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THE GLOBAL STANDARD FOR LIVESTOCK DATA

Network. Guidelines. Certification.

ICAR Technical Series no. 23

COOPERATION, NETWORKING AND GLOBAL INTERACTIONS IN THE ANIMAL PRODUCTION SECTOR

Proceedings of the ICAR Conference
held in Auckland, NZ,
10-11 February 2018

Editors: J. Bryant, M. Burke, R. Cook, B. Harris,
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June 2018

Preface

The ICAR community met in Auckland (New Zealand) in February 2018. The main two-day conference was preceeded by two days of meetings for its Sub-Committees, Working Groups, and Task Forces (ICAR's Groups) and followed by two days of joint meetings with WCGALP and one day of joint field trips. The main Conference included two days plenary and specialized workshops covering recent developments relevant to the activities of ICAR'S members. This also provided an ideal opportunity to celebrate 20 years of progress since ICAR meet in January 1998 in Rotorua (New Zealand). It was a good time to reflect on the growth of ICAR over the last 20 years which is primarily the result of the dedicated and motivated efforts of the Chairs and Members of ICAR's Groups. The Groups have provided the leadership, the design and overseen the implementation of the services which are now core ICAR functions and deliver a wide range benefits to its members.

ICAR's Groups enable world-wide cooperation on the specialist activities that make-up animal recording. They are organized to cover the full range of animal recording from identification, through milk analysis to functional traits and genetic evaluations for cattle, sheep, goats and camelids. Each Group brings together experts from around the world. By sharing their knowledge and expertise they are able to develop guidelines and services which are valuable to the animal recording community.

The work of the Sensor Devices Task Force is one example amongst the 40 papers published in these proceedings. Steven Sievert (page 21), provided a progress report on the work of the Task Force which is focused on establishing a framework and guidelines to enable ICAR's members to make well informed decisions on the use of the data arising from the explosion in development of new sensor devices.

ICAR in these last 20 years has become the major organization in the animal production sector with establishing rules and standards, specific for the purposes of: identifying animals, the registration of their parentage, recording their performance and evaluating their genetics.

ICAR also encouraged the use of animal records for the purpose of assessing the value of animals and farm management systems and provides a platform for cooperation and collaboration in all activities related to animal performance.

The services provided by ICAR now include: Milk Analyser Certification, the Certificate of Quality, accreditation of genetic laboratories for parentage testing by SNP and STR, Interbeef international genetic evaluations, the GenoEx PSE service, and ICAR accreditation as SNP data interpretation centres.

ICAR is the ISO Registrant Authority for animal ID devices, with more than 170 certified devices. It has granted certification to more than 110 milk meters, helping assure comparable data globally. Its recent Proficiency Test for milk laboratories is an activity where 70 participant laboratories from 35 countries of the five continents compare their performances, reinforcing the international role of ICAR in helping ensure data comparabiltiy and the flow of information around the world.

Genomics technology first burst onto the scene at ICAR conferences in 2008 at the Niagara Falls, USA meeting. Ten years later it has matured to become a routine part of dairy cattle breeding and is now expanding to beef, sheep and the developing world with a number of papers on this topic.

Ensuring the data used in animal recording is of high quality has been a recurring them over all ICAR conferences. 2018 was no exception. These proceedings contain papers discussing data quality in milk testing (Schwartz p. 23), and in a milk recording organization (Kyntäjä p. 179).

One of ICAR's goals is to extend its benefits to more parts of the world. It is gratifying to have papers in these proceeding which come from regions species and farming systems not traditionally part of ICAR. See for example, Monteiro et al page 135, Singh et al page 147, and Duangjinda et al on page 155.

These proceedings are limited to the papers presented during the main ICAR conference. The papers presented during the dedicated Interbull sessions in Auckland are available in Interbull Bulletin[1] No 53 (2018): Proceedings of the 2018 Interbull meeting, and the papers jointly with WCGALP are available on the WCGALP website[2] .

A final acknowledgment to the voluntary commitment of the professionals involved in ICAR who through their efforts and collaboration have demonstrated that the whole is much greater than the sum of its parts.

[1]https://journal.interbull.org

[2]http://www.wcgalp.org/proceedings/2018

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Increasing dairy farm profitability in a changing world of herd demographics

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Summary

The number of dairy farms in the United States has seen a steady decrease from 70,375 herds in 2003 to 41,809 in 2016 while the number of dairy cattle has stayed relatively the same or even slightly increased from 9,103,000 milking cows in 2001 to 9,328,000 in 2016. Wisconsin statistics mirror the national statistics. While cow numbers have also stayed the same or slightly increased, milk production per cow has increased at a rapid pace. Average milk production in Wisconsin has increased from 7,793 kg of milk in 2001 to 10,683 kg in 2016. Herd management, animal health, nutrition, technology and genetic improvements have been and continue to be the main drivers of production increases. Following the national and state trends AgSource herd demographics are rapidly changing as well, herds with 500 Holstein cows or higher represented 27% of cows processed in 2007, while in 2017, this percentage has grown to 55%. Managing herd expansion, implementation of new technologies, feeding rations, etc. requires dairy farms to have a reliable and consistent method of measuring performance and benchmark these numbers against previous years or other dairy farms of equal size or production levels for example.

At the beginning of each calendar year AgSource calculates annual benchmarks based on percentile rankings, herd size, production level and cattle breed. Analyzing the change in these benchmarks over time can provide valuable information about how the change in herd demographics are expressing themselves in herd performance data. Information obtained from these benchmarks are incorporated in herd information management services provided back to the AgSource members. The AgSource Profit Opportunity Analyzer (POA) is the premier AgSource herd analysis tool that utilizes the annual benchmarks and expresses management improvement opportunities on a dollar basis. POA benchmarks used for comparison are customized based on the demographics of the herd. To obtain maximum value, a POA is hand delivered and reviewed by the dairy farm management team in consultation with a trained AgSource outreach specialist.

Although milk recording costs represents a small percent of the overall costs of operating a dairy farm, it is important for producers to be reminded what level of return on investment can be obtained. Analyzing various AgSource herd management performance measures shows that from 2015 to 2017, the average AgSource herd increased production on an annual basis by $40.42/cow, however herds using the majority of herd management reports obtained a $55.37/cow. Herds using the POA were able to obtain a $97.23/cow improvement which is significantly higher than all other AgSource herds.

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Utilizing the data obtained through milk recording to calculate annual benchmarks and expressing differences between a herd with the benchmark as a financial opportunity allows producers of all herd sizes to monitor herd performance and seek opportunities that will increase profitability.

Keywords: profitability, milk recording, demographics.

Introduction

The US Dairy industry has seen rapid changes in the demographics of the dairy industry. The United States Department of Agriculture National Agricultural Statistics Service (USDA-NASS) provides annual data on number of herds, herd size and cow numbers. Figure 1 shows the trend in number of licensed US dairy herds and number of dairy cows by year. In the past 14 years the number of herds has shown a steady decline from over 70,000 herds to now just over 40,000 dairy herds. The number of milking cows has seen some fluctuations but has stayed around 9 million cows with a slight increase in the past 7 years.

Based on this trend, one can question if this has impacted the milk recording industry and how these trends are impacting the services milk recording organizations offer to dairy producers? To answer that question data from AgSource herds was used. Figure 2 shows the change in herd size distribution for AgSource herds comparing 2007 to 2017.

Using the data from Figure 2 and 3, it clearly shows that the herd size distribution has moved towards the larger dairy farms, however the majority of herds (75%) are still less than 200 cows. When reviewing the number of cows processed, the number of cows processed for herds under 200 cows has declined from 52% to 25% and herds over 500 cows now represent 10% of the herds but 55% of the cows that are processed.

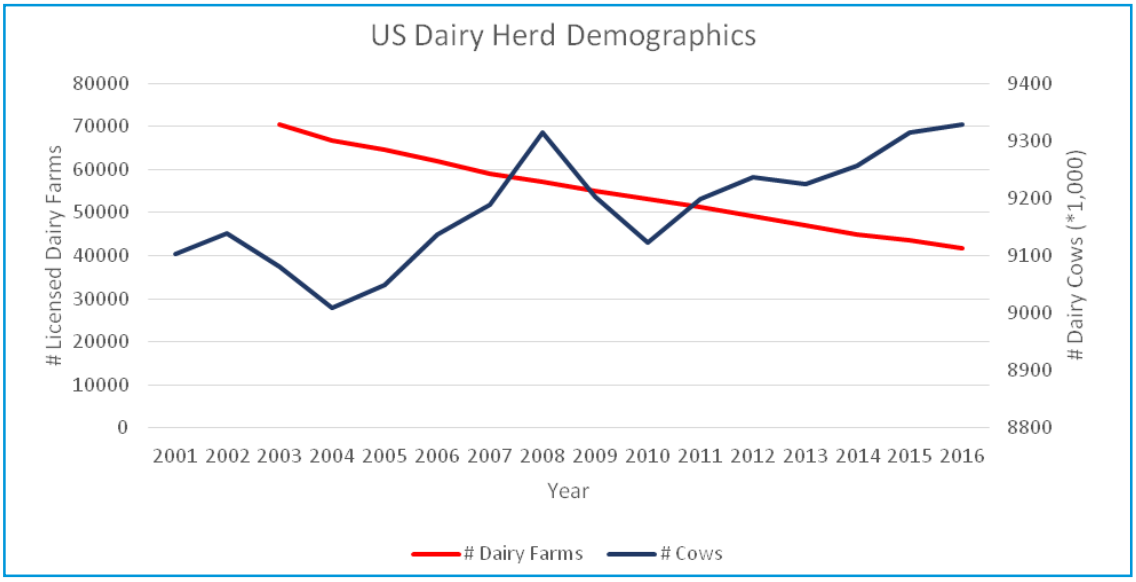

Figure 1. US Herd Demographics (Source: USDA-NASS).

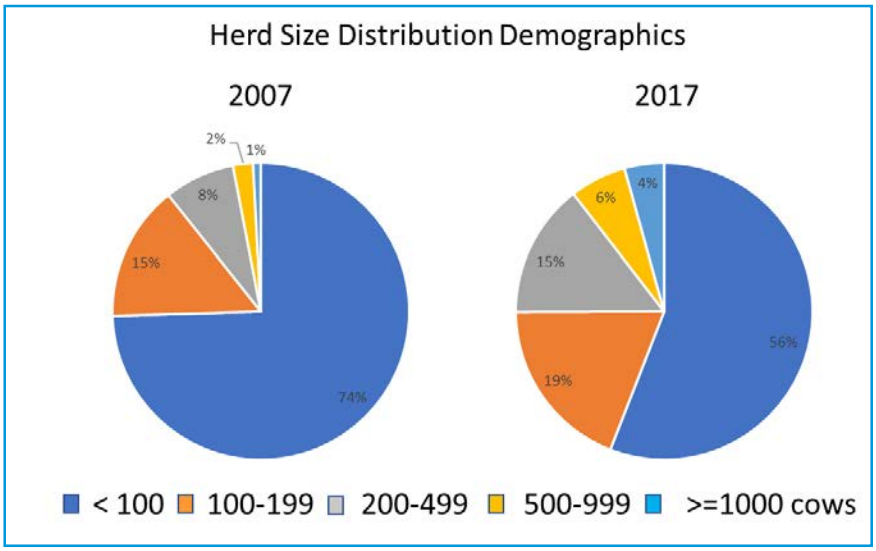

Figure 2. AgSource Herd Size Distribution Demographics 2007-2017.

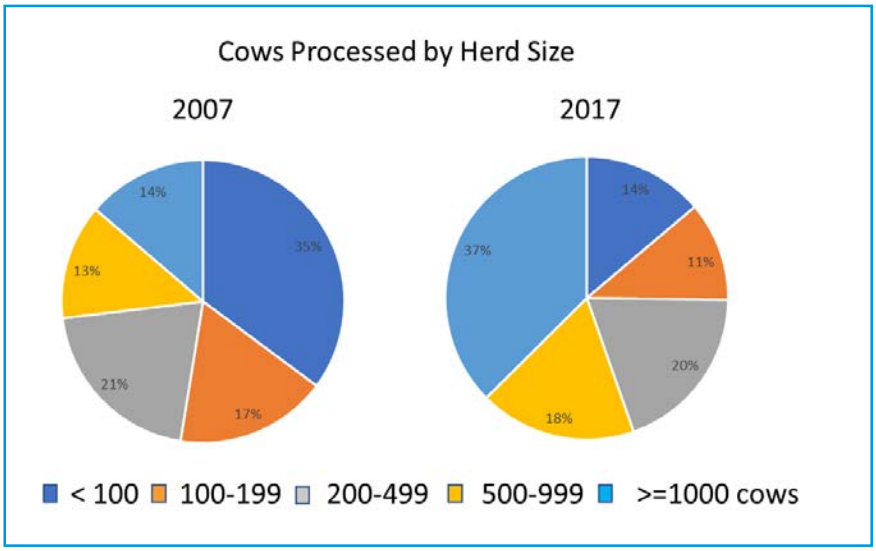

Figure 3. AgSource Cows Processed by Herd Size 2007-2017.

Coinciding with the change in herd demographics, production levels have sharply increased. Figure 4 shows the increase in average annual milk production in kg/cow for the United States and Wisconsin (USDA-NASS Statistics). AgSource 365 day average milk production for Wisconsin herds shows similar trend only at a higher production level compared to all Wisconsin herds.

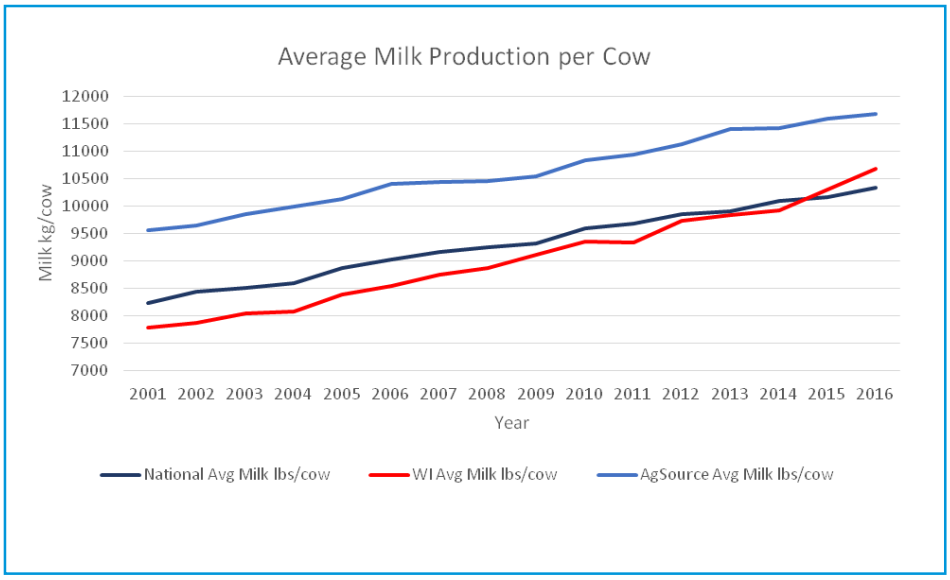

Figure 4. Average Milk Production per cow (Source: USDA-NASS and AgSource).

Key Performance Area Measurements

Since 2012 AgSource has been calculating an annual set of reports that provides summarized values for key performance areas (KPA's). This process is run at the start of each calendar year by taking a snaphot of the past year's performance of the herds that AgSource serviced. Information is summarized based on breed, herd size or production level. Within each of these groups there is a further breakdown showing the top 80th percentile, average or bottom 20th percentile. The herd size and production level statistics are currently only calculated on herds that have Holstein cattle. Depending on the herd size or production level, producers can use these numbers to compare their performance with herds that are of equal size or production level. The overall report uses 114 individual measures broken out into 18 different areas. Table 1 shows an example of the Udder Health KPA's for herds producing greater than 13,608 kg of milk.

One can argue that when a herd finds itself at the top production level they will find little opportunity to make improvements. Considering that changes in herd management or external factors can have a significant positive or negative impact on overall herd performance from year to year, monitoring where a herd ranks can pay dividends and make one aware of possible changes. Another consideration is that as other herds continue to make improvements these KPA's continue to improve over time as well. A herd may be in the 80th percentile but a year later they may learn that peer herds have made more improvements and although the herd's performance is the same, they have slipped down compared to others. An example can be found in how the Udder Health KPA's have changed in a short period of time. In 2012 a herd producing over 13,608 kg of milk would have to be below 144,000 weighted average SCC to be in the 80th percentile, in 2017 a herd would have to have improved udder health and be below 124,000 in order to stay in the same 80th percentile group. This is a 13% improvement in only 5 years.

Table 1. AgSource Udder Health Key Performance area example.

Udder health	80th Percentile	Average	20th Percentile
Weighted average SCC 1	24	178	227
Bulk tank weighted average scc	118	175	214
Percentage of of herd >= 200000 scc	12	16.9	20
Percentage of of herd new infections	6	8.8	11
Percentage of infected cows that are chronic	48	55.1	61
Herd linear score	1.9	2.3	2.6
1st lact linear score	1.6	1.8	2.1
2nd & >lact linear score	2.1	2.5	2.8
Percentage of heifers infected at first test	9	12.9	17
Percentage of fresh cows - new infections	7	12.1	16
1st lact weighted average scc	79	111	136
2nd & >lact weighted average scc	154	226	286
Percentage of dry cows cured	85	77.8	71
Percentage of dry period failure to cure	15	22.3	29

Turning Key Performance Area data into 9rofit opportunities

Using the annual KPA's, AgSource developed a product called the Profit Opportunity Analyzer (POA). The POA utilizes the summarized values as benchmarks and expresses the difference between the herd's performance and the peer group selected as a dollar opportunity value. The POA is customized for each herd by using financial information specific to that herd. In addition, the POA is hand delivered and an AgSource representative explains the results to the farm team. Typically the management team may have follow up questions that require digging deeper into the herds data using monthly management reports or using the online reports in MyAgSource. The example below in Figure 5 shows how the KPA values shown in Table 1 were incorporated as benchmarks in the Udder Health Management analysis and how the dairy producer has a $74,454 opportunity in additional revenue if they can make the improvements that will allow them to perform similar to the top percentile herds.

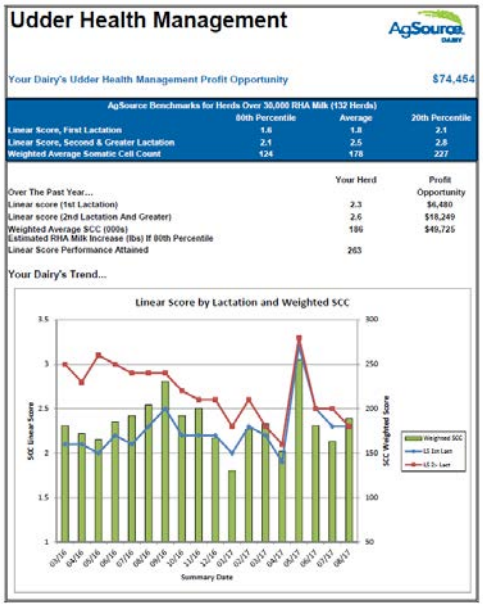

Figure 5. POA Udder Health Management Summary.

Return on investment

As US dairy herds have to face a volatile milk market and deal with increasing costs related to supplies and equipment it is important to realize that participation in the US milk recording program is a choice and organizations such as AgSource need to demonstrate that the informational tools provided to the dairy producers add value and have a positive impact on profitability.

AgSource offers a variety of management tools and dairy producers choose the products they wish to receive. At the basic level, dairy producers only wish to receive cow attention lists and a herd summary report, however on the opposite side of the scale there are dairy producers that want to use the full array of herd management tools that AgSource can offer including the Profit Opportunity Analyzer.

Recently AgSource staff evaluated the benefits that AgSource members are able to receive from utilizing the products and services that AgSource can provide. Benefits were measured as improvements seen over a 3 year period in several key performance areas. Economic values were based on the POA. Since there is no information readily available on herds that do not participate in a milk recording program, the control group that was chosen comprises all AgSource herds. Two groups were defined for comparison, the first group are AgSource herds that use the majority of available herd management reports (or MyAgSource if electronic) and the second group are the herds that utilized the majority or reports and also obtain a POA on an annual basis. Table 2 shows the results.

