K., Sale, M. and Chen, W.M., 2010. Robust relationship inference in genome-wide association studies. Bioinformatics, 26(22), pp.2867-2873.

Miesenberger, J. and Fuerst, C., 2006. Experiences in selecting on a total merit index in the Austrian Fleckvieh breed. Biotechnology in Animal Husbandry, 22(1-2), pp.17-27.

Misztal, I., Legarra, A. and Aguilar, I., 2009. Computing procedures for genetic evaluation including phenotypic, full pedigree, and genomic information. Journal of dairy science, 92(9), pp.4648-4655.

Sadeghi-Sefidmazgi, A., Moradi-Shahrbabak, M., Nejati-Javaremi, A. and Shadparvar, A., 2009. Estimation of economic values in three breeding perspectives for longevity and milk production traits in Holstein dairy cattle in Iran. Italian Journal of Animal Science, 8(3), pp.359-375.

“MastiMIR” - A mastitis early warning system based on MIR spectra

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Abstract

At farm level the mastitis disease appearance decrease the milk production, produce veterinary costs and welfare issues, increase the culling rate or cause lower milk payment (Bastin el al., 2013, Guimarães *et al*., 2017). Because mastitis is associated with a wide range of characteristics that can be measured in milk and with recent advances in estimation of milk components using mid-infrared spectrometry (Soyeurt *et al*., 2009), it is now possible to have the composition of several additional milk components such as fatty acids, lactoferrin (Leclercq *et al.*, 2013), minerals, negative energy balance, non-esterified fatty acids and beta-hydroxybutyrate or citrate (Grelet *et al*., 2015), etc. The objective of this study was to build a spectrometric tool for Alsace, such as MastiMIR in LKV B.W. and to work out if it is possible to determinate by means of the milk quality the animal health status. Furthermore, the aim was to evaluate the usability of farmer observations and MIR indicators for the improvement of early mastitis prediction. The dataset contains 303,650 spectral data from 123 herds between 2014 and 2018. The first trial is composed from 70% of data in calibration and 30% in validation, while the second trial from 70% and 30% respectively of farms in calibration and validation datasets.

To identify animal variables that were positively or negatively associated with mastitis, the spectral data was first standardised, then pre-processed by first derivative and the legendre polynom model was applied for days in milk correction. For the ill class, spectral data with mastitis diagnosis for a given cow within -14 to +14 days was chosen, while for the healthy class no diagnosis data was registered. As fix effects were considered the sampling moment, lactation stage, and breed. For this model no somatic cell filter was used. The MastiMIR calibration model showed 77.7% sensitivity and 68.4% specificity for the final calibration model. The external validation showed 67.1% sensitivity and 68.1% specificity. For the same period if the somatic cell SCC filter was done: 400,000 SCC filter for non-healthy data and 50,000 SCC filter for healthy data the sensitivity and specificity are increased to 85.5% and 72.7% respectively. The MastiMIR model provides four classes of mastitis warning such as not, moderately, significantly and severely endangered. The moderately endangered class is a signal for the farmers to keep an eye on the affected animals and contact a vet if applicable in order to prevent the mastitis diseases.

Keywords: mastitis, spectrometry, MIR milk spectral data, dairy cow, cow health

Introduction

The mastitis definition is well known; mastitis is an inflammation of the mammary glands and can be caused by more than 50 different organisms. Usually, mastitis is diagnosed by somatic cell number (SCC) and laboratory diagnostic methods. At farm level the mastitis disease appearance decreases the milk production, produces veterinary costs, welfare issues, and increases culling rate or causes lower milk payment. Mastitis is associated with a wide range of characteristics that can be measured in milk with recent advances in the estimation of milk components using mid-infrared (MIR) spectrometry.

Also if a cow has mastitis, the composition of the milk will be affected and with it the MIR-milk-spectrum. The important message from a previous project (OptiMIR) was that not only the main components can be analysed with the MIR spectrometer, but also fatty acids (Grelet *et al.*, 2014), minerals, lactoferrin (Soyeurt *et al.*, 2011), beta-hydroxybutyrate (BHB), acetate and citrates (Grelet *et al.*, 2015), etc. Complex features could also be identified, for example models for ketosis (Grelet *et al.*, 2016), energy deficit (McParland *et al.*, 2011, Smith *et al.*, 2018), energy balance (Dale et., 2019) and methane emissions (Dehareng *et al.*, 2012). Nowadays working on mastitis tools could help farmers for the herd management and better production.