Based on these results, herds that utilize more AgSource products and services have been able to achieve bigger gains in production and other herd management areas. Herds that use the POA and work closely with the AgSource education and outreach staff made twice the gains the average AgSource herd made over the same three year time span.

Table 2. Annualized overall revenue increase comparison.

Annualized Overall Revenue increase based on production and SCC			
	All Herds	**Reports**	**Reports + POA**
Production Improvement	$40.42	$55.37	$97.23
SCC Bonus	-$0.02	$0.12	$0.46
Annualized Improvement by Management Area			
Peak Milk	$92.08	$106.75	$222.17
Udder Health	$3.04	$7.28	$10.49
Reproduction			
Pregnancy Rate	$4.17	$6.07	$13.10
Days Open	$4.59	$4.67	$10.16
Transition	$5.17	$6.33	$29.17
Dry Period	$3.33	$3.10	$7.36
Genetics			
Cow	$41.67	$49.00	$53.67
Service Sire	$73.67	$85.67	$85.33

Conclusions

The US dairy industry will continue to consolidate and herds will continue to getter bigger. The adoption of new technologies such as animal monitoring systems, diagnostics and greater use of robotic milking systems will drive the evolution of the traditional milk recording organizations. Inclusion of other data sources with the traditional milk recording systems will further enhance the products and services that milk recording organizations can offer to dairy producers. Tools such at the AgSource Profit Opportunity Analyzer have shown that they can utilize large numbers of individual cow data and turn these into benchmarks and combine with research results and financial data to present comprehensive but easy to understand herd management decision support tools.

Independent validation and certification of analytical methods

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Summary

Routine testing of milk and milk products on quality and compositional parameters is often performed with alternative methods. An independent validation of an alternative method is key to demonstrate that the method is suitable for application in testing for regulatory purposes, with milk payment and/or with milk recording. Independent validation is an assessment of the performance of the method by an organisation not involved in its development. During the validation process it is established whether the alternative method complies with beforehand stated requirements. Subsequent certification comes with a tangible declaration on the suitability of the analytical method for the intended purpose. It serves to find adoption of the method and its results with laboratories, public authorities, food industry and other relevant stakeholders.

Keywords: milk, analytical methods, validation, certification, independent

Introduction

Milk composition and quality are important parameters in global dairy production. In many milk producing countries it is required that laboratory methods for milk testing comply with criteria published in international documents, such as ISO|IDF international standards, AOAC methods, national or regional regulations, etc.

Routine testing of the compositional parameters in raw milk, such as fat, protein, lactose and urea content, and other parameters, such as total bacterial count and somatic cell count, is in many countries performed with alternative analytical methods. The use of an alternative method, often high-throughput instrumental methods, is acceptable when the method is fully validated by an independent party, including a comparison against the reference method. Since independent validation is a critical factor for the acceptance of an alternative method, the validation criteria are described in official documents, following the requirements published in international standards, e.g. ISO|IDF standards. The validation process and results can be made subject to evaluation by a certification organisation, providing a formal statement on the analytical performance of the alternative method and its fitness for purpose.

Independent validation

Independent validation is a process of establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled (ISO 16140-1:2016).

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Independency can be assured via conducting the validation activities by a separate organisation which did not contribute to the development of the analytical method. A major advantage of an independent validation is that the group performing the evaluation is unbiased and emotionally or economically not involved in the method (White paper, 2001). In complex validations it may be difficult for the manufacturer to objectively evaluate the obtained validation results. An independent third party might easier identify issues that have escaped the attention of the developer.

Often the process of an independent validation is monitored by a certification organisation, best in conformity with internationally accepted and documented protocols. A certification organisation relies usually on several entities, e.g. a board, a general secretariat and a network of sub-contractors: laboratories, reviewers and auditors. The certification organisation works with appointed expert laboratories having traceable competence, which prepare and execute the validation, as well as analyse, report and often evaluate the results. The validation procedure and results are reviewed by an (international) technical committee of experts in the field. A certification board closely follows and monitors the whole process and can declare its confidence of the findings by issuing a certificate (Figure 1).

The validation of an alternative method is assigned to an expert laboratory. The process generally consists of two stages:

- an in-house validation or method comparison study, and
- a method confirmation study or an inter-laboratory study.

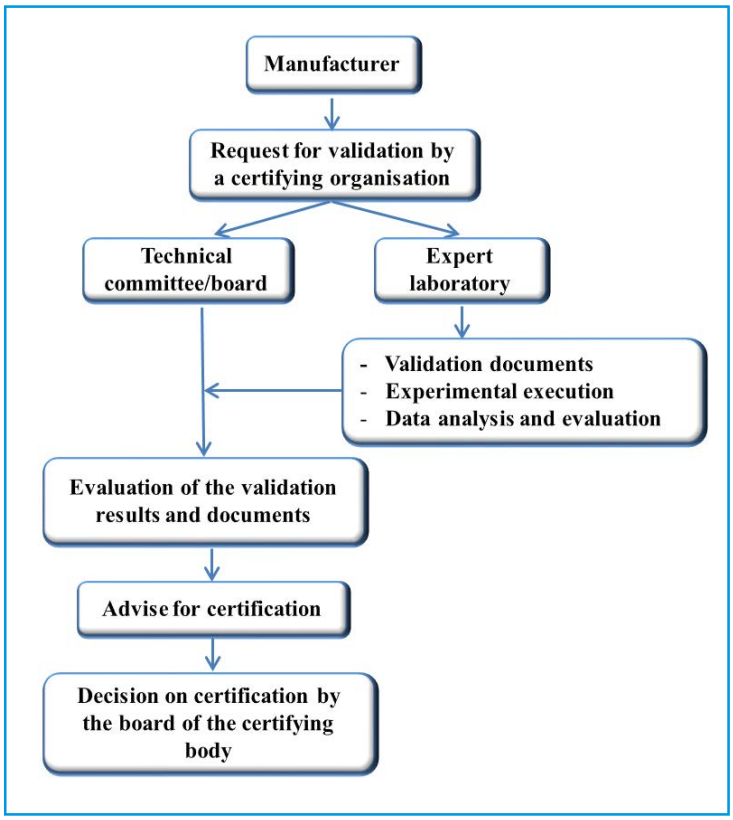

Figure 1. Schematic presentation of the general principle of a certification process (examples MicroVal, AFNOR, NordVal and ICAR).

The method comparison study demonstrates the performance of the method under validation and checks compliance with the stated acceptability limits. Different performance characteristics are evaluated for qualitative and quantitative alternative methods. For example, the relevant characteristics for qualitative microbiological methods are sensitivity, detection limit, inclusivity and exclusivity. For the evaluation of quantitative instrumental methods these are stability, linearity, repeatability, carry-over and limits of quantification. The estimation of the accuracy of the alternative method against the reference method is done with representative samples, measured with both methods. Moreover, potentially influencing factors affecting the relationship between alternative method results and reference method results are examined.

The precision characteristics of the alternative method, when executed at different user laboratories, are demonstrated by either a method confirmation study or an inter-laboratory study. By a method confirmation study the alternative method is operated under routine conditions for several weeks at several laboratories. Results are obtained with representative routine samples as well as with pilot samples and, if relevant, other check samples. The performance of the method in terms of repeatability, reproducibility and stability is evaluated in each laboratory separately and overall. During this part of the validation study the method is also evaluated for general convenience aspects such as speed, consumables, user-friendliness, security and robustness as well.

When the alternative method is already in routine use, its performance could be demonstrated by an inter-laboratory study. Sample sets are prepared by a organising laboratory, usually the expert laboratory, and measured at several laboratories where the alternative and (when required) the reference method are operational. The performance characteristics, e.g. repeatability and reproducibility of the alternative method as well as the agreement of the results with results obtained with the reference method, are evaluated by the expert laboratory.

The expert laboratory collects and analyses the results of the method comparison and method confirmation/inter-laboratory studies and prepares a validation report. The validation report is evaluated by a committee of experts appointed by the certifying body. With a positive advice from the expert committee a certificate for performance may be granted for the alternative method. The certificate demonstrates to the end users that the alternative method has been thoroughly tested using an approved and standardised procedure. It means that the method can be used confidently and with the knowledge that the results of that method will be accepted by the national and international authorities (Zegers, 2012).

Examples of independent validations for milk testing

For regulatory purposes and milk payment total bacterial count and somatic cell count are common parameters. National and international authorities in many geographies require proof on proper functioning of the applied methods through independent validation. An example is in the validation of alternative methods for the enumeration for total bacteria and somatic cells as required by EU Regulations 2074/2005 and 1664/2006. The test procedures for these validations are described in two documents issued by the European Reference Laboratory for Milk and Milk Products (EURL MMP document 2011; EURL MMP document 2013), following the relevant ISO standards (ISO 8196-3|IDF 128:2009; ISO 13366-2|IDF 148-2:2006; ISO 16140-2:2016; ISO 16297|IDF 161:2013). Recently, several instruments for total bacterial count and somatic cell count have been granted with MicroVal certificates (www.microval.org).

For the purpose of milk recording, the ICAR Certificate of Quality program requests independent validation of alternative methods for measurements of milk compositional parameters. The ICAR certification process follows the ISO protocol for validation of alternative methods (ISO 8196-3|IDF 128-3:2009) and the obtained results should comply with requirements in the relevant ISO standards (ISO 9622|IDF 141:2013, ISO 13366-2|IDF 148-2:2006).

Conclusion

The globalising milk market requires confidence in an uniform, reliable and traceable way of testing of milk characteristics all over the world. Independent validation of analytical methods demonstrates and assures that alternative methods are fit for purpose. Subsequent certification provides tangible proof of adequate performance. It paves the way for acceptance of a method and the results by public authorities, food industry, laboratories and end users.

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France Génétique Elevage management quality system: an example of multi companies system, to serve a community of organizations, based on ISO 9001: 2015 standard

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Summary

During the last 15 years, France Génétique Elevage (FGE) has developed a quality management system in compliance with ISO 9001 standard, in process mode, involving more than 180 independent organisations and 6 different professional activities, in the field of cattle genetic improvement. FGE has developed a robust quality system structured with: a quality manager employed by FGE; six pairs of process managers; one quality correspondent in each organisation. Every year, each activity is subjected to a process review. The conclusions of these reviews are compiled for evaluation by FGE management and the synthesis is then presented to the FGE Board. Despite a long-lasting deployment phase with, in the end, a somewhat smaller scope of extension than expected, the resulting scheme has enabled participating organisations to adopt quality measures at a reasonable cost. FGE has thereby been able to develop harmonisation and security of data processing methods to produce accurate breeding values. The system has brought positive results in benchmarking, building trust between partners, and providing incentive for participating organisations' top management. Merging of organisations and management of common subcontractors were therefore greatly facilitated. This system has been officially recognised by the French Ministry in charge of Agriculture and by ICAR (International Committee for Animal Recording) through its Certificate of Quality.

Keywords: genetic improvement, quality, management, ISO 9001, collective.

Introduction

Estimations on the genetic merit of animals is a fundamental piece of information in the management of populations and in the selection of cattle for reproduction. Elaborating these estimations is a long, complex procedure and requires the interaction of many processes: animal identification, pedigree recording, performance recording, management, verification and sustainability of data, estimation and computation methods as well as the process of making the results available to users.

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At every stage, the accuracy of the data is crucial (Patry, 2011). These processes must therefore be controlled and monitored to guarantee the accuracy of the genetic values for users and clients while at the same time building trust between operators.

In France, each process is deployed by specialised organisations over a specific geographic area. There are over 200 of these, varying greatly in size (from 1 to more than 500 employees), complementary and interdependent, sharing one same data system. This results in a genetic architecture for livestock that is mutual, multibreed and multi-species, powerful yet complex. The "Dispositif Génétique Français" (French livestock genetic scheme-hereafter abbreviated to "DGF") needed to develop its own quality management system (QMS) as it brings together 180 organisations (Journaux et al, 2017).

Organisation of the quality management scheme

The year 2006 saw the creation of the umbrella organisation France Génétique Elevage (FGE) in parallel with the French Ministry of Agriculture retiring from operational management of the DGF. In this context, the industry professionals decided to maintain the collective structure of the DGF and to strengthen its function with a collective QMS in compliance with ISO 9001 standard, at a reasonable cost and with optimal efficiency. In this perspective, FGE decided against certifying its QMS, preferring to acquire, while still referring to the ISO 9001 standard, recognition by the ICAR Certificate of Quality (ICAR, 2017). The architecture of the QMS in FGE was of an "associated organisations" type, where the organisations are not individually certified but agree under contract (with FGE) to apply a package of quality measures. FGE could then implement its collective QMS which is compliant with requirements, efficient and in continuous improvement.

A pilot phase was rolled out over 3 years (2006-2008). This enabled the elaboration of a complete tool package: professional guidelines, a training standard for the role of Quality Correspondent (QC) within each organisation, communication with local management, training and qualification of internal auditors, the internal audit procedure and its frequency, audit monitoring and the self-declaration of compliance procedure, managing the flow of exchanges between FGE and the partner organisations, the QMS review process undertaken by management, with the support of the quality manager (QM) during the initial review of the processes by profession and the initial review of management.

Late 2008, 139 organisations had signed their contract. The deployment of the scheme continued with gradual commitment from the other structures, the introduction of continuous improvement measures and the extension of the field of action.

In addition, from 2010 to 2016, two audits of the system were undertaken in order to guarantee the overall efficiency of the scheme and its compliance with the ISO 9001 standard, as well as to facilitate the acquisition and renewal of the ICAR Certificate of Quality.

In 2017, the QMS covered 6 professions: identification, parentage recording an verification, performance recording in beef and dairy cattle, genetics data system, evaluation and publication of breeding values. It was deployed by 183 different organisations. This translated, for the professionals involved in the QMS, as 5 000 participants: 2 500 operators, 1 200 advisors, 650 administrative staff, 350 engineers or managers.

Results

For 10 years, the FGE quality scheme has proved itself and has become a long-term management tool as well as a tool for progress, thanks to its organisational achievements, real improvement in quality of service, performance improvement within organisations and the recognition within the scheme as well as externally.

The organisational achievements involved the drafting of the professional guidelines, structured by process, which are regularly updated and which form the basis of the individual commitment of all the participating organisations.

The professional processes are reviewed annually and indicators are calculated for each organisation involved.

The annual management review looks at the results of the process reviews and decides on appropriate orientations for the future work of FGE.

This organisation has led to a steady and significant reduction in the number of non-compliances recorded as well as a substantial improvement in performance, measured objectively.

Furthermore, the participation of the organisations and their commitment to the scheme has helped to obtain recognition of their compliance with standards from clients, interested parties (French Ministry of Agriculture) and external bodies such as ICAR.

Finally, these successes were achieved at a very reasonable cost. Investment in the drafting of the professional guidelines was estimated at 792 000 • to be paid off over 10 years, that is 396 • per organisation per year. The average collective running costs is 350 000 • per year, that is, thanks to the pooling of all cross-cutting activities, 1 800 • per organisation per year. An annual average sum of 10 000 • per organisation is added for individual running costs.

Discussion

Beyond the general advantages of implementing a QMS with the aim of continuous improvement and the consideration of clients' expectations, this quality approach means that organisations share a common quality policy and benefit from better defined interrelationships as each organisation makes a commitment to apply the policy and the quality objectives set by the collective management of FGE.

The QMS helps those structures which, due to their size or their internal organisation, would not have taken the initiative of a quality approach on their own. This is only possible thanks to the motivation of the major participants (directors, managers, quality correspondents) as well as to improved communication across all levels.

The QMS of FGE also allows organisations the freedom to implement their own quality management system of ISO 9001 standard while at the same time giving them access to technical indicators and necessary audit elements for the collective steering of the project.

The DGF, organised by profession, is quite specific. However, quality schemes in compliance with ISO 9001 standard are generally quite well known to our international contacts. In this way, thanks to the QMS, FGE has presented the organisation of the DGF in a more accessible way and has helped to assure potential clients of the quality of work carried out in France.

The adjustment from drafting rules in the form of protocols towards an architecture of processes necessitated more clarity and increased responsiveness thanks to a simplified update of the professional guidelines (change from strictly imposing a method

to requiring results through monitoring). The guidelines were produced by a writer from the industry and a writer from the Livestock Institute (Institut de l'Elevage), both drawing on a group of industry experts, which guarantees the efficient integration of regulatory developments as well as field requirements. Finally, the centralised method of managing the professional guidelines via intranet greatly assisted in keeping the documentation up-to-date and accessible.

The process reviews gave the organisations access to a comparative analysis of data and results for the management of their activity, as well as the homogenisation of practices and an anticipation of new challenges and opportunities. Clearly stated conclusions and decisions as well as the follow-up of action plans quickly became new and indispensable tools for the managers steering the scheme. At first, the managers of the individual organisations were not heavily involved in the deployment of the QMS but as they gradually saw the advantages in this way of functioning, they took ownership to the point of integrating it as a management tool for their own particular organisation.

The management review, carried out annually, is an important factor in motivating the industry partners to adopt this methodology and to deploy it over their professional sector. It increases understanding of the different professions between the respective industries.

By analysing the results of process reviews and by networking amongst QCs, FGE has created a community of shared practices, supplemented by the contributions of other interested parties. FGE has built trust between the partners of the DGF and reinforced the coherence between the collective management (vision, strategy, policy, objectives) and "production" performance (indicators, results, action plans, improvement).

It is also important to underline the indirect advantages of this scheme. Since its implementation, the QMS has facilitated the merging of organisations: when organisations share the same professional guidelines, merging has been greatly simplified not only for the operators on the ground but also for management.

Certain subcontractors were tracked and evaluated in a coordinated manner by a large number of organisations. This coordinated tracking helped to reformulate mutual requirements and expectations in a much faster and efficient way compared to uncoordinated individual actions.

However, this type of organisation presents certain limits. Due to its deployment over 200 organisations, the QMS required about 5 years to define itself and to be distributed on the ground. The stages of commitment for an organisation up to the first declaration of compliance took on average 3 years in each profession.

The actual organisation of the DGF, divided into activities by type of organisation, does not facilitate the concept of products and clients because the structures involved are clients and suppliers with each other, where each organisation contributes by way of intermediary products. The final product, which is the genetic evaluation, is not the direct product of the organisations on the ground. This caused difficulty in organising efficient customer service for the end client.

In the same way, this multi-professional organisation does not facilitate the systematic and standardised recording of complaints: the causes may be complex to analyse or may be outside the scope of responsibility of the organisation that identified them. This does not take anything away from the efficiency in treating complaints, in fact well managed on a day-to-day basis, but it did prevent the collective system from having the means to carry out a global analysis for the improvement of customer satisfaction.

Finally, it has not been possible to implement the QMS of FGE in all sectors or professions. The reasons are varied. The different families of organisations who committed first were the ones that had regulation requirements or were under pressure internationally. Then followed the groups that were in subcontracting positions with a large number of operators. In contrast, the families that were more autonomous in their activity, with no regulatory constraint to adopt a QMS or that faced major organisational changes, did not take the step to commit. For the small ruminant sectors, the limited number of operators, their smaller size and a more centralised decision-making framework resulted in a lack of commitment to quality management beyond animal identification.

Conclusion

The structure of livestock breeding in France is marked by a strong collective culture which has enabled the substantial pooling of financial and human resources and tools. This has helped to bring French genetics to its current level of international recognition.

The QMS of FGE has been running for 10 years with the aim of improving the management of this organisation and its effectiveness has been proven. It is recognised, on a national level, by the Ministry in charge of Agriculture, and on an international level, where it was fundamental to FGE obtaining the ICAR Certificate of Quality.

After investment since 2003 and deployment since 2008, the QMS has succeeded in the two objectives that were assigned to it: to demonstrate the professionalism and effectiveness of the DGF through its umbrella organisation FGE and all the participants, and to ensure optimal investment and running costs.

The QMS of FGE is a successful example of the declination of the ISO 9001 standard to a large multi-enterprise organisation. The 183 companies involved in the QMS have all gained a large quality capital in the form of knowledge, experience and know-how, which constitutes a precious asset in their restructuring within the framework of the European zootechnical Regulation.