The objective of this study was to build a spectrometric tool, such as MastiMIR in LKV B.W. for Alsace and to see if it is possible to determinate true the milk quality the animal healthy status. The aim was to evaluate the farmers observations usability and MIR indicators for the improvement of early mastitis prediction.

Material and methods

The dataset contains 303,650 spectral data from 123 herds between 2014 and 2018. The observations were documented by the farmers with the help of 86-part diagnostic keys. The gold standards to create the MastiMIR model were the mastitis observations together with the spectral data. The observations used for the model were: chronic, acute and subclinical mastitis, as well as coli mastitis. The model is based purely on standardized spectral data since all spectra registered at the MRO Agricultural Chamber Alsace level have been standardised starting from January 2012, due to the OptiMIR project participation.

All data editing, modelling and calculations were done using the R statistical language and environment. To identify animal variables that were positively or negatively associated with mastitis determination, the spectral data set was first pre-processed by Savitzky-Golay first derivative in order to remove the offset differences between samples for baseline correction, before performing Legendre polynomial transformation based on days in milk. Then the data was submitted to logistic regression in combination with LASSO variable selection and regularization and 10 fold cross validation using the "glmnet" R package. For the non-healthy class, spectral data with mastitis diagnosis for a given cow within -14 to +14 days was chosen, while for healthy class no diagnosis data was registered. As fix effects were considered the sampling moment, lactation stage, breed. No somatic cell filter was used for this model. What comes after the mastitis diagnostic was not taken into account for modelling. For the healthy group only spectra which had no diagnosis associated within ±60 days were used.

For "glmnet" model were considered as fix effects the sampling moment (with three variants: standard, morning and evening), lactation stage (if lactation number was greater than 5 it was taken as 5) and usage of breeds (milk, mix and meat) and a 212 OptiMIR wavenumbers subset of the pre-processed spectral data. The calibration data set contained 303,650 spectral data from 123 herds. The first validation approach was based on a random split of data, 70% of data was used for calibration model and 30% for validation model. The second validation model was based on a lot of 8 farms for an external validation in order to exclude animal and farm effects. These 8 farms were the farms with the highest diagnosis registration rate and had to cover the important breeds e.g. 4 Holstein farms, 2 Brown Swiss farms and 2 Simmental farms from Agicultural Chamber Alsace were registered. For this two validation models the same data cleaning approach as for calibration model was used. Due to the external validation with the extreme values diagnosis cases, a third validation model is proposed with production data from a whole production year. Data from 1st October 2017 till end of September 2018 in combination with diagnosis data was aimed to verify if the proposal model could be afterwards used or not in production. From a research and statistical point of view, production data approach could show what in the reality exists and if the model will be working in reality.

Results and discussions

Mastitis can only be predicted to a limited extent via the number of cells. Therefore a model based on spectral data, animal parameters, and mastitis observations such as MastiMIR has been developed. After modelling with GLMNET in R, a sensitivity (the percentage of sick cows that were correctly identified as having the condition) of more than 63% in calibration and 72% for the validation and external validation model could be obtained. The specificity (the percentage of healthy cows that were correctly identified as not having the condition) is more than 74% for calibration model and 1st validation model and 64% for the external validation model, 2nd validation model (Table 1).

It can be underlined that until now no information of direct use of spectral data to predict the mastitis treat has been found in the literature before 2017 (Dale and Werner, 2017). Regarding the 3rd validation model with production data, it can be seen that the sensitivity is just 71.6% while the specificity is 66.2%. This can be explained by the probable presence of untreated mastitis cases, subclinical mastitis and missing registration of diagnosis events in the production data.

The idea was to cover this group of data by means of a mastitis risk probability provided by a presumed logistic-linear relationship (S-curve) between MastiMIR probability and the mastitis danger. This model allowed by using different thresholds to distinguish 4 danger/risk classes. The class limits were determined by using statistical methods such as cumulative probability and Cox event time analysis. The class size was negatively correlated with the mastitis class. It can be seen in the distributions of the MastiMIR and the SCC classes over the lactation week, that the mastitis class distribution has the shape of the lactation curve on both models.

The MastiMIR class distribution on whole population from Agricultural Chamber Alsace for the year 2017 is more pronounced than SCC class. Regarding the animals with MastiMIR danger or risk it can be pointed out that mastitis can occur also when the cows have less SCC and animals with higher SCC may still have other diseases as it was underlined in the literature (Dale *et al.*, 2019). There is also a difference between the healthy classes and moderately endangered and also significantly endangered. The size of the group decreases while the SCC classes with higher mastitis risk increas. T

Table 1. MastiMIR calibration and validation statistics.

MastiMIR Model	Sensitivity	Specificity
Calibration	63.9%	74.1%
1st Validation	72.4%	68.6%
2nd Validation	64.6%	64.8%
3rd Validation	71.6%	66.2%

he Cox event time analysis improved the classification. If an animal has mastitis observations, it can be seen earlier with the MastiMIR model than with the SCC class model. The transition from significantly endangered to severely endangered was better differentiated. The transition from healthy to moderately endangered class was displayed earlier. If a cow has health problems due to mastitis, it has not only a lower amount of milk or higher SCC but it also reacts with a change of the main milk components: the lactose content is negatively correlated with mastitis and the protein content and the fat-lactose ratio are positively correlated. A positive correlation also applies to the milk fine components sodium, Lactoferrin and BHB, as the literature has already confirmed.

In the Agicultural Chamber Alsace, the MastiMIR Alsace model is taken into the 65 pilot farms for testing. The criteria for the selection of pilot farms were: farms with advisors specializes in milk quality, also robotic farms, farms with SCC problems, farms with mastitis problems. Each advisor have different approachs, such as: sorting the parameters to understand how the algorithm works, communication with the farmer to understand the classification of the animals or feedback from farmers should be standardized in order to raise awareness among the model. Each advisor receives weekly emails from LKV B.W. IT center with the PDF and excel documents for each farm. Regarding the interpretation of the reports, each advisor works individual and different.

One of the advisors is working direct with farmers the other one has another approach such as sending the reports and take an appoiment with the farmer later in the same week. Thus the farmer can be prepared and informations can be noted. It is very important to pursue the history of each cow with high risk and to get information from the farmers about those animals and to note if the cow gets mastitis or not. Moreover there is a need to document if an animal had abacteriological analysis (differences between e.g. coli (a rapid development) and staphylococcus). It is very important to sensitize the farmers to document also the untreated udder infections and cows with mild symptoms.

Therefore it was very important to select farmers who know their animals well and have knowledge of mastitis. There are still a lot of questions such as how does the farm determine that a cow does not have mastitis (flakes, no symptoms...)? Could it be a subclinical cow? The Agricultural Chamber Alsace, pointed out that it is essential to inform the participating farms about the MastiMIR report, various milk parameters and their relationship. Indeed, farmers often have difficulty identifying mastitis. Some farmers give their respond quickly, others not, therefore it is challenging because until now no bacteriological analyzes have been made if mastitis is suspected. Therefore it is important to analyse affected cows because it is known, that coli bacteria cause a rapid infection. This pathogen comes from the environment not from the metabolism of the cow, thus the MastiMIR project may not be able to find the animal at risk.

Furthermore there are similar markers in the spectra that could point out not just if a cow has mastitis, there is also cow with lameness (Bonfatii *et al.*, 2020), but in the report for the MastiMIR, it can be pointed out if the index of mastitis is low and the cow has high SCC value, it should be check if a cow has or not lameness problems. Moreover, it turned out, that interaction with other diseases need to be taken into account, because poorly planned stable buildings, stress, hoof problems, milk fever, milking hygiene or feeding can be recognized in the spectral data.

Conclusions

The model provides four classes of Mastitis warning such as not, moderately, significantly and severely endangered. MastiMIR can be a good threat to mastitis. The moderately endangered class is a signal for the farmer. In that case the farmer would contact the veterinary and a control could be made in order to prevent the mastitis diseases. Compared to the SCC model, the MastiMIR model shows an earlier occurrence of the ‘slightly at risk’ classification. The MastiMIR model is a complementary tool for the SCC model, therefore it can supplement the SCC classes. The evaluation in the field within the framework of the HappyMoo project is currently being prepared.

List of references

Bonfatti, V., Ho, P. N., and Pryce, J. E. 2020. Usefulness of milk mid-infrared spectroscopy for predicting lameness score in dairy cows. Journal of dairy science, 103(3), 2534-2544.