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ICAR and sensor devices - a progress report on the development of ICAR guidelines for certification, routine maintenance/calibration and data usability standards for sensor devices for livestock

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Summary

After the theoretical work of the Accuracy Task Force was completed in 2016, ICAR established a Sensor Devices Task Force (SD-TF). This task force is charged with development of methodology to classify or quality sensor devices; determine certification and routine maintenance or calibration procedures for these devices; and finally disseminate new ICAR guidelines for the suitability or usability of data collected by these devices. The initial work of the SD-TF is focused on measures of milk volume and quality but the scope of the SD-TF is not limited to either measure on milk nor is it limited to dairy cattle. To understand the needs of both ICAR members and device manufacturers, the SD-TF conducted a survey of both industry groups to identify challenges and develop specific plans of action moving forward.

The SD-TF will present a summary of work completed, the proposed timeline moving forward and the limitations as perceived at the present time. Working with five key groups of measurements as identified by the industry surveys, development of new ICAR guidelines and supporting best practices for data collection continue with the goal of addressing the challenges and opportunities associate with sensor devices that face member organizations. These key groups of sensor measurements include:

- Milk yield and composition.
- Milk flow rate and duration.
- Live body measurements.
- Live activity measurements.
- Feed efficiency measurements.

Contributions from other ICAR subcommittees and working groups will be important to completing this work in a cooperative and timely fashion.

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Quality assurance tools in milk-testing laboratories: The view of an instrument manufacturer

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Summary

The objective of this work is to provide an overview on a) the validation and certification of FOSS's milk analysers, b) working with FTIR technology/data and standardisation as part of quality assurance as well as c) quality assurance for new milk-testing parameters.

Besides extensive internal as well as field testing of milk analysers, FOSS seeks for thorough validation and certification of its instruments according to internationally accepted standards, like ISO 8196, performed by independent organisations. In terms of the Fossomatic™, legislation dictates that only certified milk analyser are approved for enumeration of somatic cell count in payment samples in the EU and the USA. Beyond that, additional national approvals might be required in some countries. In terms of the analysis of the composition of milk primarily approvals on national level are required so far. The newly available ICAR certification service for milk analysers, however, covers the validation of both somatic cell counter and milk component analyser. It is further thought to replace national approvals with the ICAR certification and thus contribute to the optimisation of the validation and certification process of milk analysers around the world. Besides fulfilling regulatory requirements, the validations and certifications can generally be used to demonstrate the performance of an instrument. Furthermore, the international ICAR validation would allow laboratories to implement new instruments by simply verifying them according to ISO 17025 using reference materials and proficiency tests.

Fourier Transform InfraRed (FTIR) spectrometry as applied on MilkoScan™ instruments is nowadays a commonly used technique for analysis of milk samples on fat, protein, and lactose and more recently other minor components such as urea, BHB, and acetone. Beyond that, the spectra data are more and more utilised to describe a dairy cow's health and welfare status and possibly other conditions as precisely as possible. In this context, the standardisation of spectra is of outmost importance to make data comparable and transferable. Furthermore, actual possibilities and limitations of FTIR technology need to be considered.

The implementation of new parameters on high-throughput milk analysers for laboratories often requires the availability of appropriate reference methods to allow confirmation of accuracy of results generated on the high-throughput instrument. In the example of ketosis screening, which is based on the prediction of BHB (and acetone) using MilkoScan™, an official reference method is not available.

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However, in Canada and France, quality assurance programmes based on wet-chemistry methods were developed and are used successfully since. In the example of the new differential somatic cell count (DSCC) parameter a reference method is lacking. But initial work on this subject has begun within the International Dairy Federation (IDF).

In conclusion, FOSS helps to allow quality assurance working with its milk analysers by obtaining different certifications, offering a standardisation concept for FTIR analysers, and supporting the development of analytical methods, reference materials, and proficiency testing programmes. Raw milk samples hold a wealth of valuable information that can help us to make significant improvements in the dairy milk supply (both milk quality and dairy herd management). Hence, the development of new parameters, associated quality assurance tools, and effective communication of data are clearly in the interest of and supported by FOSS.

Keywords: Daniel Schwarz, quality assurance, instrument certification, milk analysis.

Introduction

Laboratories providing analytical services need to be able to demonstrate to their customers that the results provided are precise, accurate, and equivalent. In this context, regulatory requirements need to be fulfilled and quality assurance procedures are key. According to ISO 9000 quality assurance is defined as "part of quality management focused on providing confidence that quality requirements will be fulfilled". Quality assurance is further described as the systematic measurement, comparison with a standard, monitoring of processes and an associated feedback loop that confers error prevention.

The objective of this work is to provide an overview on how FOSS supports milk-testing laboratories in terms of meeting regulatory requirements as well as establishing quality assurance procedures. Specifically, the paper provides information on a) the validation and certification of FOSS's milk analysers, b) working with FTIR technology/ data and standardisation as part of quality assurance as well as c) quality assurance for new milk-testing parameters.

International certifications of milk analysers

Various different international certifications/approvals of milk analysers are available. Briefly, the EU RL (European Union Reference Laboratory) certification in the EU as well as the NCIMS (National Conference on Interstate Milk Shipments) approval in the USA are required for Somatic Cell Count (SCC) analysers when they are used for payment purposes. In terms of milk component analysers, specific national requirements do not to be fulfilled, e.g. in France. Instruments for bacteria counting must be approved by EU RL as well as NCIMS for official/regulatory use in EU and USA, respectively, too.

Somatic cell counter - Fossomatic

The certification procedure is carried out by the organisation Microval in the EU. In this context, a laboratory with EU expert laboratory status is testing the somatic cell counter according to ISO 13366-1, ISO 13366-2, and ISO 8196-3 (IDF 148-1, IDF 148-2, IDF 128-3). The test results for Fossomatic 7 and Fossomatic FC and the respective specifications of the International Dairy Federation (IDF) are summarised

in Table 1. All IDF specifications were met and both Fossomatic 7 and Fossomatic FC got certified (Fossomatic 7 DC currently pending). The certificates and test reports are available on the following website: *http://microval.org/en/issued-certificates/*.

In the USA, somatic cell counters are mainly tested for their accuracy and repeatability. As a results of the NCIMS approval, the so called 2400 form is published on the following website: *http://ncims.org/forms/*. Laboratories doing payment testing are operating their instruments according to the 2400 form. The instruments Fossomatic 5000 and FC are approved (Fossomatic 7 and Fossomatic 7 DC currently pending).

Table 1. Overview on test parameters and results for Fossomatic 7 as well as Fossomatic FC and IDF specifications.

Item	Fossomatic 7	Fossomatic FC	IDF specification
1. Repeatability (r) in % per cell count level			
low (100 k)	11	16	< 17
medium (500 k)	5	11	≤ 11
high (1,500 k)	3	8	< 8
2. Carry-over (CO) in % per cell count level			
low (500 k)	$CO_{H/L}$ = 0.14; $CO_{L/H}$= 0.48	$CO_{H/L}$ = 0.45; $CO_{L/H}$= 0.28	< 2
medium (1,000 k)	$CO_{H/L}$ = 0.07; $CO_{L/H}$= 0.14	$CO_{H/L}$ = 0.21; $CO_{L/H}$= 0.05	< 2
high (3,000 k)	$CO_{H/L}$ = 0.05; $CO_{L/H}$= 0.32	$CO_{H/L}$ = 0.13; $CO_{L/H}$= 0.14	< 2
3. Linearity (r_c) in %	1.8	1.7	< 2
4. Lower limit of quantification	17 k cells/ml	37 k cells/ml	-
5. Upper limit of quantification	10.000 k cells/ml	10.000 k cells/ml	-
6. Intra-laboratory reproducibility ($R_{intra-lab}$) in % per cell count level			
low (50-200 k)	11	16	< 19
medium low (201-400 k)	9		≤ 19
medium (401-650 k)	9	11	< 14
medium high (651-1,000 k)	7		≤ 14
high (1,000 -1,500 k)	10	7	< 11

Milk composition analyser - MilkoScan

Milk composition analysers must be validated according to ISO 8196-3 (IDF 128-3) and the CNIEL (Centre national interprofessionnel de l'économie laitière) specifications in France. MilkoScan 7 RM and MilkoScan FT+ were tested for accuracy, repeatability, linearity, carry-over, and stability (parameters fat and protein each) and all results obtained were conform with the specifications (Table 2).

Bacteria counter - BactoScan

The BactoScan FC/FC+ was tested in the EU as well as the USA. All test results were within the specifications of ISO 4833-1, ISO 4833-2, ISO 16140-2 (Table 3). While a certificate and a summary of the test report are available on Microval's website (*http://microval.org/en/issued-certificates/*), a 2400 form for BactoScan FC/FC+ is published on NCIMS's website (*http://ncims.org/forms/*).

Table 2. Comparison among milk composition analysers.

Item	Milkoscan 7 RM	Milkoscan FT+	IDF specification
1. Accuracy ($s_{y,x}$) in g/l			
Fat	0.37	0.45	< 1.03
Protein	0.49	0.43	≤ 1.03
2. Repeatability (S_r) in g/l			
Fat	0.08	0.10	0.14
Protein	0.08	0.04	0.14
3. Linearity (r_c) in %			
Fat	0.68	0.8	1
Protein	0.26	0.2	1
4. Carry-over (CO) in %			
Fat	0.28-0.34	nd*	1
Protein	0.21-0.45	nd*	1
5. Stability according to ISO 8196-3	Yes	Yes	

*= not determined

ICAR milk analyser certification

ICAR is offering a new services for certification of milk analysers since 2017 (*http://www.icar.org/index.php/certifications/milk-analysis-laboratories-certifications/milk-analysers-icar-certified/*). The service entails certification of instruments for somatic cell count and milk composition analysis according to the ICAR protocol for evaluation of milk analysers and the ISO 8196-3. Above described certifications are primarily focused on payment analyses, whereas the ICAR certification is rather dedicated on analyses of individual cow milk samples in the context of milk recording testing. The key objective of the ICAR certification is to apply a harmonised protocol that serves the interest of milk recording worldwide. This, in turn, should be sufficient to fulfil the various requirements and possibly certifications required on national level in many countries. The procedure for obtaining the ICAR certification of the CombiFoss instrument was initiated.

Spectral analysis of milk

Fourier Transform InfraRed (FTIR) spectrometry is nowadays a commonly applied technique for analysis of milk samples on fat, protein, and lactose. More recently minor components such as urea as well as acetone and β-hydroxybutyrate (BHB) (de Roos *et al.*, 2007) were developed in order to provide additional valuable information for optimising dairy herd management. FTIR technology is more and more used to extract extra information that could be used for improving dairy herd management further. In this context, the prediction of numerous new parameters like lactoferrin or major minerals based on milk spectra and different phenotypes was described in the literature (see Figure 1).

FOSS has developed global models (prediction models) for the parameters fat, protein, lactose, SNF, casein, urea, fatty acids, BHB and acetone. These models have been validated thoroughly over many years. However, in terms of indirect parameters, meaning parameters that cannot be measured directly using FTIR spectrometry due to their low concentrations, such as lactoferrin, correlations between milk spectra and reference values could be established. Nevertheless, such correlations depend on the actual local conditions (e.g., feeding, breeding, etc.) and further validation would be required when applying such calibrations even under slightly different conditions.

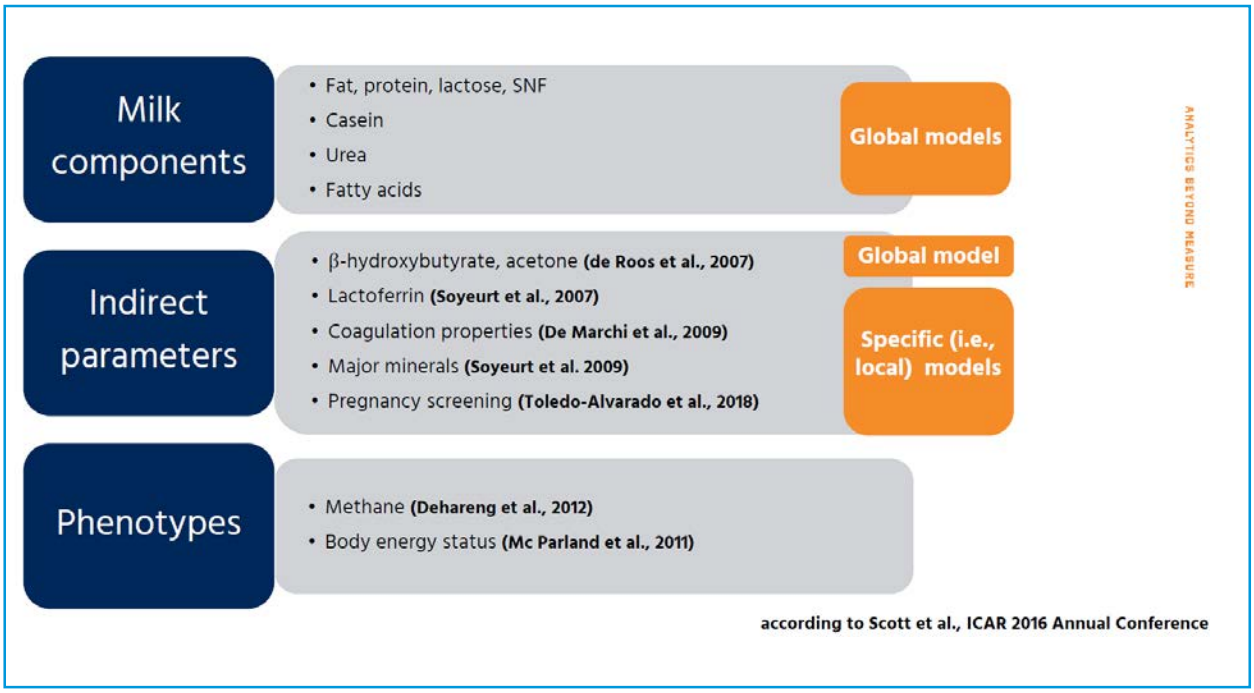

Figure 1. Overview on milk components, indirect parameters, and phenotypes that can be predicted using FTIR spectrometry.

A prerequisite for the global transfer of prediction models is the standardisation of the FTIR instrument. In this context, FOSS introduced a procedure for standardisation of MilkoScan instruments based on the monthly analysis of the so-called FTIR Equalizer in 1995. The procedure is described in detail elsewhere (*https://www.fossanalytics.com/-/media/files/documents/papers/dairy-segment/standardization-of-ft-ir-instruments_gb.pdf*).

New parameters for milk testing and reference methods

Milk samples hold a wealth of valuable information about dairy herds as well as individual cows. Two examples for new parameters unveiling more information from milk samples are BHB and acetone used for ketosis screening, as well as FOSS's new Differential Somatic Cell Count (DSCC) parameter for mastitis screening. However, in case of both applications official reference methods are not yet available today.

Example 1: BHB and acetone - Ketosis screening

Ketosis screening based on the prediction of milk BHB and acetone was introduced in 2006. Milk BHB and acetone values predicted using FTIR spectrometry showed good correlations with results generated using a wet chemistry method (de Roos *et al.*, 2007). However, there is no official (e.g., IDF recommended) reference method for milk BHB and acetone. Hence, the milk-testing organisations working with milk BHB (and acetone) in Canada and France developed their own quality assurance programmes involving wet chemistry methods as described elsewhere (Schwarz,

2017a). Apart from that, the IDF Action Team S03b: New applications of IR spectrometry is working on a guideline describing, among other things, how to work with prediction models used for ketosis screening (to be published in 2018).

Example 2: Differential somatic cell count

DSCC, representing the combined proportion of polymorphonuclear neutrophils and lymphocytes in percent, was introduced in 2016 and is a new biomarker for mastitis management (Damm *et al.*, 2017; Schwarz, 2017b). DSCC is beyond the scope of the current reference method for total somatic cell count (ISO 13366-1). However, the IDF Action Team S15 Bulletin on improvement of the reference method for somatic cell counting started mapping possibilities/technologies for and improved SCC reference method including the capability of DSCC. A first bulletin describing outcomes of that work is supposed to be published in 2018.

Conclusions

FOSS helps to allow quality assurance working with its milk analysers by obtaining different certifications, offering a standardisation concept for FTIR analysers, and supporting the development of analytical methods, reference materials, and proficiency testing programmes. In general, raw milk holds a wealth of valuable information that can help us to make significant improvements in the dairy milk supply (both milk quality and dairy herd management). Hence, the development of new parameters, associated quality assurance tools, and effective communication of data are clearly in the interest of and supported by FOSS.

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Moving from approval to certification for recording and sampling devices by ICAR - a dynamic approach to connect member organizations and manufacturers while encouraging innovation and testing of new devices

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Summary

Traditionally, successful ICAR testing of recording and sampling devices results in a lifetime approval from ICAR for the specific device combination. A recording and sampling device may have many components - milk meter, controller, keypad, sampler, firmware, and software. The approval approach has served the milk recording industry well for many devices, particularly mechanical milk meters. However, changes in one of more of the components of a complete device may affect the accuracy of either the milk yield prediction or the delivery of a representative milk sample. While the current ICAR Guidelines state that manufacturers are required to report these changes to the Subcommittee for Recording and Sampling Devices (RSD-SC), some modifications are not reported in a timely fashion. Further, device installation protocols or routine calibration procedures, which are reviewed during the ICAR testing process, may be altered by manufacturers after the ICAR approval is awarded. Validation of these changes by the RSD-SC along with timely communication to ICAR member organizations of such changes has been identified as an area in need of improvement.

The current ICAR Guidelines include language for annual reporting by both device manufacturers and member organizations. Building on these existing reporting Guidelines, the RSD-SC is moving to an annual review of certification for all recording and sampling devices. This dynamic approach is designed to increase the responsiveness of the RSD-SC to member organizations' challenges or concerns as well as facilitate timely resolution by device manufacturers. Further, this certification plan is desirable when compared to re-testing and re-certification of every recording and sampling device after a specific time frame or certification period expires. Rather, manufacturers are encouraged to invest resources into ICAR testing of both modified and new devices rather than in testing of current devices that have not undergone any changes in design or components. This approach to device certification is also expandable to include sensor devices in the future. The RSD-SC is committed to meeting the needs of ICAR member organizations and building strong relationships with device manufacturers.

Keywords: milk recording, recording devices, sampling devices, certification, milk sampling

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Background and perspective

As one of the standing subcommittees of ICAR, the Recording and Sampling Devices Subcommittee (RSD-SC) is responsible for the testing of milk recording and sampling devices at various ICAR-qualified test centres. Currently these test centres are based in France, Germany and The Netherlands and work cooperatively under the direction of the RSD-SC and Service-ICAR. Testing of devices involves both laboratory and field (farm) testing in accordance with Section 11 of the ICAR Guidelines (*https://www.icar.org/index.php/icar-recording-guidelines/*), whose review and maintenance is also the responsibility of the RSD-SC. In addition to testing of the device with respect to specific metrics outlined the in the Guidelines, an evaluation of both the installation and routine calibration procedures for the recording and sampling device is conducted. After completion of an ICAR test, a full report of the recording and sampling device is reviewed in detail by the RSD-SC with a resulting recommendation for approval that is forwarded to the ICAR Board for endorsement. Finally, this approval is published on the ICAR website as a reference for all ICAR member organizations.

Traditionally this ICAR 'approval' has been for the lifetime of the recording and sampling device. The RSD-SC does offer the opportunity for device manufacturers to report and apply to modification testing or desk review(s) of changes to devices over time. Some manufacturers embrace this opportunity as modifications or improvements to the originally approved device are brought to the marketplace. Other manufacturers provide only limited updates to the RSD-SC, mostly in response to queries or concerns raised by ICAR members. A lifetime approval for a recording and sampling device is not practical nor in the best interest of ICAR and its members, noting that changes or improvements in devices occur through the normal business practices of device manufacturers. The challenges noted by the RSD-SC include, but are not limited to the following:

- Change in design of meter and/or sampler.
- Change in firmware and/or software.
- Availability of original components.
- Changes or deviations in specific parts.
- Change in installation or routine calibration procedures.
- Quality control issues.
- Change in branding, device name, or mounting position.