Dale, L. M., Werner, A., and Gollé-Leidreiter, F. 2019. “MastiMIR”-A mastitis early warning system based on MIR spectra. New traits and adding new value to the recording and id services in the animal production, ICAR Tech. Ser, 24, 131-135.

Dale, L.M., and Werner, A. 2017. “MastiMIR” – Ein Mastitis – Frühwarnsystem basierend auf MIR-Spektren, Vortragstagung der DGfZ und GfT Aus der Arbeit der Forschungsstätten für Tierwissenschaften, A12, Stuttgart, Deutschland.

Dale, L. M., Werner, A., Spiekers, H., Stamer, E., Au, M., and Onken, F. 2019. Prediction of evaluated energy balance (NEL and ME) in dairy cows by milk mid-infrared (MIR) spectra. ICAR Tech. Ser, 24, 137-141.

A. Dehareng, F., C. Delfosse, E. Froidmont, H. Soyeurt, C. Martin, N. Gengler, A. Vanlierde and P. Dardenne. 2012. Potential use of milk mid-infrared spectra to predict individual methane emission of dairy cows. Animal, 6(10): 1694-1701.

Grelet, C., J.A. Fernández-Pierna, H. Soyeurt, F. Dehareng, N. Gengler, and P. Dardenne. 2014. Creation of universal MIR calibration by standardization of milk spectra: example of fatty acids. EAAP – 65th Annual Meeting, Copenhagen. p.108.

Grelet, C., C. Bastin, M. Gelé, J.B. Davière, R. Reding, A. Werner, C. Darimont, F. Dehareng, N. Gengler and P. Dardenne. 2015. Milk biomarkers to detect ketosis and negative energy balance using MIR spectrometry. In Book of Abstracts of the 66th EAAP-Annual Meeting (p. 354). Wageningen Academic Publishers.

Grelet, C., C. Bastin, M. Gelé, J.B. Davière, M. Johan, A. Werner, R. Reding, J.A. Fernandez-Pierna, F.G. Colinet, P. Dardenne, and N. Gengler. 2016. Development of Fourier transform mid-infrared calibrations to predict acetone, beta-hydroxybutyrate, and citrate contents in bovine milk through a European dairy network. J. Dairy Sci., 99(6): 4816-4825.

Lainé, A., C. Bastin, C. Grelet, H. Hammami, F.G. Colinet, L.M. Dale, A. Gillon, J. Vandenplas, F. Dehareng, and N. Gengler. 2017. Assessing the effect of pregnancy stage on milk composition of dairy cows using mid-infrared spectra. J. Dairy Sci. 100:2863–2876.

McParland, S., G. Banos, E. Wall, M.P. Coffey, H. Soyeurt, R.F. Veerkamp, and D.P. Berry. 2011. The use of mid-infrared spectrometry to predict body energy status of Holstein cows. J. Dairy Sci. 94:1222–1239.

Smith, S. L., M. P. Coffey, and E. Wall. 2018. Association of milk MIR-derived body energy traits with fertility parameters in cows. Proc. 11th WCGALP: 1121.

Soyeurt, H., C. Bastin, F.G. Colinet,V.R. Arnould, D.P. Berry, E. Wall, F. Dehareng, H.N. Nguyen, P. Dardenne, J. Schefers, and J. Vandenplas. 2012. Mid-infrared prediction of lactoferrin content in bovine milk: potential indicator of mastitis. Animal. 6(11):1830-8.

Implementation of differential somatic cell count as a new parameter for improved udder health monitoring through routine milk recording

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Abstract

The aim of this study is to describe the experience and the status quo on implementing the new Differential Somatic Cell Count (DSCC) parameter as a new service to milk-testing clients of Qnetics through routine milk recording.

Milk samples from all dairy herds enrolled to milk recording in the federal state Thuringia, Germany, were analysed on CombiFoss 7 DC. A total number of 416,198 test days was available for data analysis in our study. The udder health status of cows was categorised into four different groups depending on SCC and DSCC results: Udder Health Group A – healthy, SCC <200,000 cells/ml and DSCC $\leq$ 65%, B – onset of mastitis, SCC <200,000 cells/ml and DSCC >65%), C – (subclinical) mastitis, SCC >200,000 cells/ml and DSCC >65%, D – chronic mastitis, SCC >200,000 cells/ml and DSCC $\leq$ 65%. Linear mixed effect models were applied for statistical analysis of the data.

Briefly, the performance of cows in the different Udder Health Groups was investigated and cows in group A revealed the highest performance (e.g. milk weight, fat and protein). While the performance was lower in group B, it decreased significantly in group C. Cows in group D turned out to be evidently less productive compared to cows in groups A-C. The distribution of cows among the four different Udder Health Groups was investigated for the whole population as well as for each individual farm. Interestingly, the distribution varies hugely between farms, indicating and revealing different farm management practises.

In a next step, multiple dairy farms were selected and their farm management practises were further investigated. Beyond that a pilot phase, where the selected farms regularly receive a new udder health report based on SCC and DSCC results, was initiated. First feedback confirms new possibilities for udder health management and optimising herd health and performance based on the new udder health report. The learnings from the pilot phase confirm the added value of DSCC for udder health management and will be used to further roll out the new service.

Keywords: DSCC, udder health, milk recording.

Introduction

The company Qnetics offers various services to dairy farmers in the German federal state of Thuringia. Among other things, these include the organisation and execution of DHI testing for dairy cows, ewe, and dairy goats, milk payment analysis, and advisory services for herd management, dairy cow nutrition and milk quality challenges. Beyond that, breeding services such as raising of own bulls, sperm production and artificial insemination services are offered.

Dairy farming in Thuringia

A total number of 94,548 dairy cows was kept on 282 dairy farms in Thuringia in 2020. The vast majority (98.2%) of these farms are utilising dairy herd improvement services on a regular basis. The average annual production per cow was at 9,978 kg milk, 404 kg fat, and 345 kg protein in 2020. The average herd size is at 335 cows and approximately 60% of all cows are kept on 25% of all farms.

The average productive lifetime of cows is a key figure investigated every year and was at 2.3 lactation in 2020 and at comparable levels in previous years. Delving into when and why cows are culled revealed that nearly 50% of cows are culled in their first or second lactation and udder health challenges was the top 2 culling reason (13.8%) after reproduction. Given that this is critical from an economic and ethic perspective, CombiFoss 7 DC technology including the DSCC parameter was acquired. The key motivation was to be able to provide farmers with better DHI-based services for managing udder health, in particular earlier detection of udder health issues.

Udder health group concept

A data base consisting of 416,198 test days generated between August 2019 and January 2020 has been used for initial data analysis. More specifically, cows were categorised into four different udder health groups (UHG) depending on their actual SCC and DSCC test day result as follows:

- A – healthy: SCC $\leq$200,000 cells/ml and DSCC $\leq$65%,
- B – onset of mastitis: SCC $\leq$200,000 cells/ml and DSCC >65%
- C – (subclinical) mastitis: SCC >200,000 cells/ml and DSCC >65%
- D – chronic mastitis: SCC >200,000 cells/ml and DSCC $\leq$65%

The SCC cut-off of 200,000 cells/ml used is according to IDF recommendations (IDF, 2013). The DSCC cut-off of 65% has been identified as optimal cut-off in a study where the actual udder health status of cows as defined by bacteriological testing was available (Schwarz *et al*. 2020a).

Interestingly, the analysis of the data base revealed that the dairy cow performance of cows differed significantly between cows in the four different udder health groups. These differences, in turn, indirectly confirm the interpretation of the four groups. Cows in group A showed the highest performance, which was to be expected as they are healthy based on their low SCC and low DSCC test day results. Cows in group B showed a significantly lower performance than those in group A, which is explainable based on inflammatory processes (i.e. high DSCC values) consuming energy that is then not available for milk production anymore. This confirms that cows in group B might be in the early stage of mastitis. Presences of mastitis pathogens and inflammatory processes has been described in such cows before (Schwarz *et al*., 2011a,b; Pilla *et al*., 2012; Schwarz *et al*., 2020a). Lower performance of cows with elevated SCC have been described before, but we could observe significant differences between cows in groups C and D. Those in group D performed significantly worse than cows in group