Approval versus Certification

While some organizations or businesses may use the terms approval and certification interchangeably, the differences that exist in the application of these terms for positioning in the marketplace are notable. The traditional term 'approved' as used by the RSD-SC denotes that the recording and sampling device has met minimum standards after testing. While in accordance with the specific tests or metrics outlined in the ICAR Guidelines for laboratory and field testing. The term 'certified' is more appropriate in the case of ICAR whose mission involves continuous improvement in systems and practices. Certified or certification implies that not only has the device met minimum testing standards but is also in compliance with all of the ICAR Guidelines including manufacturer reporting and device labelling. Further, certification is a recognition of continued compliance and quality with a specific time frame associated with said certification.

Embracing that the role of ICAR is certification for devices, the ICAR Subcommittee for Animal Identification moved from identification device approval to certification in 2015. This change, as outlined at previous ICAR sessions and on the ICAR website, designates a five-year certification period for an identification device with a specific expiry date. With the goal of maintaining clarity of ICAR certification services for various device categories, the RSD-SC reviewed this approach in the context of retesting and recertification of recording and sampling devices. Noting that the lifespan of these devices is longer and not practical in many situations due to device availability, the RSD-SC concluded that a different approach was warranted. Further, the investment of testing recording and sampling devices made by manufacturers should be focused on new or innovative devices rather than retesting of either current or out of production devices where none of the previously described challenges exist.

The dynamic approach to recording and sampling device certification

Working with existing sections of the ICAR Guidelines and recognizing the needs of ICAR members, the RSD-SC has implemented a dynamic approach to continued certification of recording and sampling devices. It should be noted that no changes to the application or testing of new or modified recording and sampling devices will be implemented. Those processes will continue as described though manufacturers will note potential restructuring of the ICAR Guidelines as part of a larger effort by ICAR to modernize the functionality of all ICAR Guidelines. This dynamic approach was developed using the reporting options that currently exist with a focus on ICAR member engagement and device manufacturer responsibility. The certification of all recording and sampling devices will be reviewed by the RSD-SC on an annual basis. The documentation for this review will be the ICAR member reports and manufacturer reports on ICAR-certified devices in the marketplace. Both of these reports are described in the current ICAR Guidelines. To aid in this reporting, the RSD-SC has developed templates for both groups to complete and submit.

ICAR members are not required to complete an annual report of satisfaction but are strongly encouraged to communicate their concerns to the RSD-SC using this tool. With completion and submission of a member report, the RSD-SC acknowledges a desire for timely resolution to known and relevant issues. In addition to ICAR member reporting on an annual basis, device manufacturers are required to submit a report on ICAR-certified devices in the marketplace. This report will include devices sold and in which countries they are sold, modifications in any and all device components, and alternative market names including private-labelling or branding of said devices.

Using the information provided in the annual reports, the RSD-SC will review all recording and sampling devices for continued certification. For any device with no noted concerns from the member aspect and no reported modifications or changes, ICAR-certification will continue. For those devices that do continue as certified, the RSD-SC will suspend the certification of the device until the concern is resolved. The time frame required for resolution will be at the discretion of the RSD-SC and mutually agreed upon by the manufacturer and the RSD-SC, noting that each case needs to be evaluated on both the merits of concern and scope of the response from the manufacturer. In the case where resolution cannot be achieved, either after attempts by the manufacturer or by the unwillingness of the manufacturer, the certification of that recording and sampling device, including those devices manufactured or marketed under alternative names, will be withdrawn.

As previously noted, certification requires compliance with all the ICAR Guidelines. In addition to the required manufacturer report, a critical component of the Guidelines is labelling of ICAR-certified devices with an appropriate label. This requirement exists

in the current Guidelines (Section 11.5.4 - *https://www.icar.org/index.php/icar-recording-guidelines/*) and will continue moving forward. To aid manufacturers and ICAR members alike, the RSD-SC has redesigned the ICAR label to include device name, year of initial certification, species, and use or mounting position (high-line, low-line or AMS). The application of labels to ICAR-certified devices not only adds value to the device but assists the users of the device in proper installation and use.

The change from lifetime approval of recording and sampling devices to a review and certification process is designed to provide benefits to both ICAR member and device manufacturers. Central to this process is communication between the RSD-SC, building synergy between device manufacturers and device users, resolving issues of concern, and encouraging manufacturers to invest in testing new and innovative devices rather than retesting of devices that have not changed but are currently in the marketplace.

Strategic udder health monitoring and benchmarking based on national SCC data in Germany

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Summary

Udder health still is one of the most problematic areas in dairying, although a lot of efforts have been spent over the last fifty years to improve the situation. As udder health management is a complex task, depending on a multitude of influencing factors, a strategic approach is needed to effectively control the situation, both on single farm level and on a population or national level. In any case reliable, standardized and globally available data are needed to facilitate strategic approaches, whatever the relevant management level may be.

In Germany a new udder health monitoring report, based on SCC data from the national DHI system, has been introduced in 2015. For this report six new key figures are being computed and summarized to help the farmer keeping an objective eye on important risk factors in the life of his dairy cows. These key figures show the proportion of cows with healthy udders in the herd, the new infection rate during lactation, chronically ill cows with poor prognosis, the new infection rate and the cure rate during dry period as well as the rate of heifer mastitis in the herd.

These key figures are presented for the herd level, for the regional and the national level to facilitate benchmarking as a management tool. Fact sheets and checklists, developed in the same frame of *milchQplus* (*www.milchqplus.de*), a national project funded by the Federal Ministry for Food and Agriculture and DLQ e.V., the national umbrella association of all regional DHI organizations, support the dairy farmers and advisors in identifying the relevant influencing factors and selecting the right measures and actions to be taken.

Keywords: Udder health, strategic management, monitoring, benchmarking, SCC data, key figures, milchQplus, DLQ.

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Comparison of individual cow SCC estimates using an on-line SCC analyser and conventional herd tests

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Summary

The ICAR Recording Guidelines include provision for on-line milk analysis. However the guidelines are inconsistent with some of the practical realities of on-line milk analysis. Consequently, no milk analyser has been able to achieve ICAR approval to date. In the case of somatic cell count (SCC), the use of percentage error limits at low SCC makes compliance practically unattainable. More generally, the guidelines do not recognise the importance of cow-specific bias (CSB), which should be the main focus of technology evaluation for on-line milk analysers. This study investigated the level of CSB exhibited by a commercially available on-line SCC analyser. CellSense® obtained on-line SCC results at each cow milking. Conventional laboratory SCC results were obtained from herd tests conducted at twenty consecutive milking sessions (i.e. ten days). CellSense and herd test SCC results were compared in two ways: paired single samples; and cow-averaged samples. For context, cow-mean herd test results from one day were compared with cow-mean herd test results from ten days. CellSense provided a better estimate of ten-day cow-mean SCC than a single-day herd test. Yet this technology falls well short of the accuracy limits for SCC analysers specified in the ICAR Guidelines. This is a compelling demonstration that the ICAR accuracy limits for on-line SCC analysers need to be reviewed.

Keywords: on-line milk analysers, CellSense, mastitis, somatic cell count, cow-specific bias.

Introduction

ICAR has recognised the increasing use of on-line milk analysers and their potential to undermine farmer participation in official milk recording programmes. Accordingly, the ICAR Recording Guidelines include provision for on-line milk analysis (ICAR, 2017a and b). To date, no milk analyser has attained ICAR approval. This is partly because the ICAR guidelines for on-line milk analysers are inappropriate, given the practical realities of on-line milk analysis. In the case of SCC specifically, the use of percentage error limits at low SCC makes compliance practically unattainable. A low SCC sample can have a large percentage error at a level that is inconsequential for mastitis management or animal evaluation. Another area of concern is that the guidelines fail to recognise the importance of cow-specific bias (CSB) in technology evaluation. CSB occurs when a measurement is consistently under- or overestimated for a given cow relative to the rest of the herd (Anderson *et al.*, 2016). The random component of measurement error will average out with multiple measurements, whereas the cow-specific component will not. CSB therefore limits the ability to

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accurately rank animals within a herd, which is crucial for animal evaluation. It is this aspect of on-line milk analysers that should be the main focus of technology evaluation, and was the subject of this study.

In order to overcome some of the practical constraints of on-line milk analysis, CellSense does not collect a representative proportional sample. Instead it sucks a 'spot-sample' from the milk tube at a predetermined time during the milking (Whyte *et al.*, 2004). This sampling method is acceptable for detecting and monitoring mastitis, but can be substantially different to the SCC measured from a proportional representative milk sample. If these differences contribute to CSB, they will limit the ability to obtain a good estimate of cow-mean SCC from the analyser over multiple measurements.

The aims of this study were to:

- Test whether the differences introduced by CellSense's sampling method are cow-specific or random.
- Evaluate the ability of CellSense to estimate the short-term cow-mean SCC, and ultimately whether CellSense is suitable for the purpose of animal evaluation.
- Review the suitability of the current ICAR error limits for SCC analysers.

Materials and methods

Data were collected from equipment installed at Livestock Improvement Corporation's Innovation Farm, Rukuhia, New Zealand during the 2014-2015 milking season. The milking herd was 345 spring-calved cows, milked twice per day in a 34-bail rotary milking system. CellSense on-line SCC analysers (LIC Automation, Hamilton, New Zealand) were installed at 17 milking positions (50%), measuring SCC from individual cows at each milking. An earlier version of this technology was reported by Whyte *et al.* (2004). Herd tests were conducted at twenty consecutive milking sessions, spanning a ten-day period from the afternoon of 2 November until the morning of 12 November 2014. Samples were collected using Tru-Test Wide Bore Field Collection mechanical meters (Tru-Test, Auckland, New Zealand). The samples were analysed for SCC using flow cytometry at LIC Sample Processing North (Hamilton, New Zealand).

Cows with greater than four CellSense results in the period, and with two herd test results on 7 November were included in the analysis (259 cows). The distribution of tests per cow is shown in the histograms in Figure 1. All SCC averages described in this paper were calculated using the geometric mean. No attempt was made to adjust for AM-PM differences. CellSense results were compared with herd test SCCs for individual measurements (Single-test Comparison) and after averaging the results for each cow (Aggregated Comparison). For the Aggregated Comparison, all available herd test results in the period were averaged, by cow, to produce aggregated herd test SCC values for each cow. These were the gold standard cow-mean SCC values for the period. CellSense results were processed in the same way to produce aggregated CellSense values for each cow. These were the CellSense estimates of cow-mean SCC for the period. To put these comparisons in context, the 7 November (middle day) cow-mean herd test SCC was compared with the ten-day cow-mean herd test SCC (Herd-test Comparison).

Performance was quantified in three ways. First, the correlation (r) between $\log_2$ SCC estimates was calculated. Second, the animal SD (accuracy) defined in the ICAR Guidelines (2017a and b) was determined by calculating the standard deviation of relative error (SDRE) for cows or samples with herd test SCC values less than or

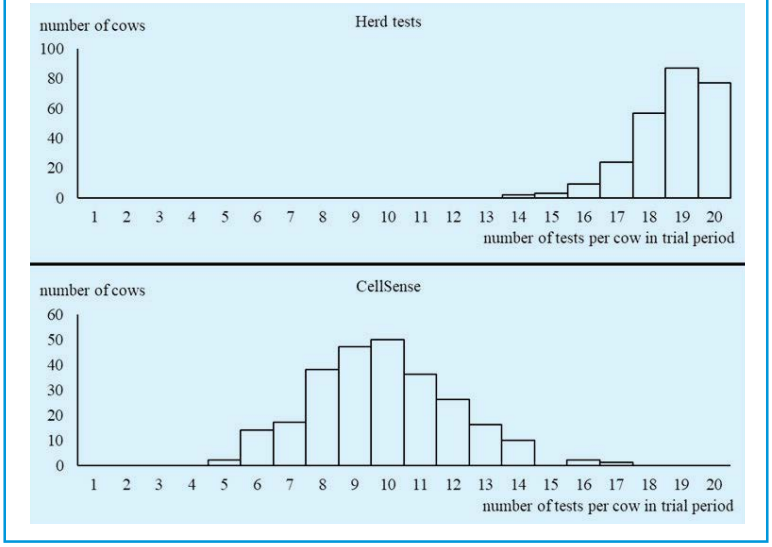

Figure 1. Histograms showing the distribution of tests per cow in the trial period for herd tests (upper) and CellSense (lower).

equal to 2000 kcells/mL (ICAR Method). To calculate the SDRE, the difference between CellSense and herd test values was first calculated. This difference was then divided by the herd test value for that cow or milking to obtain the relative error. The sample standard deviation of relative errors across all cows or milkings was the SDRE. Third, the data were divided into two groups according to herd test SCC. Cows or milkings with SCC less than 200 kcells/mL were in the low SCC group, for which the standard deviation of error (SDE) was calculated. SDE was calculated as the sample standard deviation of the differences between CellSense and herd test SCC values. For the remaining cows or milkings in the high SCC group, the SDRE was calculated (Banded Method).

Comparisons between herd tests and CellSense for SCCs outside the range 100-1500 kcells/mL are problematic for a number of reasons discussed later in this paper. Therefore the analysis was repeated after bounding all individual SCC results within the range 100-1500 kcells/mL. In this case, SCCs lower than 100 kcells/mL were set to 100 kcells/mL, and those greater than 1500 kcells/mL were set to 1500 kcells/mL.

Results

The plots in Figure 2 and statistics in Table 1 illustrate the effect of aggregating SCC data by cow. The correlation of the Single-test Comparison was 0.493, which improved to 0.732 in the Aggregated Comparison and 0.929 with bounding. This correlation was similar to the (unbounded) Herd-test Comparison (0.942). Bounding the data for the Herd-test Comparison did not improve its correlation (0.914). Using the ICAR method, the SDRE values for the Single-test Comparison (548%) and Aggregated Comparison (165%) were very large. The SDRE for the Herd-test Comparison (57%) was also well outside the ICAR limit of 25%. Using the Banded Method, the SDE and SDRE improved from 51.6 kcells/mL and 46.0% in the Single-test Comparison to 33.6

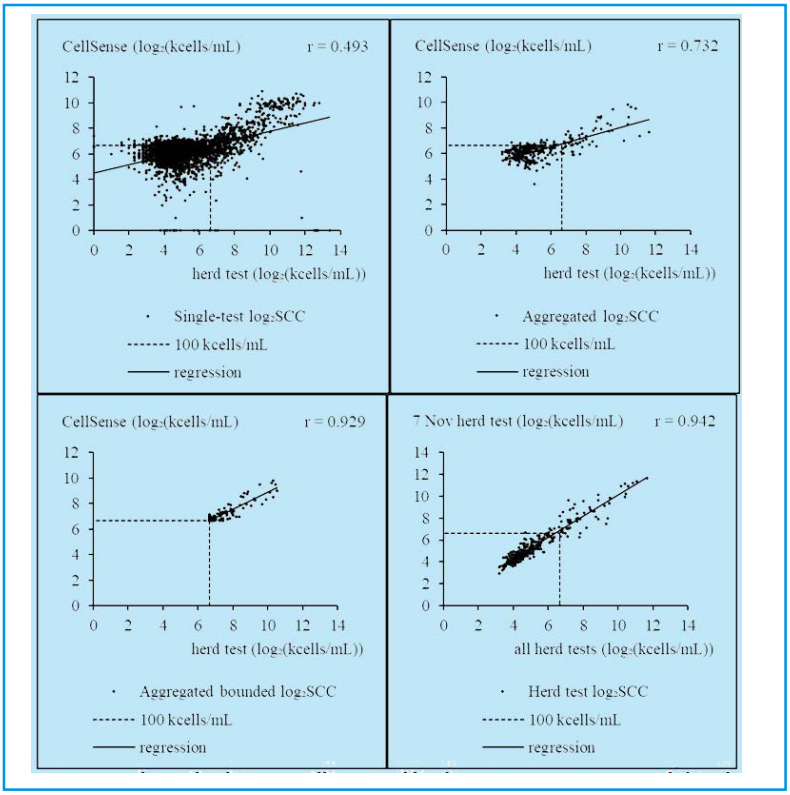

Figure 2: Relationship between CellSense and herd test SCC estimates. Upper left: individual milking results. Upper right: aggregated ten-day mean SCC. Lower left: aggregated ten-day mean SCC after bounding. Lower right: single-day aggregated herd test versus ten-day aggregated herd test.

Table 1. Performance metrics for each comparison method. The number of cows or tests included in the statistic is shown in parentheses.

	Correlation (r)	SDRE	SDE (kcells/mL)	SDRE
SCC range (kcells/mL)	All	0-2000	<200	≥200
Single-test comparison	0.493 (2332)	548% (2293)	51.6 (2042)	46.0% (290)
Aggregated comparison	0.732 (259)	165% (257)	33.6 (227)	29.5% (32)
Bounded aggregated comparison	0.929 (259)	17%[1] (259)	12.0[1] (224)	24.5% (35)
Herd-test comparison	0.942 (259)	57% (257)	64.1 (227)	55.9% (32)
Bounded herd-test comparison	0.914 (259)	34%[1] (259)	50.5[1] (224)	55.4% (35)

[1]These statistics are distorted from bounding low SCC results to 100 kcells/mL.

kcells/mL and 29.5% in the Aggregated Comparison for the low and high SCC groups respectively; and in the Herd-test Comparison, the SDE and SDRE for the low and high SCC groups were 64.1 kcells/mL and 55.9% respectively.

Discussion

There are two primary sources of systematic CellSense error compared with a herd test. First, the on-line analyser uses a different measurement principle to the flow cytometry used by laboratory SCC analysers. Flow cytometry involves staining the DNA of somatic cells and counting the number of stained DNA particles, whereas the detergent used by the on-line analyser lyses somatic cells and interacts with the unwound DNA molecules to increase the viscosity (Whyte *et al.*, 2004). We hypothesise that the differences between the two methods would be clearest at low SCC, where dead epithelial cells constitute the largest proportion of the total SCC. The second primary source of error is the spot-sampling method used by CellSense, because SCC is known to vary markedly within a cow milking (Sarikaya and Bruckmaier, 2006). Error from this source would be greatest at high SCC, where SCC fluctuates the most within a cow-milking. Although both sources of error could produce CSB in theory, the results of the current study show that CSB is relatively small for CellSense as evidenced by the marked improvement in performance metrics after aggregation.

Until now, the primary use of an on-line SCC analyser has been for mastitis management. A useful on-line SCC analyser will perform well in the 100-1500 kcells/mL range. This allows subclinical mastitis to be detected and provides a measure of severity (DairyNZ, 2012). The same rationale can be applied to laboratory SCC analysers. Indeed, laboratory instruments such as the Fossomatic 7 do not claim accuracy outside the range 100-1500 kcells/mL (Foss, 2017). Animal evaluation schemes use a log transformation for all SCC computations (e.g. DairyNZ, 2017a). Using the log scale has advantages, but highly exaggerates errors at low SCC. This is particularly alarming, given that most cows have SCC less than 100 kcells/mL (69% of all NZ cow tests in the 2016-2017 season, from unpublished data). The implication is that the majority of cows are evaluated on the basis of samples with SCC outside the claimed Foss accuracy range, at an SCC level where errors are exaggerated on the $\log_2$ scale. Despite this, continued improvement in SCC attributed to genetic gain has been observed (e.g. DairyNZ, 2017b). This suggests it is the differentiation between animals with SCCs greater than 100 kcells/mL that is driving genetic gain, and that there is little value in accurately ranking cows with SCC less than 100 kcells/mL.

There are also implications for the evaluation of on-line SCC analysers. The animal SD (accuracy) defined in the ICAR Guidelines (2017a&b) surprisingly stipulates the use of SDRE even for samples with 0 cells/mL. At low SCC, the relative error is highly exaggerated, resulting in inflated SDRE values. Most samples fall outside the claimed accuracy range of the reference method (79% of milkings in the current study), at an SCC level where relative errors are exaggerated. There is therefore a strong risk of new SCC technology being unfairly disadvantaged. Rather than use SDRE as the performance metric across the whole SCC range, it would be fairer to use the Banded Method, with SDE at low SCC and SDRE at higher SCC, similar to the ICAR error limits for milk yield (ICAR, 2017a).

The evidence from the present study exemplifies this. When evaluated using the correlation on the log2 scale and eliminating the influence of errors at low SCC by bounding the data, CellSense provided an estimate of the cow-mean SCC in the ten-day period with similar accuracy to a single-day herd test. When evaluated using

the Banded Method, CellSense provided a substantially better estimate of the cow-mean SCC in the period than a single-day herd-test. From these analyses, we conclude that CellSense is suitable for animal evaluation purposes, exhibiting minimal CSB.

The advantage of CellSense is even greater given its ability to monitor cows across the entire lactation. A mastitis event is far more likely to coincide with a CellSense test than an occasional herd test (Zhang *et al.*, 2018). Figure 3 shows one of the more extreme cows in the current trial. This example shows the 7-November herd test coinciding with a brief mastitis event, and causing a very large error in the single-day herd test estimate of cow-mean SCC. It is easy to envisage the opposite occurring, with the herd test missing the mastitis event altogether. The high day-to-day variation typically exhibited by SCC, limits the ability of a single-day herd test to estimate the true cow-mean SCC, while the frequent measurements from analysers like CellSense do account for day-to-day variation. This is one reason why CellSense can provide a better estimate of the ten-day cow-mean SCC than a single-day herd test.

Despite the compelling evidence in favour of CellSense, when evaluated according to the current ICAR Guidelines, CellSense was 22 times worse than the ICAR error limit. The poor performance when measured in this way was mainly due to the exaggeration of small errors at low SCC (the majority of milkings), and does not reflect the ability of CellSense to detect the presence and severity of mastitis.

Conclusions

CellSense provided a better estimate of ten-day cow-mean SCC than a single-day herd test. This implies that the errors due to CellSense's sampling method are sufficiently random and CSB sufficiently small, that CellSense is suitable for animal evaluation purposes. Yet this technology falls well short of the accuracy limits for SCC analysers specified in the ICAR Guidelines. The findings from this trial demonstrate that the ICAR accuracy limits for SCC need to be reviewed. The performance metric for SCC error limits should not exaggerate errors at low SCC, as the current metric does. One option could be to use SDE at low SCC and SDRE at higher SCC, as demonstrated in this trial. Results should also be averaged by cow as part of the evaluation to provide an estimate of CSB.

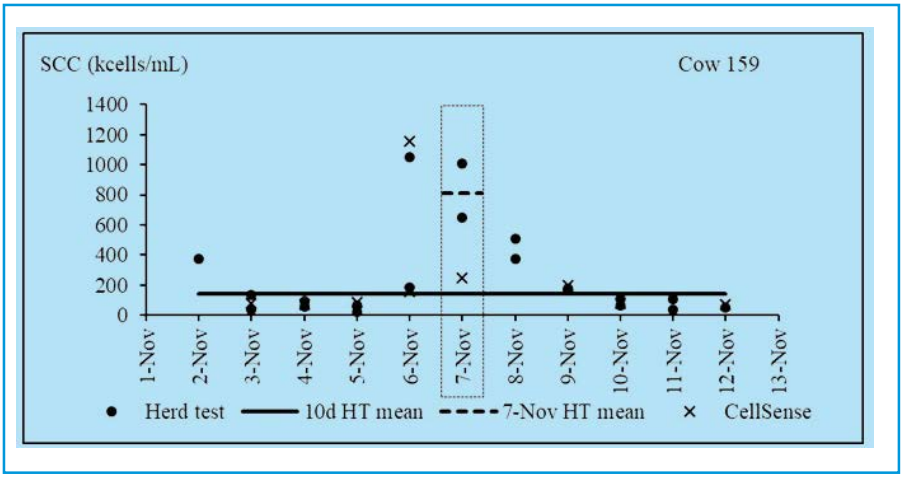

Figure 3. Example SCC results for an individual cow with a large difference between single-day and ten-day herd test values. The dashed box highlights 7 November, which was the single day chosen for the Herd-test Comparison.

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Update on development of criteria, protocols and guidelines for sensor devices

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ICAR Sensor Devices Task Force

Summary

The ICAR Board has initiated a Sensor Devices Task Force (SD-TF) with the task to provide guidelines and methodology to help classify and qualify on-farm sensors and sensor data in relation to milk production. This to create better safeguards on their suitability for herd management and breeding decisions. In developing guidelines and establishing potential performance criteria for sensor devices it is essential to consider sensor measurements as part of a whole process towards decision making. It is the aimed at quality in decision making that should determine the required quality level in each of the preceding steps. Developing sensor validation criteria for possibly occurring situations requires to address questions like:

- What is the trait?
- What is the purpose of the sensor measurement?
- What type of data does the sensor produce?
- What is the anchor?
- Single measurement or multiple measurements?

As part of this paper the approach and the challenges of the SD-TF are illustrated with an example on two different types of udder health monitoring devices.

Keywords: Sensor devices, performance criteria, precision, accuracy, guidelines

Introduction

ICAR has the role of supporting farmers and breeding organisations in their effort to collect valid data for daily management, and to deliver proven and validated information for genetic evaluations. In a rapidly increasing extent sensor technologies are currently being implemented in dairy farms to electronically monitor livestock, their environment, and to collect real-time data to make more informed decisions on aspects such as reproduction activities, herd and individual animal health status, feeding and nutrition, milking and milk production data and others. However, as yet data from such sensors is mostly unqualified with respect to accuracy levels, calibration and/or validation status. Consensus statements on minimum required performance criteria as well as standardized protocols for validation, maintenance and calibration are largely lacking.

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As a consequence, unqualified data may be transferred from on-farm computer systems into central databases and/or data processing centres, either intentionally or unintentionally at the present time.

In 2013 ICAR convened an 'Accuracy Task Force (A-TF) with the goal of developing a sound philosophical basis for ICAR to use in establishing required accuracy for the collection of animal recording data that is incorporated into information services. The A-TF presented their final report to the ICAR Board and General Assembly in 2016 (ICAR, 2016).

In order to move from the theoretical basis to a more practical phase, the ICAR Board initiated a new Sensor Devices Task Force (SD-TF) with the task to:

- Provide guidelines/methodology to help classify and qualify non-ICAR certified sensors and sensor data that measure:
  - milk volume;
  - milk speed;
  - milk constituents;
  - mastitis and
  - other traits

so as to determine their suitability for the respective management and breeding decisions;

- Disseminate new guidelines, so members and practitioners are aware of and educated in the approach for determining suitability of data from non-ICAR certified sensors.

This paper is intended to give insight in some of the encountered challenges by the SD-TF and the intended approach in dealing with these. This is illustrated with some examples on udder health monitoring.

The process

The process from sampling and sensor measurements to farm management decisions is schematically depicted in figure 1.

Robust data collection starts with proper identification of the animal, which is either right or wrong.

Sampling is to a large extent decisive for the ultimate representativeness of the collected data. During milking a single point (spot) sample of the excreted milk may signal an abnormality with the milk or the animal but will not allow to determine values for compositional or somatic cell count values representative for the whole milking. For that purpose a representative sample of the whole milking has to be collected or

Figure 1. The process with the use of sensor data.

repeated measurements during milking have to be done with the use of an adequate calculation algorithm. Whatever the type of sampling, a standardized and well controlled sampling procedure is a prerequisite to arrive at valid and useful data.

After sampling and possible sample pre-treatment, the sample is measured with a sensor device. It is noted that in the frame of this paper the term sensor device encompasses the whole range from simple temperature and conductivity sensors to more complex in-line or at-line analytical devices. With sensor measurements the question arises what the target of the measurement is. How well is the target defined? Is there a 'gold' standard, a reference method or another well-defined entity to anchor against? How secure is the anchor? Furthermore, sensor measurements require a certain degree of stability and robustness.

Data processing is about converting data into information that is to be used in decision making. As a part of this step, currently collected data may be combined with other animal data available from other sources or from other moments in time. Data processing also involves the handling of missing values or outliers and may include algorithms for data smoothing. Biomodels can be used to create proxies useful in herd management or in genetic selection. Measurement errors may to some extent be compensated for during this step, at the same time additional estimation errors may be introduced due to model inaccuracies.

Effective decision making is facilitated by well thought formats for information delivery. Warning lists help to pinpoint animals needing specific attention. Clear graphs can provide easy to grasp insights in trend-based courses.

The general approach for setting performance criteria

In the depicted process it is the performance level with each of the steps that determines the quality of the information that is used for decision making. In defining criteria for the subsequent steps of the process, this statement must be inverted. What is the aimed at quality of the information in decision making and what does this mean for the required performance level in each of the steps? So setting criteria for sensor devices starts from the question what the targeted use of the data is. Is it individual animal management, signalling animal health and welfare issues, herd management and/or genetic evaluation?

Key performance parameters with qualitative and quantitative parameters

The current focus of the SD-TF is on sensor devices and the quality assurance with sensor data. The SD-TF has started to build a database of sensor devices on the market with identification of their key characteristics such as aimed at trait, purpose of the measurement, availability of an anchor ('gold' standard, reference method, other), measurement matrix, method principle and available performance information from literature or other sources.

For each of the identified traits one can distinguish between sensors delivering qualitative and quantitative data. When examining the performance or setting performance criteria, separate performance parameters apply. The identified key performance parameters for each type of sensor device are summarized in Table 1.

Other relevant performance parameters may relate to the measurement range (lower limit, upper limit, capability of detection) and carry-over (the effect on a sample on the measurement result with a successively measured sample).

Table 1. Overview of key performance parameters with sensor devices producing either qualitative or quantitative data.

Qualitative data	
Sensitivity	The ability to detect the analyte compared to the reference = true positive rate
Specificity	The ability not to detect the analyte when it is not detected by the reference = true negative rate
Quantitative data	
Repeatability r (expressed as standard deviation of repeatability s_r, $r = 2.8 * s_r$ with > 20 measurements in replicate)	Standard deviation of values under a set of repeatability conditions of measurement, that is conditions where independent measurement results are obtained with the same device on identical test samples in the same place by the same operator within short intervals of time. In other words, the ability to produce the same result over and over under the same conditions.
Reproducibility R (expressed as standard deviation of reproducibility s_R, $R = 2.8 * s_R$ with > 20 measurements in at least replicate)	Standard deviation of values under a set of reproducibility conditions of measurement, that is conditions where independent measurement results are obtained with another copy of the same device on identical sample in different places and/or with the same device at different times by different operators. In other words, the ability to produce the same result at different places and/or at different times, e.g. under different conditions.
Accuracy (expressed as standard deviation of accuracy $s_{y,x}$)	Closeness of agreement between a measured quantity value and a true quantity value of a measurand, which is a result from both random error (precision) and systematic error of the measured quantity value. It is only the latter part that is also known as trueness and expressed as bias.
Robustness	Vulnerability to interactions and environmental interferences (shocks/vibrations, humidity/water, cleaning chemicals, temperature, milk flow speed).

Examples for udder health sensors

Udder health is one of the focal traits with the application of sensor devices. Many devices based on different principles have found their application on farms, i.e. milk conductivity sensors, LDH measurement, ATP measurement, NAG-ase measurement, thermal imaging, automated CMT/WMT (viscosity measurement) and somatic cell counting devices. The purpose of these type of sensor measurements in general is to timely signal animals that need closer attention from the farmer and/or a veterinarian. Applying on-farm sensors mainly serves animal health and welfare and herd management purpose, but it is imaginable that so collected data are used for determining breeding values for udder health, provided the quality of the data is adequately assured.

Qualitative data

Udder health sensors delivering only qualitative data have a typical on-farm purpose, that is to signal animals with (potential) udder health problems. With that, the trait, the purpose and the type of sensor data are clear. A well-defined and traceable anchor for the measurement itself is lacking, the goal is to signal animals with an (upcoming) clinical or subclinical mastitis for further examination. In that, it is relevant to miss not too many problematic animals, even if that comes at the expense of signalling a few extra non-problematic animals. At the same time every false positive indication comes with unwanted hassle for the farmer. The SD-TF is therefore considering whether guidance should state to aim for at least 80% sensitivity and 99% specificity when scored against internationally accepted mastitis definitions as proposed by Hogeveen et al., (2010) for alerting on clinical mastitis. With multiple measurements, these criteria would apply for the combined sensor output during one milking.

Quantitative data

Let us now assume we are dealing with an installed in-line somatic cell counting device which measures the somatic cell count in a representative aliquot of the milking. The purpose is on the one hand to signal problematic animals but also to use the data for breeding value estimates on udder health. In fact the same purpose as with traditional milk recording where the quantitative somatic count values are determined in representative samples in a central laboratory. Therefore in this situation also equivalence in the quality of the data is sought. The reference method for somatic cell count is the direct microscopic cell count, although also a well calibrated laboratory somatic cell counter can serve as the anchor.

The two main performance parameters are precision (repeatability and reproducibility) and accuracy. Precision is determined by non-systematic error and is part of the accuracy. Precision can be reduced by multiplicative measurements and using the average results. The other part of accuracy is trueness, the systematic deviation due to the error with calibration or due to a systematic influence of the matrix. In other words, milk from different animals behaving differently with different methods. Due to the systematic character, lack of trueness cannot be compensated for by repeated measurements.

For this situation the ICAR Sub-Committee on Milk Analysis developed guidelines on on-farm milk analysis, which are now part of the ICAR Guidelines (ICAR, 2017). The aim of these guidelines is to promote consistency and correspondence between different measuring systems with regard to measurement uncertainty and through that enabling comparison within time and place:

- For milk producers to manage day-to-day milk production.
- For milk recording organisations to maintain sufficient accuracy in estimating genetic indicators.

The rationale behind the establishment of performance criteria for on-farm milk analysers is that the accuracy of the sensor device must allow for an adequate monitoring of significant day-to-day production changes of an individual animal with representative sampling and when measured with reference methods. For that, the accuracy of the analytical device should be better than the natural day-to-day fluctuation of the measured parameter to allow the signalling of the relevant variation. The indicated limits for precision and accuracy for on-farm analytical devices are based on the ratio between the limit of the standard deviation of analytical measurement at the farm and the limit of the standard deviation of analytical measurement at the laboratory, expressed in a so-called equivalence factor (FE). Starting from the day-to-day fluctuation in fat concentration as being the maximum value for accuracy of analytical measurement on-farm, the statistical limits for precision and accuracy were calculated from those stated for laboratory analysers, see Table 2. For somatic cell counting devices the stated limit values for the relative standard deviations of repeatability and reproducibility are dependent on the level and range from 20% to 5% and 25% to 6% respectively. The indicated limit value for accuracy over the whole range is 25%. The SD-TF is currently considering the adequateness of these limits. Moreover, it is intended to precise how multiple measurements should be accounted for in the limit performance values of a single measurement.

Table 2. Precision and accuracy limits for test bed evaluation of milk analysers in milk recording (ICAR, 2017)

Component		Fat	Protein	Lactose	Urea	SCC
Units		g/ 100 g	g/ 100 g	g/ 100 g	mg/ 100 g	10^3 cells/ml
Range	Total			4,0 - 5,5	10,0 – 70,0	0 – 2000
	Low					0-100
	Medium	2,0 - 6,0	2,5 - 4,5			100-1000
	High	5,0 - 14,0	4,0 - 7,0			> 1000
Sample number	Animals (Na)	100	100	100	100	100
	Herds (Nh)	5	5	5	5	5

Milk analytical devices		Laboratory			On-farm At-line			On-farm In-line		
Equivalence Factor	FE	x 1			X 2			x 2,5		
Component		F-P-L	Urea	SCC	F-P-L	Urea	SCC	F-P-L	Urea	SCC
Units		g/ 100 g	mg/ 100 g	percent	g/ 100 g	mg/ 100 g	percent	g/ 100 g	mg/ 100 g	percent
Repeatability								[a]	[a]	[a]
Standard deviation *(sr)*	- Total range			4%			8%			10%
	- Low			8%			16%			20%
	- Medium	0,014	1,4	4%	0,028	2,8	8%	0,035	3,5	10%
	- High	0,028	2,8	2%			4%			5%
Within lab reproducibility										
Standard deviation *(sR)*	- Total range			5%			10%			13%
	- Low			10%			20%			25%
	- Medium	0,028	2,8	5%	0,056	5,6	10%	0,069	6,9	13%
	- High	0,056	5,6	2,50%	0,056	5,6	5%	0,070	7,0	6%
Accuracy										
Animal sample SD *(sy,x)*	- Total range			10%			20%			25%
	- Low									
	- Medium	0,10	6,0		0,20	12,0		0,25	15,0	
	- High	0,20			0,20 [b]			0,25 [b]		
Calibration [c]										
Mean bias *($\bar{d}$)*	- Total range		± 1,2	± 5 %		± 2,4	± 10 %		± 3,0	± 13 %
	- Medium	±0,05			±0,10			±0,13		
	- High	±0,10			±0,20			±0,25		
Slope *(b)*		1±0,05	1±0,10	1±0,05	1±0,10	1±0,10	1±0,10	1±0,13	1±0,10	1±0,13

[a] Where relevant i.e. for in-line differed time analysis.
[b] No larger tolerance by the usual factor 2 for sheep and goat to maintain accuracy with no more numerous records.
[c] Compared to manufacturer calibration.

Next steps

Based on the outlined purpose-based approach, the next steps in the work of the ICAR SD-TF can be identified as:

- Extending the sensor device database, to be completed with missing sensors and missing information on available and/or applied sensors and to provide an overview with key characteristics on the ICAR website.
- Developing sensor validation criteria for different purposes, based on working through a checklist for a structured evaluation on:
  - What is the trait?
  - What is the purpose of the sensor measurement?
  - What type of data does the sensor produce?
  - What is the anchor?
  - Single measurement or multiple measurements?
- Developing ICAR validation protocols and ICAR approval protocols, first for udder health sensors, then for other traits;
- Dissemination of recording guidelines using sensor data;
- Dissemination of best practices for data collection from sensor devices and related quality assurance.

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Characterization of milk composition and somatic cell count estimates from automatic milking systems sensors

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Summary

Adoption of automatic milking systems (AMS) is increasing rapidly in Canada. Some AMS are equipped with sensors to estimate milk components (i.e., fat, protein and lactose) and somatic cell count (SCC). However, the accuracy of the estimates produced by these sensors is unknown. For these data to be used for herd management, benchmarking and genetic evaluations, it is important to get a better understanding of how this data compares with traditional milk recording laboratory analyses.

Milk samples were collected from all milkings and from all the AMS in use on each farm (2.7 ± 1.0 milkings per cow) during a period of 24-h each on 10 farms using Lely Astronaut A4 AMS. The manufacturer's automatic sampling device was used to collect samples. Samples were analysed for fat, protein, lactose and SCC (n=10, 10, 7 and 6 farms, respectively). All herds were comprised of mostly Holstein cows and herd size was on average 74 ± 15 milking cows. Samples were analysed in the Valacta laboratory for milk components and SCC (CombiFoss FT+, Foss, Hillerød, DK). Data on milk production, milk composition estimates and number of milkings were also extracted from the AMS T4C software for the corresponding period. Milk composition derived from laboratory analyses was calculated as 24-hr average weighted by milk yield at the corresponding milking and was compared to the 24-hr estimate provided by the AMS. Only records comprised of three or more samples were considered in the comparison, leaving 501 records (i.e., cows) from the original 939 for statistical analysis.

On four farms with DeLaval VMS equipped with OCC sensors for SCC, sensors were programmed to measure samples of all milkings during a 12-h period. As visits were scheduled the same day of the monthly DHI test, comparisons were made between the AMS estimates and the DHI test results, matching the samples by the time they were taken (n=199). Since SCC estimates were available for each milking, only one sample was collected for each cow, as per regular DHI testing protocol, and results from the AMS sensors and the milk-recording laboratory for the corresponding milking were compared for each cow.