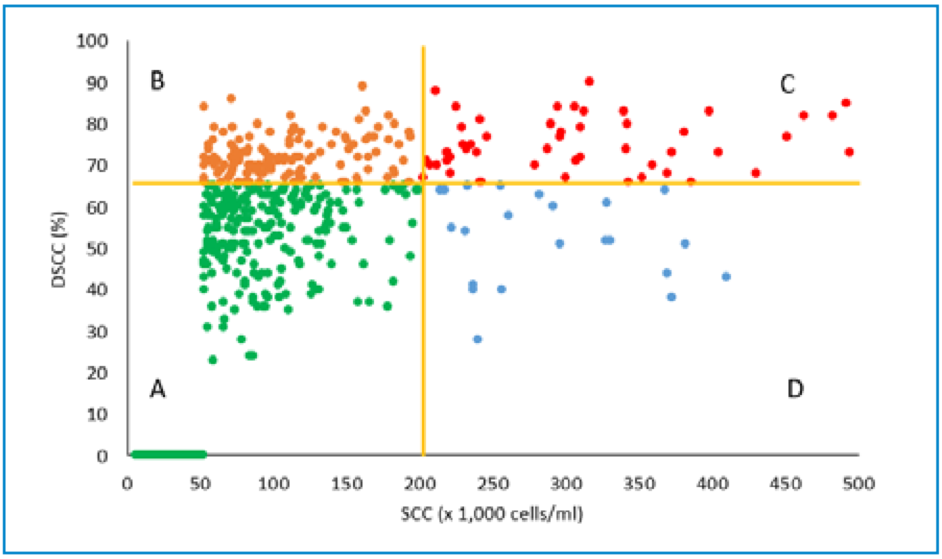

Figure 1. Dairy herd improvement results of one dairy farm (random example) to illustrate the four different udder health groups: A – healthy, B – onset of mastitis, C - (subclinical) mastitis, D – chronic mastitis.

C. Cows in group D are considered to experience chronic mastitis and it is well-know that such chronic infections lead to destruction of milk producing tissue explaining the low performance.

This study including more results from Austria, China, Estonia and Spain has been published elsewhere (Schwarz *et al*., 2020b).

Pilot project to build up practical experience

Based on our data base it became evident that the distribution of cows among the four different UHG varied hugely between farms. We selected farms with different proportions of cows in the four groups and visited them to better understand management practises and associations with the UHG concept. At the same time, we introduced the new DSCC parameter and a new SCC and DSCC-based udder health report to the farms and invited them to join a pilot project where they would get access to the new report for a period of 1 year.

A total number of 11 dairy farms with 150-900 lactating cows agreed to join. Observed changes in terms of proportions of cows in the UHG over time where discussed with the herd managers and clear associations with herd management practises were found. Particularly cubicle management and hygiene as well as milking procedures and hygiene were identified as key factors.

Experiences from pilot phase and next steps

All of the participating dairy farms agreed at the end of the pilot phase that "*the information provided through the new report and the arising possibilities aid in improving animal health and, at the same time, lead to better usage of resources and higher animal performance." Versatile application of the new udder health report was observed as can be seen based on the following quote of herd manager*: "..we already introduced