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On average, the mean differences between the results of the Lely AMS MQC sensors and the laboratory for fat and protein percentage were small (-0.05 ± 0.5 % and -0.001 ± 0.23 %, respectively). However, mean absolute errors (MAE) were larger (0.38 and 0.18 %, respectively). Moreover, differences among herds were greater for fat percentage, ranging from -0.22% to 0.14% (MAE = 0.47 to 0.28 %). Similar variations were found within herds, where the average difference for a 24-h period was small, but differences between cows were larger. Results from a concordance correlation coefficient analysis (CCC) between milk component estimations from the AMS and the laboratory analysis also showed variability in the level of agreement between the two measurements (CCC= 0.43 to 0.74 and 0.31 to 0.71, for fat and protein, respectively). In general, differences of milk components followed the same trend, i.e., when the fat was underestimated, there was an underestimation for the other components.

Differences between SCC laboratory measurement and (x1000) for DeLaval or Lely sensors were similar (-66 ± 364 and -61 ± 255, respectively), as well as the MAE (101 and 99, respectively). However, concordance with laboratory measurement of SCC differed between Lely and DeLaval sensors with a CCC 0.52 and 0.91, respectively. Likewise, the range of correlations within herd also varied for DeLaval and Lely farms (CCC= 0.84 to 0.98 and 0.14 to 0.80, respectively). These differences can be explained by the fact that sensors are using different technologies to measure SCC.

Future research will be important to better understand the influence of calibration procedure of AMS sensors, and evaluate the benefits of performing calibration using individual cow samples on a regular basis. It would be then possible to make recommendations to producers with AMS regarding calibration procedures and frequency. Finally, as AMS generate large amounts of data and information, it will be necessary to establish validation mechanisms and thresholds according to the different possible data usages (e.g., farm management, genetic evaluations), in order to enhance data usage from these systems.

Keywords: automatic milking systems, milk composition, sensors.

Introduction

In Canada, the number of farms implementing automatic milking systems (AMS) is increasing rapidly. The implementation of AMS affects milking management on the farm, where the two major aspects that change are the number of milkings per day and the frequency of milkings. Whereas in a conventional system, cows are milked two or three times a day with a fixed frequency, in the AMS cows enter voluntarily, so they can be milked at any time. Although permissions to be milked can be established by the producer for each cow, the consequence is that intervals between milkings are irregular. The range of milkings per day per cow reported in the literature goes from 1.5 to 5.0 with an average of 2.8 ± 0.2 (Bouloc *et al.*, 2001, Tremblay *et al.*, 2016).

Milk composition and somatic cell count (SCC) are of crucial importance for cow and herd nutritional and health management, genetic evaluation and milk payment. Milk composition and SCC can be affected by numerous factors including nutrition, genetics, management, health, stage of lactation and environment (Seegers *et al.*, 2003, Jenkins and McGuire, 2006, Quist *et al.*, 2008, Forsbäck *et al.*, 2010). Studies have shown that milking frequency and milking intervals affect milk composition and SCC. Increasing milking frequency from two to three times result in a fixed increase of 3.5 kg/day in milk yield and 92 g/day of fat yield independently of parity (Erdman and Varner, 1995). Increasing milking frequency also has shown to reduce SCC (Smith *et al.*, 2002, Dahl *et al.*, 2004). Studies on AMS indicated that milk yield could increase by 5 to 10%

(Bach *et al.*, 2007, Bijl *et al.*, 2007). Results comparing AMS with conventional systems showed that SCC increase by implementing AMS (Klungel *et al.*, 2000, Hovinen and Pyörälä, 2011). Milk composition (i.e., fat, protein and lactose contents) does not seem to be influenced by the type of milking system (Jacobs and Siegford, 2012); it appears that the length of the interval since the previous milking and the variation of milk yield per milking are more important factors (Friggens and Rasmussen, 2001).

Milk recording is challenging in irregular time intervals for milk sampling and 24-h predictions. Furthermore, some AMS are equipped with sensors to estimate milk components (i.e., fat, protein and lactose) and SCC. There are no published reports of the accuracy of the estimates produced by these sensors. For these data to be used for herd management, benchmarking and genetic evaluations, it is important to get a better understanding of how this data compares with traditional milk recording data based on regular milking time intervals and laboratory analyses. The aim of this study was to characterise and compare the results from the AMS sensors and the milk-recording laboratory.

Material and methods

On ten farms equipped with Lely AMS, milk samples were collected from all milkings (2.7 ± 1.0 milkings per cow) during a period of 24 hours starting at midnight on all AMS in use on each farm. The manufacturer's automatic sampling device was used to sample each cow for fat, protein, lactose and SCC (n=10, 10, 7 and 6, respectively).

On four farms with DeLaval AMS equipped with OCC sensors for SCC, OCC sensors were programmed to measure SCC of all milkings during a 12-h period. As sampling was scheduled to occur on the same day of the monthly DHI test, comparisons were made between the AMS estimates and the DHI test results, matching the samples by the time they were taken (n=199). All herds were comprised of mostly Holstein cows and herd size was on average 74 ± 15 milking cows.

Samples were analysed in the Valacta laboratory for milk components and SCC (CombiFoss FT+, Foss, Hillerød, DK). Data on milk production, milk composition estimates, number of milkings and milking description were extracted from the AMS software for the same time period.

For Lely farms, milk composition derived from laboratory analyses was calculated as 24-hr average weighted by milk yield at the corresponding milking and was compared to the 24-hr estimate provided by the AMS (i.e., calculated as the weighted average of the last five milkings for each cow). As the estimate of SCC by the AMS is the geometric mean of the last three milkings, for comparison purposes, the geometric mean of SCC from the laboratory analyses was also calculated. Only records comprised of three or more samples were considered in the comparison, leaving 501 records (i.e. cows) from the original 939 records for statistical analysis.

Data from the AMS was exported in Microsoft Excel. Differences were calculated as the results given from the AMS minus the laboratory results. Mean absolute errors (MAE) were computed, and concordance correlation coefficient (CCC) and Bland Altman analysis were done using the R program version 3.4.1 (R Development Core Team, 2017). The CCC and the Bland Altman analyses quantify the agreement and the reliability between two quantitative measurements, and help establishing the validity of a new technique (Kwiecien *et al.*, 2011; Giavarina, 2015). The CCC analysis was preferred over the Pearson correlation because the latter only provides a measure of the extent to which the points conform to the best fit line. The CCC analysis modifies

the Pearson correlation coefficient by assessing not only how close the data is from the best fit line, but also how far that line is from the perfect agreement line (i.e., 45-degres line through the origin) (Watson and Petrie, 2010).

Results

Lely AMS MQC sensors

On average, mean differences between the results of the Lely MQC sensors and the laboratory for fat, protein and lactose percentages and fat and protein yields and linear score were small (Table 1). However, mean absolute errors (MAE) were larger for fat and protein percentages, but not for lactose (Table 1).

Results from the Bland Altman analysis indicate that the fat percentage had the highest mean bias of the milk components estimated by the AMS, followed by protein and lactose (0.05, 0.04, and 0.001, respectively). The mean bias for the SCC and linear score was 61 and -0.05, respectively. Similarly, the results of the CCC analysis between milk component estimations from the AMS sensors and the laboratory analysis showed the variability in the lack of agreement between the two measurements. On average, the CCC were 0.61, 0.59, and 0.69 for fat, protein and lactose percentages, respectively. The CCC for SCC and linear score was 0.52 and 0.32, respectively.

Table 1. Mean differences and standard deviations of milk components generated for the ten farms by the Lely AMS MQC sensors and the milk recording laboratory.

Item	% fat	% protein	% lactose	Fat yield (kg/d)	Protein yield (kg/d)	SCC (cells/mL)[1]	Linear score
AMS	3.76 (0.57)	3.19 (0.21)	4.65[2] (0.11)	1.63 (0.40)	1.40 (0.33)	71[3] (145)	2.11 (0.87)
Laboratory	3.81 (0.55)	3.19 (0.28)	4.68 (0.16)	1.67 (0.40)	1.39 (0.30)	133 (340)	2.11 (1.68)
Differences	-0.05 (0.50)	-0.001 (0.23)	-0.04 (0.1)	-0.04 (0.23)	0.01 (0.10)	-61 (255)	0.01 (1.56)
MAE[4]	0.38	0.18	0.09	0.17	0.08	99	1.31

[1] Geometric mean of the last three milkings (x1000)
[2] Data available only for seven farms
[3] Data available only for six farms
[4] Means absolute error

Differences among herds (Table 2) were larger for fat percentage than from protein and lactose percentages, ranging from -0.22% to 0.14% (MAE = 0.47 to 0.28%). The same pattern was seen for fat and protein yields. Similar variations were found within herds, where the average difference for a 24-h period was small, but differences between cows were larger. The variation between farms was also seen on the results from the Bland Altman analysis by the range of the bias. For example, for fat percentage the bias range was between -0.14 to 0.25.

Similarly, CCC analysis for each farm also showed variability in the level agreement between sensor results and laboratory measurements. The CCC analyses of fat and protein percentages by farms are presented in Figures 1 and 2. In general, differences of milk components followed similar patterns, i.e., when the fat was underestimated, there was an underestimation for the other components.

Table 2. Accuracy of milk components and SCC estimates by Lely AMS MQC on ten farms.

	Farms									
Item	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Fat %										
AMS	3.54	3.80	3.57	3.61	3.80	3.82	3.64	3.74	3.85	4.09 .
	(0.62)	(0.55)	(0.61)	(0.52)	(0.49)	(0.53)	(0.53)	(0.49)	(0.53)	(0.53)
Laboratory	3.60	4.02	3.63	3.60	3.94	3.75	3.63	3.93	3.74	3.94
	(0.58)	(0.47)	(0.46)	(0.50)	(0.42)	(0.43)	(0.59)	(0.56)	(0.55)	(0.67)
Difference	-0.06	-0.22	-0.06	0.004	-0.14	0.08	0.01	-0.17	0.11	0.14
	(0.43)	(0.40)	(0.58)	(0.37)	(0.38)	(0.50)	(0.46)	(0.47)	(0.44)	(0.59)
MAE[1]	0.30	0.35	0.47	0.28	0.33	0.40	0.37	0.34	0.34	0.45
Protein %										
AMS	3.23	3.31	3.07	3.14	3.12	3.09	3.18	3.15	3.16	3.33
	(0.18)	(0.18)	(0.18)	(0.14)	(0.21)	(0.19)	(0.24)	(0.23)	(0.13)	(0.23)
Laboratory	3.25	3.29	3.09	3.18	3.14	3.13	3.20	3.16	3.18	3.26
	(0.16)	(0.27)	(0.23)	(0.27)	(0.25)	(0.25)	(0.39)	(0.27)	(0.27)	(0.34)
Difference	0.02	-0.02	-0.02	-0.03	-0.02	-0.04	-0.02	-0.01	-0.02	0.07
	(0.20)	(0.20)	(0.20)	(0.25)	(0.24)	(0.23)	(0.25)	(0.20)	(0.24)	(0.24)
MAE	0.16	0.16	0.15	0.20	0.19	0.18	0.17	0.15	0.20	0.22
Lactose *%a*										
AMS	4.61	4.70	n.a.[2]	4.64	4.54		4.61		4.62	4.71
	(0.09)	(0.09)		(0.10)	(0.09)	-	(0.09)	-	(0.10)	(0.08)
Laboratory	4 70	4.70	4.64	4.75	4.60	4.67	4.71	4.68	4.66	4.68
	(0.12)	(0.18)	(0.17)	(0.15)	(0.15)	(0.18)	(0.12)	(0.14)	(0.15)	-0,15
Difference	-0 09	-0.02		-0.11	-0.06		-0.09		-0.04	0.01
	(0.08)	(0.10)	-	(0.09)	(0.09)	-	(0.09)	-	(0.10)	(0.09)
MAE	0.098	0.083	-	0.12	0.081	-	0.11	-	0.083	0.073
Fat yield (kg/d)										
AMS	1.29	1.61	1.86	1.57	1.55	1.28	1.38	1.63	1.81	1.77
	(0.30)	(0.30)	(0.47)	(0.36)	(0.35)	(0.26)	(0.19)	(0.30)	(0.37)	(0.40)
Laboratory	1.34	1.71	1.96	1.60	1.63	1.27	1.40	1.71	1.78	1.72
	(0.33)	(0.33)	(0.44)	(0.42)	(0.32)	(0.24)	(0.23)	(0.30)	(0.43)	(0.33)
Difference	-0.05	-0.10	-0.10	-0.03	-0.08	0.01	-0.02	-0.08	0.03	0.05
	(0.15)	(0.19)	(0.29)	(0.19)	(0.18)	(0.18)	(0.18)	(0.18)	(0.22)	(0.26)
MAE	0.11	0.16	0.25	0.13	0.15	0.14	0.15	0.15	0.16	0.20
Protein yield (kg/d)										
AMS	1.18	1.41	1.62	1.37	1.28	1.04	1.22	1.37	1.51	1.47
	(0.22)	(0.25)	(0.39)	(0.29)	-27	(0.18)	(0.21)	(0.24)	(0.39)	(0.30)
Laboratory	1.19	1.40	1.62	1.38	1.28	1.05	1.23	1.37	1.50	1.44
	(0.21)	(0.23)	(0.36)	(0.26)	(0.23)	(0.18)	(0.22)	(0.25)	(0.31)	(0.26)
Difference	-0.01	0.01	0.004	-0.005	0.0001	0.01	-0.01	-0.003	0.01	0.03
	(0.07)	(0.09)	(0.11)	(0.10)	(0.09)	(0.08)	(0.10)	(0.09)	(0.12)	(0.12)
MME	0.06	0.068	0.083	0.083	0.074	0.062	0.07	0.066	0.097	0.098
SCC (cells/mL)										
AMS	139 (67.8)	42.3	51.9	-	83.7	-	-	66.2	-	
		(27.9)	(36.7)		(106.5)			(24.2)		
Laboratory	80.5	119	156	114	209	393	105	96.3(178)	86'7	134
	(90.6)	-176	-260	-254	-630	-675	-275		-131	-440
Difference	58.22	-77.01	-103		-126.1			-21.28		-40.5
	(78.2)	-161	-252	-	(535.9)	-	-	-146	-	-227
MME	84.8	94.2	123	-	160	-	-	63.2	-	86.1
Linear Score										
AMS	3.34	1.72	1.90		2.32			2.29		2.28
	(0.62)	(0.71)	(0.72)	-	(0.90)	-	-	(0.62)	-	(0.60)
		2 19	2.50	2.19	2.16	3.68	1.65	1.91	1.84	1.81
Laboratory	2.0 (1.39)	(1.68)	(1.68)	(1.46)	(1.93)	(1.85)	(1.61)	(1.52)	(1.56)	(1.70)
Difference	1.34	-0.57	-0.54		0.16			0.34		0.48
	(1.03)	(1.60)	(1.58)	-	(1.48)		-	(1.38)	-	(1.38)
MAE	1.48	1.43	1.32	-	1.24	-	-	1.18	-	1.25

[1]Mean absolute error
[2]Data was not available on the AMS

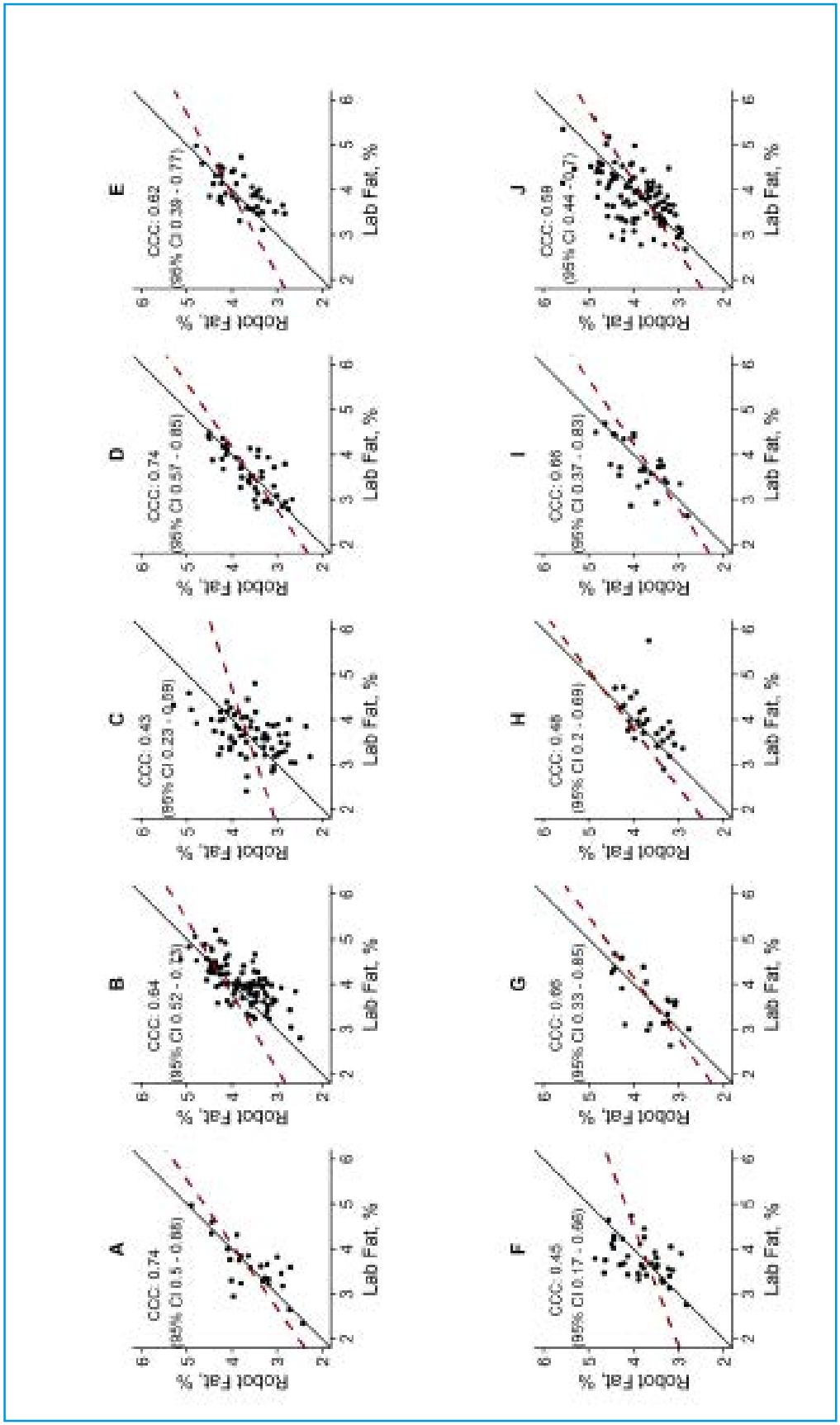

Figure 1. Concordance correlation coefficient (CCC) between milk fat percentages from the AMS sensors and the laboratory analysis of the 10 farms.

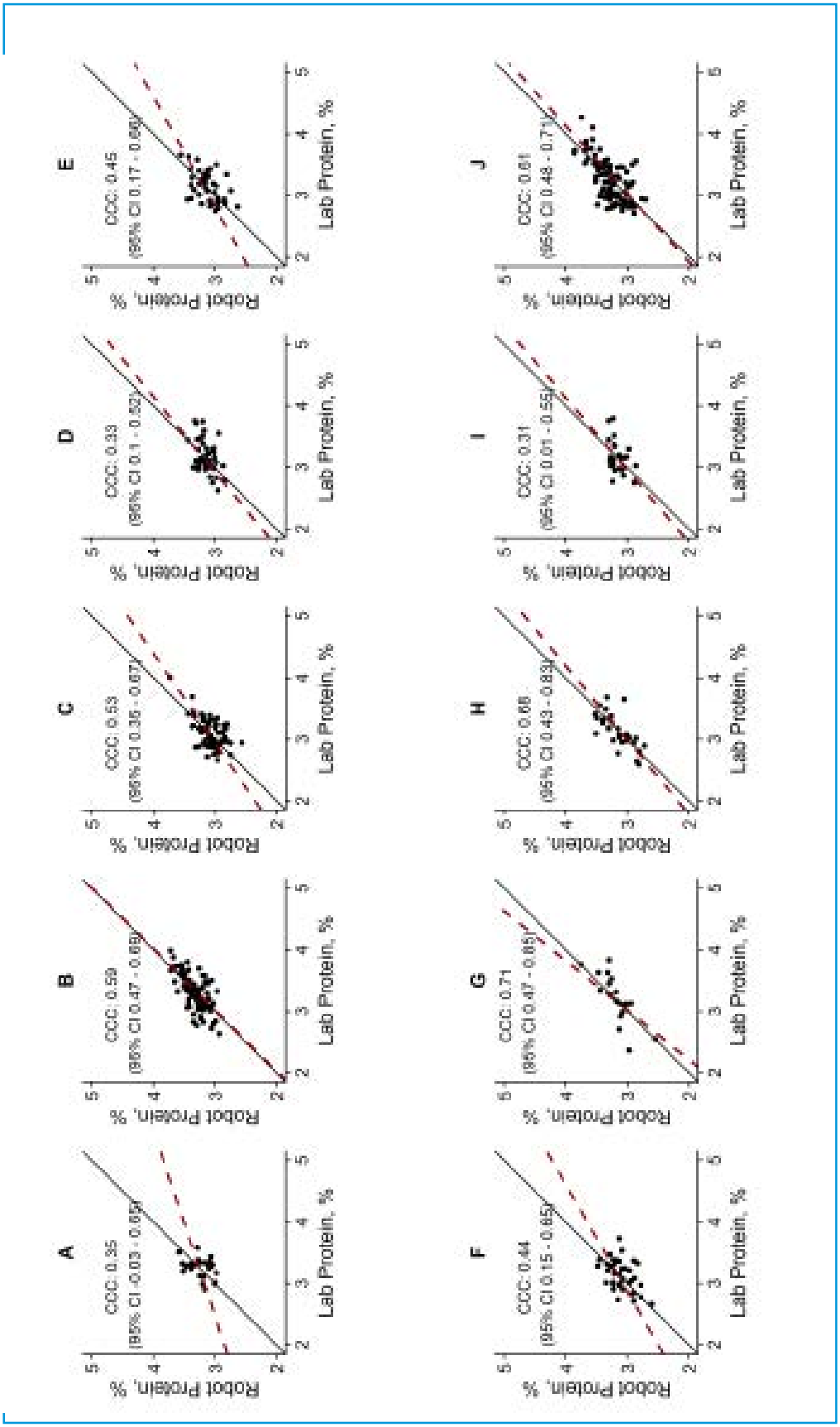

Figure 2. Concordance correlation coefficient (CCC) between milk protein percentages from the AMS sensors and the laboratory analysis of the 10 farms.

DeLaval AMS OCC sensors

Differences between the SCC (x1000) for DeLaval farms were -66 ± 364, and the MAE was 101. The average CCC between SCC estimations from the AMS sensors and the laboratory analysis showed good agreement between the two measurements (CCC= 0.91). Results for each farm are presented on Table 3. Within herds CCC ranged from 0.84 to 0.98.

Table 3. Accuracy of SCC estimates by the DeLaval AMS OCC sensors on four farms.

	Farms			
Item	**W**	**X**	**Y**	**Z**
SCC (cells/mL)				
AMS	98 (181)[1]	262 (825)	190 (741)	333 (829)
Laboratory	126 (234)	320 (1008)	241 (999)	452 (1404)
Difference	-28 (75)	-58 (190)	-51 (259)	-120 (635)
MAE[2]	38	66	58	128
Linear Score				
AMS	2.14 (1.35)	2.64 (1.73)	2.12 (1.65)	3.03 (2.02)
Laboratory	2.40 (1.43)	2.76 (1.84)	2.16 (1.77)	3.13 (2.14)
Difference	-0.26 (0.68)	0.13 (0.54)	-0.03 (0.34)	-0.11 (0.32)
MAE[2]	0.49	0.39	0.26	0 .25

[1] Number in parentheses is the standard deviation
[2] Mean absolute error

Discussion

Estimates of milk components provided by Lely AMS MQC sensors showed only moderate agreement with laboratory measurements. Furthermore, sensor-based estimates tended to overestimate component levels below average and underestimate high components concentrations.

Within-herd level of agreement differed between farms. One hypothesis to explain the large inter-herd variations between data from the Lely AMS MQC sensors and milk recording laboratories may be the way producers calibrate the sensors. The calibration of the Lely AMS sensors can be done in two ways:

1. Ccalibration at the cow level: using the results of the DHI.
2. Calibration at the herd level: using components results for the bulk tank.

The method of calibration will probably lead to different results but, unfortunately, we did not find publicly available studies on the impact of the calibration method on the results. All farms in the present study calibrated the AMS sensor using bulk tank results.

Another possible explanation for the variation between herds could be the frequency at which the calibration is performed. In the present study producer-declared calibration frequency ranged from every bulk tank pick-up (every other day) to biweekly. However, it was not possible to retrieve records of previous calibrations since only the last calibration date is reported in the AMS software. Another source of variation among herds might be the number of robots at that farm. Most farms that participated in the present study (9 out of 10) had two AMS. Since calibration is based on a single bulk tank value, milk composition of the bulk tank, which is a mixture of the milk from the two AMS the calibration process does not account for differences between the sensors of each AMS. Since the AMS software does not provide the milk components data for each AMS, it is not possible to assess if there are differences in milk components provides by the sensor of each AMS when farms have more than one.

Future research will be important to better understand the influence of calibration procedure of AMS sensors and evaluate, for example, the additional benefits of performing calibration using individual cow samples, on a regular basis. It would be then possible to make recommendations to producers with AMS regarding calibration procedures and frequency.

Milk composition (i.e., fat, protein and lactose) of each milking was not available in the AMS software. The data available for milk composition was, the average of the five last milkings, as calculated at the end of the day. It is known that milking frequency can significantly change from a cow to another and as the lactation progresses (Bouloc *et al.*, 2001). Studies report that milking frequency ranges from 1.5 to 5 milkings per day with an average of 2.8 ± 0.2 (Bouloc *et al.*, 2001, Tremblay *et al.*, 2016). The lactation period covered by each mean can be from one day (with cows with five milkings per day) to 3.3 days (for cows with 1.5 milkings per day). For this study, it would have been necessary to take milk samples during 1.8 days (average milkings was 2.8 ± 0.92). However, sampling was done over a period of 24-hours. Subsequent analyses should be done using sensor data produced at each milking, when this becomes available.

There was a significant difference in the accuracy of the two sensors producing an estimate of SCC. The optical measurement of SCC by the DeLaval OCC sensor provides reasonably accurate estimation of SCC as compared to laboratory measurement (CCC = 0.91). However, the Lely MQC2 sensor, which is based on a modified California mastitis Test reaction had lower accuracy for SCC below 500 (CCC = 0.52).

Conclusions

As AMS generate large amounts of data which could contribute to central databases such as those used by milk recording and genetic evaluation organisations, it will be necessary to establish validation procedures and thresholds according to the different possible data usages (e.g., farm management, genetic evaluations) in order to enhance data usage from these systems.

Future research is required to better understand the influence of calibration procedure and frequency on the accuracy of AMS sensors and evaluate the benefits of performing calibration using individual cow samples on a regular basis. It will be then possible to make recommendations to producers with AMS regarding calibration procedures and frequency.

Acknowledgements

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The value of recording live animal and carcase scan traits for the genetic selection of lean meat yield in lamb

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Summary

The value of a carcase is highly influenced by its saleable meat yield. Direct measures of lean meat yield (LMY) are currently not commercially available due to the high cost of measurement through the 'gold standard' of computed tomography (CT). Therefore, the objective of this study was to assess the use of post-weaning scan traits, carcase traits, and a dual-energy x-ray absorptiometry (DEXA)-derived lean, as indicator traits for selection to improve LMY in lamb. DEXA-derived lean was strongly genetically correlated to CT lean (r_g = 0.75). This suggests that DEXA (which can be recorded under commercial processing conditions) has value as an indicator trait for CT lean. However, there are different implications for predicted genetic gains for ram breeding and progeny testing situations due to availability of records from different classes on animals. Since a ram breeder sells most animals as breeding stock and there is limited access to slaughter records, only 50% of potential genetic gains (compared to selection for CT lean) could be achieved through DEXA alone. The addition of post-weaning traits increased potential genetic gains to 67%. Therefore, the ram breeding industry will still need to rely on the correlated trait measured on the live animals and the rate of genetic gain will be slower. As seedstock producers will struggle to record DEXA and CT traits in sufficient numbers, resource populations such as the Information Nucleus Flock will continue to be required to validate the parameters presented in this study and improve the ability for ram breeders to make effective selection decisions for LMY improvement. Furthermore, since there are unfavourable associations between LMY and eating quality, genetic improvement of LMY will need to be managed, and eating quality included in the breeding objective for Australian lamb breeders.

Keywords: DEXA, CT, objective carcase measurements, genetic gain.

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Introduction

The value of a carcase is highly influenced by its saleable meat yield. Lean meat yield (LMY) represents the proportion of the carcase that is lean meat (muscle), with all visible subcutaneous and intermuscular fat removed. While the 'gold standard' to directly measure LMY is through computed tomography (CT), the cost of measurement through this technology is high, logistically difficult, and is not viable in the commercial processing environment. Therefore, the genetic selection for LMY is reliant on indirect selection, where gains are achieved through correlated responses from indicator traits.

Sustainable genetic gains in LMY have been achieved for terminal sheep breeds through selection indexes based on body weight, and eye muscle and fat depth from ultrasound scans on the live animal (Swan at *al.*, 2015). The search for new objective carcase measurements technologies (Brown *et al.*, 2017), such as dual-energy x-ray absorptiometry (DEXA) (Gardner *et al.*, 2015), provide potential indicator traits for LMY. The use of indicator traits can be associated with slower genetic gains and is reliant on their heritability and strength of correlations holding up across the population. Therefore, the objective of this study was to assess the value of post-weaning traits measured on-farm (weight, body condition and scan traits), measures on the carcase, and DEXA derived values of lean, for the genetic evaluation of LMY in lamb. The value of these indicator traits was assessed by predicting the potential genetic gain in LMY under a ram breeder or progeny test scenario were selection is based on a series of different indicator traits.

Materials and methods

Information Nucleus Flock data

An Information Nucleus Flock (INF) was established by the Australian Cooperative Research Centre (CRC) for Sheep Industry Innovation in 2007 (Fogarty *et al.*, 2007), which evolved into the Meat and Livestock Australia funded Resource Flock (RF) in 2012. The program was based around the annual mating of 100 sires to ~4500 dams (van der Werf *et al.*, 2010), with flocks located at eight research sites across Australia. A key objective of the INF program was to measure and estimate genetic parameters for a diverse range of traits, many of which either cannot be recorded on-farm or are prohibitively expensive, or are not widely or systematically recorded off-farm (such traits are often referred to as "hard-to-measure"). The LMY indicator traits assessed in this study were measured on the live animal, on the carcase, and by CT and DEXA (Table 1) in the INF and/or RF.

*Table 1. Summary of traits**

Trait	Units	Records	Sires	Mean	SD	CV
Post-weaning weight	kg	41,698	1,427	30.53	8.26	27
Post-weaning eye muscle depth	mm	22,411	1,330	25.09	4.83	19
Post-weaning fat depth	mm	22,414	1,330	2.94	1.20	41
Post-weaning condition score	(1-5 score)	14,152	1,106	2.78	0.47	17
Hot carcase weight	kg	24,709	1,409	22.43	3.76	17
Carcase eye muscle depth (C site)	mm	22,879	1,407	29.82	4.72	16
Carcase fat depth (C site)	mm	22,643	1,406	4.11	2.37	58
CT lean	%	2,340	526	57.47	3.59	6
DEXA-derived lean		546	163	83.04	6.54	8

*CT: computed tomography;
DEXA: dual-energy x-ray absorptiometry (unit-less);
SD: standard deviation;
CV: coefficient of variation (%)

Live animal traits were measured post-weaning, and were available for the lambs born between 2005 and 2016 from terminal, maternal and Merino sires. These were measured at an average age of 213 ± 46 days. Carcase traits were measured on lambs slaughtered at commercial abattoirs at an average age of 272 ± 76 days (Mortimer *et al.*, 2014). A subset of the 2011-born slaughtered lamb carcases were chosen for CT scanning within 72 hours of slaughter, and the proportion of lean (CT Lean) was determined (Anderson *et al.*, 2015). The CT traits were expressed as a percentage of the weight of the scanned carcase. DEXA-derived lean (Gardner *et al.*, 2015) was available from 546 of the 2014-born progeny.

Statistical analysis

Genetic parameters were estimated as the average variance estimates from a series of bivariate sire models analysed in ASReml (Gilmour *et al.*, 2009). The models fitted were based on those previously developed and described in depth by Swan *et al.*, (2014). Due to the low number of records, sire by site interaction and genetic group effects were not fitted, and the models were reduced to a sire model to aid convergence.

Fixed effects included birth type (1,2,3,4+), rearing type (1,2,3+), age of dam (linear), age of dam squared, dam breed, sire breed, age at trait measurement (linear) and contemporary group (defined by breed, flock, year of birth, sex, management group, date of measurement and kill group (Swan *et al.*, 2014)). Weight was fitted as a covariate for eye muscle depth and fat depth. Random effects included the sire genetic effect and, for post-weaning traits, the dam permanent environment effect.

The expected response to selection was evaluated using the MTINDEX spreadsheet for multiple trait selection index by van der Werf (2005). Two scenarios were explored. For the ram breeding program scenario, it was assumed that each ram had its own record, as did its sire and dam, 20 half-sibs and 20 progeny for live traits, where only 10% of their progeny and half-sibs were slaughtered. For the progeny testing situation, it was assumed that records for both live animal and carcase traits were only available for 30 progeny. Genetic gain for LMY under the different scenarios was expressed as a proportion of the gain that could be achieved if all animals had a recorded LMY phenotype. This reflects a situation where LMY is the only trait we are aiming to change by genetic selection (not the case in reality).

Results and discussion

Genetic parameter estimates for all traits are presented in Table 2. The heritability estimate of CT lean was 0.24 ± 0.03 (± SE). This was slightly lower than the 0.34 ± 0.05 estimate reported by Mortimer *et al.* (2010) for the 2007- and 2008-born INF progeny, but certainly providing substantial scope for changing the trait by selection.

DEXA-derived lean was strongly correlated to CT lean, phenotypically (0.80 ± 0.01) and genetically (0.75 ± 0.10). While more data are required to validate these results, DEXA-derived lean provides a good indicator of both the phenotypic and genetic variation that exists for LMY. It is also viable to obtain DEXA-derived measures under commercial processing conditions, indicating it is a potential option as a correlated trait as part of a selection criterion to improve LMY.

The genetic correlations between CT lean and post-weaning traits ranged from -0.54 to 0.10. Similar correlations have been observed between live scan traits & LMY (Mortimer *et al.,* 2010). These correlations were weaker than observed between CT lean with the carcase and DEXA-derived lean. The results indicate that the traits measurable

on live animals are moderate to strongly genetically related to CT Lean, and are heritable themselves. Which, as was also observed with the DEXA-derived lean, makes them suitable for use as a correlated trait within the selection criterion.

The predicted genetic gain in LMY by selection for indicator traits is shown in Figure 1. The expected gain is expressed as a proportion of the genetic gain that could be achieved via direct selection for LMY where all animals are assumed to have a CT lean record.

Despite DEXA-derived lean being a good indicator of CT lean, phenotypes can only be obtained on slaughtered animals. A ram breeder will sell most of their animals as breeding stock and thus only surplus animals will be slaughtered (assumed to be <=10%). Therefore, the ram breeder can only capture 50% of the potential genetic gains if they rely on DEXA records from the slaughter animals alone. The ram breeder would be able to achieve similar genetic gains if they were to base selection on the

Table 2. Genetic parameter estimates (standard error) for live animal and carcase traits, and estimated phenotypic and genetic correlation with CT lean

Trait	Phenotypic Variance		Heritability		Phenotypic Correlation		Genetic Correlation	
CT lean	8.90	(0.00)	0.24	(0.03)				
Post-weaning weight	20.61	(0.17)	0.26	(0.01)	-0.19	(0.05)	0.10	(0.12)
Post-weaning eye muscle depth	5.00	(0.05)	0.36	(0.01)	0.11	(0.05)	0.00	(0.11)
Post-weaning fat depth	0.50	(0.00)	0.22	(0.01)	-0.27	(0.04)	-0.54	(0.11)
Post-weaning condition score	0.09	(0.00)	0.20	(0.01)	-0.29	(0.08)	0.00	(0.15)
Hot carcase weight	5.10	(0.05)	0.27	(0.01)	-0.36	(0.02)	-0.11	(0.11)
Carcase eye muscle depth (C site)	9.42	(0.10)	0.24	(0.01)	0.17	(0.02)	0.35	(0.12)
Carcase fat depth (C site)	3.06	(0.03)	0.29	(0.01)	-0.35	(0.02)	-0.57	(0.09)
DEXA-derived lean	16.89	(1.05)	0.59	(0.07)	0.80	(0.01)	0.75	(0.10)

Abbreviations: CT: computed tomography; DEXA: dual-energy x-ray absorptiometry.

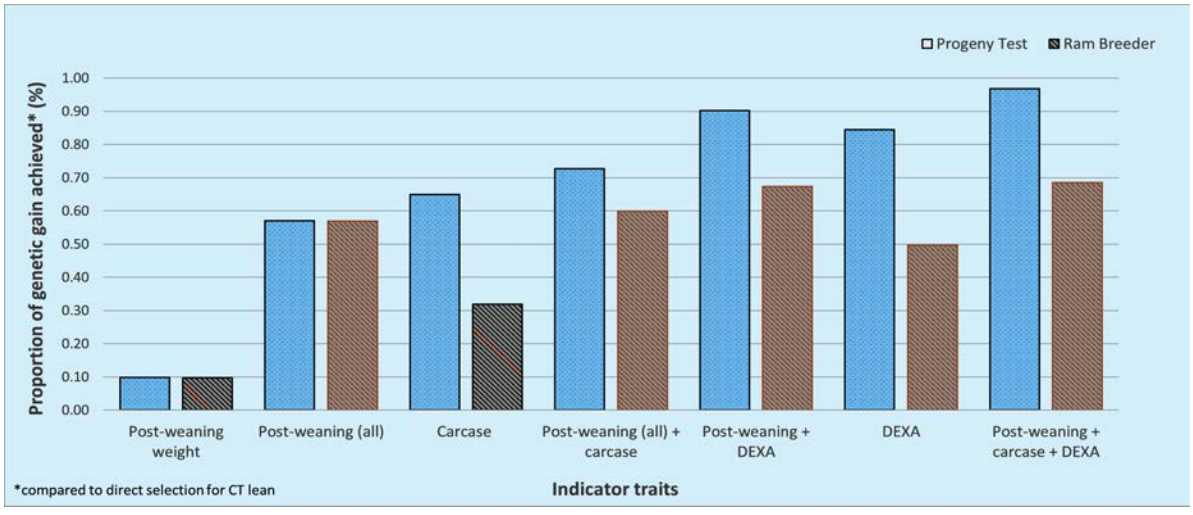

Figure 1. Proportion of genetic gain achieved in lean meat yield through use of indicator traits, compared to direct selection based on computed tomography (CT) lean, in progeny test and ram breeder situations

post-weaning traits (57% of the genetic gains). Despite the weaker genetic correlations between CT lean and post-weaning traits, post-weaning traits are useful predictors of LMY as they can provide an understanding of the genetic variation for LMY that exists within the flock without the need to slaughter animals. Including DEXA records with the post-weaning records as part of the selection criterion improve the relative genetic gain achieved to 67%.

To achieve stronger genetic gains ram breeders are reliant on either progeny testing rams in resource flocks or using genomics to create genetic links to resource flocks where hard-to-measure traits are routinely recorded. In a scenario where the progeny of the selection candidates (rams) have DEXA-derived lean records, 84% of the potential genetic gain could be achieved, compared to only 57% and 65% if the progeny are only recorded for post weaning or carcase traits. However, if post weaning, carcase and DEXA information was available on all progeny, then 97% of the potential genetic gain could be achieved.

Since DEXA measures can be implemented with minimal interruptions to processing, the combination of post-weaning and DEXA derived traits would appear to be of greatest value to breeders especially if animals of industry significance can be progeny tested in resource flocks.