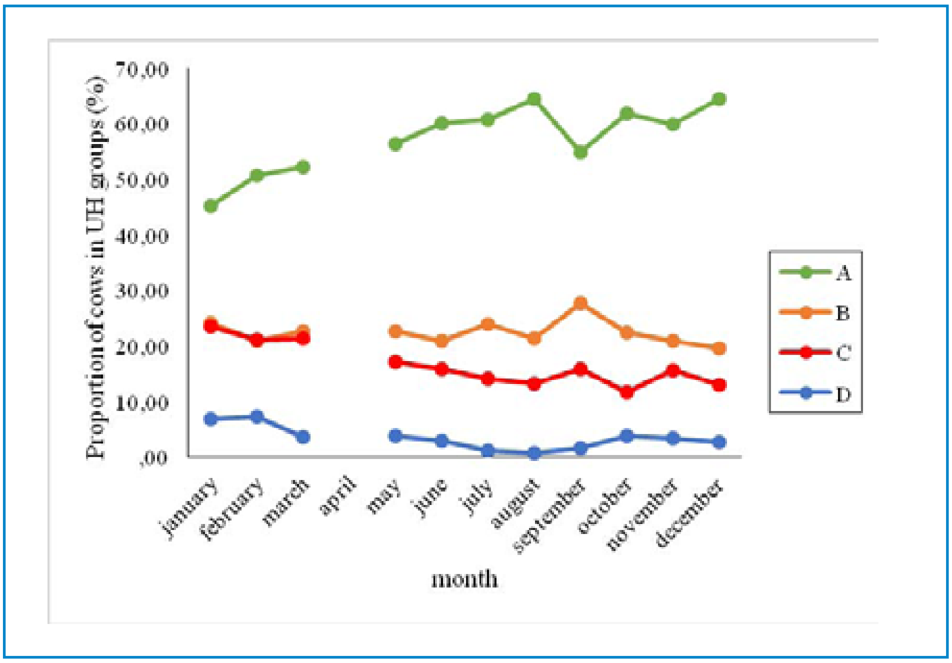

Figure 2. Development of proportions of cows in each of the four udder health groups (A, B, C, D) over the 12 months pilot phase in a herd with 850 lactating cows.

improvements to have cleaner and drier cubicles end of 2019 and could see the positive effects based on the new report in detail, praise our staff and motivate them for keeping up the extra effort. All this resulted in less antibiotic treatments, less wasted milk, better animal health and performance." Herd manager. The positive developments in terms of udder health on this farm are illustrated in Figure 2. Another herd manager reported: "..we use the report two fold: on herd level and on individual cow level. The report helped us to optimise the management of our cubicles and maintain a high level of hygiene and comfort. Daily production per cow and the amount of milk delivered increased. We further use the report to better monitor individual cows, mostly those in UHG B and D. Fresh and late-lactating cows are of particular interest. Cows with stable udder health falling in UHG A before dry-off are dried off without antibiotics." The manager of a 170 cow herd emphasized: "Particularly cows in group B are of interest for us because here mastitis can be spotted at a very early stage."

In a next step, a newly developed online tool (vit verden) for presenting the udder health report based on SCC and DSCC information is being trialled. Subsequently, the tool will be rolled out to all farms participating in DHI programmes.

Conclusions

Mastitis is still a huge challenge on dairy farms and is one of the key reasons for premature culling of dairy cows. Our pilot project on working with a new SCC and DSCC based udder health report revealed that it helps dairy farmers to better manage udder health in their herds. Improvements in terms of dairy cow health, welfare, and performance and less antibiotic treatments were already seen during the pilot project. In the long run, improvements regarding cow longevity and milk quality in general can also be expected.

List of references

IDF, International Dairy Federation, 2013. Guidelines for the use and interpretation of bovine milk somatic cell count. Bull. IDF. 466/2013.

Pilla, R., D. Schwarz, S. Konig and R. Piccinini. 2012. Microscopic differential cell counting to identify inflammatory reactions in dairy cow quarter milk samples. J. Dairy Sci. 95:4410–4420. *https://doi.org/10.3168/jds.2012-5331*.

Schwarz, D., U.S. Diesterbeck, K. Failing, S. Konig, K. Brugemann, M. Zschock, W. Wolter and C.-P. Czerny. 2011a. Flow cytometric differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J. Dairy Sci. 94:5033–5044. *https://doi.org/10.3168/jds.2011-4348*.

Schwarz, D., U.S. Diesterbeck, K. Failing, S. Konig, K. Brugemann, M. Zschock, W. Wolter and C.-P. Czerny. 2011b. Microscopic differential cell counts in milk for the evaluation of inflammatory reactions in clinically healthy and subclinically infected bovine mammary glands. J. Dairy Res. 78:448–455. *https://doi.org/10.1017/S0022029911000574*.

Schwarz, D., D.E. Santschi, J. Durocher and D.M. Lefebvre. 2020a. Evaluation of the new differential somatic cell count parameter as a rapid and inexpensive supplementary tool for udder health management through regular milk recording. Prev. Vet. Med. 181, 105079. *https://doi.org/10.1016/j.prevetmed.2020.105079.*

Schwarz, D., S. Kleinhans, G. Reimann, P. Stückler, F. Reith, K. Ilves, K. Pedastsaar, L. Yan, Z. Zhang, M. Valdivieso, M.L. Barreal and R. Fouz, 2020b. Investigation of dairy cow performance in different udder health groups defined based on a combination of somatic cell count and differential somatic cell count. Prev. Vet. Med. 183, 105123. *https://doi.org/10.1016/j.prevetmed.2020.105123*.