Conclusions

DEXA has value and is a suitable replacement for CT especially since measurement can occur at normal chain speed. The cost of implementation needs to be weighed up against the returns generated by genetic progress for LMY. However, industry genetic progress for LMY will be considerably assisted by recording and using the correlated traits measured on the live animals in ram breeding flocks. As ram breeders will struggle to record DEXA and CT traits in sufficient numbers, resource populations such as the INF/RF are required to validate the parameters presented within this study and strengthen the ability to make sensible selection decisions to improve LMY. Furthermore, since there are unfavourable associations between LMY and eating quality, the genetic improvement of LMY will need to be managed, and eating quality should be included in the breeding objective.

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Breeding for meat sheep in France

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Abstract

Selection of meat sheep in France is based on within breed collective breeding programs. Selection criteria and phenotypes collected in the national performance recording system depend on the type of breeding program. A comprehensive selection scheme includes 3 key steps with genetic evaluations: (1) on-farm evaluation of maternal abilities and meat qualities evaluations based on (2) individual testing and (3) progeny testing. On-farm evaluation of maternal abilities is based on both prolificacy and mothering ability, assessed by one weighing per lamb around 30 days of age. On the whole, 330,212 ewes had on-farm phenotypes in 2016, in 1,230 flocks from 46 different breeds. These figures represent roughly 5% of the whole meat sheep population. Meat qualities are evaluated on young rams in central test stations; the criteria are growth rate and weight at typical age assessed by weighings, fat and muscular development at typical age assessed by ultrasonic measures (fat thickness and muscle depth) and by muscle scoring (shoulders, back-loins and legs). A total of 3,794 rams were evaluated in such stations in 35 breeds in 2016. Finally, 194 rams from 9 breeds were progeny-tested for meat qualities; phenotypes are collected on lambs in fattening unit and at slaughtering at fixed age: growth rate during fattening, conformation assessed by morphological development, carcass conformation and rib eye muscle, dressing percentage, fat (external fat extent, internal fat amount, loins fat amount, back fat depth at last rib). Besides this pattern of breeding schemes and the phenotypes currently collected, different traits are on the way to be included in the selection criteria for some breeds, such as resistance to parasites, behavior, mortality/vigor of the lambs, longevity. One of the main challenges within the prospect of the new zootechnical European regulation is to reinforce the collective organization of meat sheep genetic improvement, especially through efficient breeding organizations.

Keywords: meat sheep, recording, genetics, phenotypes.

Introduction

Sheep production in France represents a total of 5,159,000 reproductive ewes (FGE, 2017). 73% out of them (3,779,000) are meat sheep, the other 27% being dairy sheep. The meat sheep are commonly divided into specialized pure meat breeds (2/3 of all meat sheep), hardy pure meat breeds (1/4) and crossbreds. Selection in France is organized within breed. 47 breeds had at least one flock in performance recording in 2016. 8 specialized meat breeds and 13 hardy meat breeds are

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considered to have a breeding program. Besides this most important breeds, 26 underutilized breeds are either in conservation or with too small population to implement a selection program. The specialized meat breeds are mainly situated in the northern part of France, in flat area, while the hardy breeds are located in the southern part of France, in non favorized mountainous or dry areas.

The organization of a typical selection scheme in French meat sheep can be drawn as follows, with 3 possible stage for the more complete scheme:

- First step: on-farm recording and evaluation (prolificacy, mothering ability, growth) are focused in the nucleus flocks. In this flocks, AI is practiced in some breeds either to produce connection between flocks or (if the AI rate is sufficient) to run a maternal progeny testing program.
- Second step: rams born from assortative matings in the nucleus flocks (sires of sires and dams of sires) enter a central test station for an individual evaluation for growth and meat quality. Only a part of the breeds have central test station.
- Third step: in some breeds, the best rams after massal selection in the central test station are progeny-tested, in order to produce elite rams.

On-farm performance recording of maternal abilities

In 2016, 330,112 ewes were recorded on-farm in 1,267 flocks (Tiphine, 2017). This figures represents 8.6% of the total meat sheep. 85% of the recorded flocks were engaged in a breed society. 149,599 ewes were inseminated by meat rams, ie 3.9% of the French meat sheep.

Three levels or method of on-farm performance recording, designed as a Russian doll system, can be chosen by the breeder. The first design, called reproduction procedure, consists in recording reproduction data and especially prolificacy, both on natural and induced oestrus. The mothering ability procedure is a design where, in addition to reproduction, the viability of the lambs and a weighing at around 30 days of age are phenotyped. Finally, the complete procedure also collects a weighing at around 70 days of age (before weaning), allowing to estimate the growth between 30 and 70 days. 70% of the flocks (increasing number) use the mothering ability procedure, whereas 20% are in reproduction procedure (stable) and 10% only are in the complete procedure (decreasing number).

The corresponding genetic evaluation and selection criteria to the different procedure are:

- Reproduction procedure:
  - The selection objective is prolificacy.
  - The EBVs are prolificacy on natural oestrus ($PROL_{NO}$) and prolificacy on induced oestrus ($PROL_{IO}$).
  - The selection criterion is PROL = $a\,PROL_{NO} + b\,PROL_{IO}$, with weightings a and b depending on the breed.
- Mothering ability procedure:
  - The selection objective is both prolificacy and ewe ability.
  - The EBVs are (in addition to prolificacy): viability of the lambs (or total weaned weight of lambs per dam) (VIAB) and weight at 30 days (WEIGHT).

- The selection criterion for ewe ability is: EWAB = *a* (1/2 WEIGHTdir + WEIGHTmat) + *b* VIAB, with weightings *a* and *b* depending on the breed.

- Complete procedure:
  - The selection objective is prolificacy, ewe ability and growth.
  - The EBVs are (in addition to prolificacy and ewe ability): growth between 30 and 70 days (ADG_{30-70}) and weight at 70 days ($WEIGHT_{70}$).
  - The selection criterion for growth is GROW = 1/2 ADG_{30-70} + ½ $WEIGHT_{70}$.

Performance recording of growth and meat quality in central test station

Individual selection for growth and meat quality is undertaken in central test station which are flock or station gathering the best young rams born from assortative matings. 35 breeds have a station where at least one batch per year is phenotyped. 13 breeds submit the rams to the whole protocol which is described below, 22 breeds are submitted to a lighter protocol.

The central test station is a major tool for the collective management of the breed with a triple objective: genetic management of the rams, sanitary control of the rams, collective and participatory dynamics of the breeders. Only scrapie-resistant rams, born from the best rams and dams, enter the station. Almost 3,500 young rams are evaluated each year.

In the complete protocol, rams enter the station just after weaning, at 70 days. After an adaptation period of 2 weeks, the rams are tested during 8 weeks, with a bunch of phenotypings, including weighing, ultrasonic scan and scoring. At the end of the test period, a transition period during which evaluations of the rams are produced and results diffused, results in the mass selection of the rams. The bottom 20% are eliminated, the top one are selected for AI, and the other are qualified as recommended rams for natural mating and diffused in both the selection and commercial flocks.

The table 1 describes the main measurements collected during the test period, as well as the criteria calculated and evaluated (Tiphine *et al*, 2011). An index is produced as a linear combination of growth, weight, fat and muscle, the weightings depending on the choice of the breed.

Table 1. Measurements collected and criteria evaluated on the rams in central test station.

Measurements	Criteria
Weighing	Growth rate Weight at typical age
Ultrasound (fat depth and rib eye area)	Fat at typical age Muscle at typical age
Scoring (shoulders, back-loins, legs)	Muscle at typical age

Progeny testing of meat quality

A few breeds are involved in a progeny testing for meat qualities. This progeny test has a dual objective:

- Assessing the meat quality of the lambs and improving the carcass quality (more muscularity and less fat).
- Decreasing the production costs, by reducing the age at selling. This leads indirectly to an increase of feed efficiency through growth and fat.

Protocol and phenotypes

The top 10 or 15 rams from the central test station enter AI center. They are inseminated with 35 to 50 females, either suckling or dairy females, in order to produce at least 30 progeny. The lambs born from the AI are gathered in a fattening station at weaning (30 days for the lamb born from dairy ewes, 70 days for the lambs born from meat ewes). There are 4 fattening station in France (figure 1). The lambs are fattened until a fixed age (33 kg for the female lambs, 39 kg for the male lambs) where they are slaughtered. This corresponds to an average age of 110 days. During the period of fattening, the lambs are measured (weekly weighings) and scored. Different appraisal and measures are realized on the carcass:

- Carcass weight.
- Conformation score.
- Fat score (external).
- Fat score (internal).
- Amount of loins fat.
- Back fat depth.
- Shoulder width.
- Rump width.
- Carcass length.
- Rib eye area.
- Muscle depth.

Evaluation and diffusion of the results

The different phenotypes are evaluated and combined in indexes:

- Growth from birth to slaughter: ADG0-slaughter
- Fat index: FAT = linear combination of fat score[external], fat score[internal], amount of loins fat, back fat depth
- Conformation index: CONFORMATION = linear combination of shoulder width, rump width, carcass length, conformation score, rib eye area, muscle depth.

A total merit index is diffused with weightings *a*,*b*,*c* depending on the breed:

$$TMI = a\,\text{ADG0-slaughter} + b\,\text{FAT} + c\,\text{CONFORMATION}$$

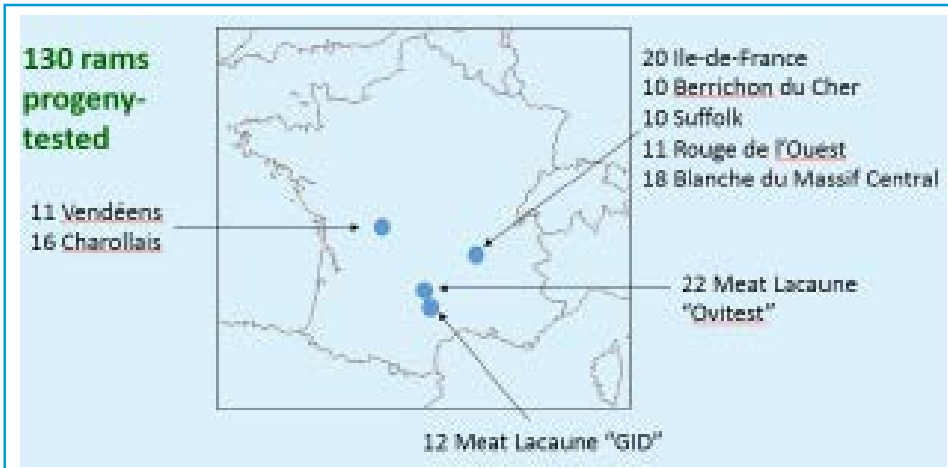

Figure 1. Progeny testing for meat qualities: fattening stations and breeds involved.

On the whole, 130 rams are progeny tested each year in 8 breeds (Cheype, 2017) as shown in the figure 1.

The different EBVs are represented in a spider chart (Cheype *et al.*, 2016) as illustrated in the figure 2.

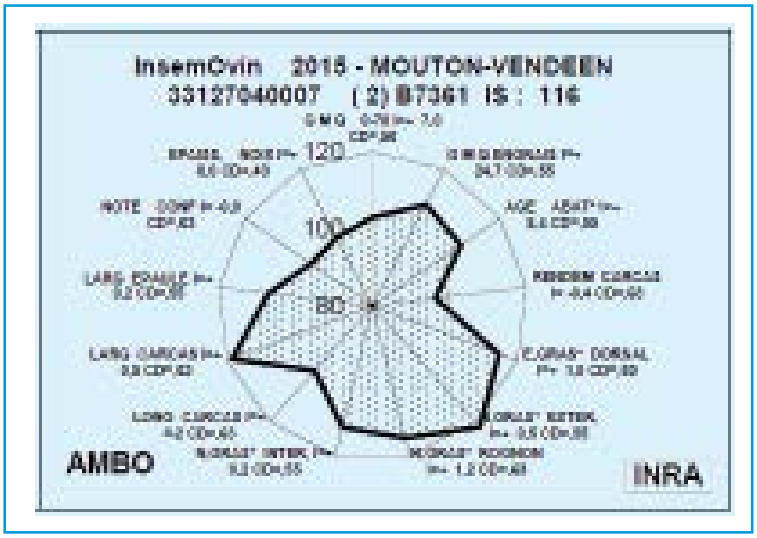

Figure 2. Spider chart of the EBVs of a ram after progeny testing for meat qualities.

Perspectives

Novel traits investigated

The current situation of meat sheep selection allows to select for reproduction traits, growth traits and meat quality traits.

Several novel traits are investigated in research & development programs with the purpose to include them as quick as possible in the breeding objectives.

- Resistance to internal parasites (nematodes). The phenotypes is collected through an experimental infestation with *Haemonchus contortus* applied on rams in central test station. 5 breeds are concerned so far. Genetic parameters are still to be estimated.
- Maternal behavior. Different in-progress study are undertaken in experimental farms with different tests (handling test, arena test) to assess the maternal behavior of the ewes. The traits have a moderate to high heritability. The interesting point is that litter survival increases as maternal behavior score increases.
- Lamb survival. Within different programs including 2 breeds, the aim is to define a common way of registration for lamb survival, before extending to the collection of the phenotypes to all breeds (Cheype *et al.*, 2016).
- Functional longevity. The breeders are more and more concerned with what they called rusticity or robustness. Functional longevity, assessed by the productive lifespan (date of last lambing – date of first lambing) is an overall trait which can synthesize all functional abilities of the ewes and thus increase the rusticity. An in-progress program aims at producing EBVs of functional longevity (Talouarn *et al.*, 2018).
- Semen production has been already collected in 9 AI centers for several years. EBVs on volume, motility, concentration and number of spermatozoa are released once a year. EBVs are used by the AI center to select the rams.

Besides these novel traits, various major genes are genotyped, collected and managed. The more important is the PrP gene (resistance/susceptibility to scrapie). More than one million animals have been genotyped since 1999 (30,990 in the year 2017). Different major genes related to prolificacy are genotyped: the mutations $FecX^{L}$ (gene BMP15), $FecL^{L}$ (gene B4GALNT2) and $FecB^{B}$ (gene BMPR1B). The mutation $FecL^{L}$ is managed in one of the two strain of the Lacaune breed where 3,900 rams and ewe lamb are genotyped each year. Finally, the second strain of the Lacaune breed has introduced the double-muscling mutation (gene GDF-8) from the Belgian Texel sheep.

Organizational issues

French selection of meat sheep is based on a large number of organizations:

- 18 breeds societies
- 63 performance recording organizations
- 9 AI centers
- Genetic evaluation was so far run by INRA whereas diffusion of EBVs were run by the French Livestock Institute.

The main features to draw meat sheep selection is the collective side of the breeding programs, which allows an actual efficiency of the selection.

The EU Animal Breeding Regulations (2016/1012) will deeply change the organization: breed societies will manage all aspects of selection, including performance recording and genetic evaluation which is new in France.

This new rule is the main **challenge** of the upcoming years, regarding the sustainability of collective breeding programs, their efficiency and their economic model.

Conclusion

Performance recording of meat sheep in France is widespread, with 330,000 ewes recorded each year, even though the trend over the last decade is a steady decrease. Moreover, it appears difficult to maintain AI and the number of inseminated ewes also decreases, rendering more fragile the programs of connection and on-farm progeny test. The central test station are at the heart of the collective breeding programs and are reinforced. So far the different selection tools have been efficient to respond to urgent matters such as resistance to scrapie issue. The “breed approach” is a king of French specificity. The commitment of both the breeders and the administration to maintain the breeds is a way to keep a strong level of biodiversity.

Regarding ICAR concerns, France Génétique Elevage (the French ICAR member) has obtained the ICAR Certificate of Quality for meat sheep in 2017. This is an acknowledgement of the quality of the tools and methods implemented in French meat sheep selection.

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Perspectives of the selection scheme of the Sarda dairy sheep breed in the era of genomics

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Summary

Over the last decade, a progressive decline of the efficiency of the breeding scheme of the Sarda dairy sheep breed has been observed. In this context, a program for implementing innovative genomic tools has been established. The current state of the selection scheme of the Sarda breed is summarized and the potential impact of innovative genomic tools taking into account their economic sustainability is drawn. A female reference population was created to identify LD o causal mutations and to trigger genomic selection. Results of QTL detection and accuracies of Herd Book rams genomic predictions realized on the basis of the female reference population show that it is a realistic option to increase the effectiveness of the current selection program. The impact of the female nucleus may be increased by an organization of the HB flocks in levels according to the application of selection tools *i.e* the incidence of pedigree known and the engagement in AI program.

Keywords: QTL, MAS, genomic selection, reliability.

Introduction

The efficiency of most dairy sheep selection schemes has been traditionally limited by three main constraints: the scarce diffusion of artificial insemination (AI), the low accuracy of the recording system and the small size of the herd book (HB) relative to the whole population. Thus only few selection programs are currently ongoing in dairy sheep breeds with large differences in terms of genetic gains and cost effectiveness (Carta *et al.*, 2009).

The Sarda is the largest Italian dairy sheep breed with around 90% of the national stock reared in Sardinia (3 million heads). In 1992, an AI program combined with a genetic evaluation based on BLUP AM methodology was implemented. This selection scheme has allowed to achieve satisfying levels of genetic gain for milk yield (Salaris *et al*, 2008b). Over the last decade, the national and local Breeders Associations involved in the official recording of production and pedigree data have been revising the organization of the program. This uncertainty produced a suboptimal application of the selection tools causing a progressive decline of the efficiency of the program

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which is negatively affecting the introduction of the recording of innovative production quality, health and functional traits. Moreover, the import in Sardinia of improved exotic breeds such as Lacaune and Assaf has been observed.

In this context, the National Breeders Association (ASSONAPA) with the scientific support of the Regional Agency for Agricultural Research (AGRIS) started a program implementing innovative genomic tools in the breeding scheme.

The objective of this study is to summarize the current state of the selection scheme of the Sarda breed and to draw the potential impact of innovative genomic tools taking into account their economic sustainability.

Current state of the selection scheme

The breeding scheme of the Sarda breed is based on the traditional pyramidal management of the purebred population (Carta *et al.*, 2009). Selection objectives are milk yield, scrapie resistance and udder morphology. Milk composition traits are recorded but no breeding value has been provided to breeders. The main feature of the Sarda scheme has been the large application of the single sire mating (SSM) and rates of AI adequate to achieve accurate genetic evaluations not only of AI rams but also of natural mating ones (Salaris *et al.*, 2008a). Milk yield during exclusive milking estimated from test day records with the monthly AC method is currently the selection criterion.

Only lactation records of ewes within 4 years old are retained for genetic evaluation. Udder morphology scoring and milk composition recording started in 1999 and 2000 respectively. Only ewes in first lactation are sampled for milk composition and scored once a year for udder morphology. The official breeding plan for Scrapie resistance started in 2005 (Salaris *et al.*, 2014). The breeding plan established that all the breeding males and the young ewes with high parent average for milk yield must be genotyped. Since 2013 only breeding males have been genotyped.

The highest number of breeding flocks and recorded ewes was registered between 2000 and 2005, when rates of ewes with known sire and from AI sire were 80% and 15% respectively (Table 1). Over the last years a progressive decline of all these parameters has been recorded. In 2016, beside the decreasing of the number of flocks and recorded ewes, the rates of ewes with known sire and from AI sire have reduced to 56% and 3% respectively. Both the number of mating sires and the number of progeny tested rams have decreased from above 1700 and 500 in 2005 to 1100 and 340 in the last years.

To evaluate the impact of this declining statistics on the effectiveness of the selection scheme, the evolution of the size of the recorded population which is actually exploitable for selective breeding was estimated. Thus, a subset of all data used for the official genetic evaluation including only the portion of the population genetically connected was retained. Practically, only the contemporary groups (CG) i.e the levels of the flock-year-age class-lambing season interaction with at least 5 lactation records of ewes with known sire were retained. Therefore only CG including daughters of sires having offspring in at least one other CG including daughters of other sires were retained. The rate respect to the official dataset of the number of recorded ewes, CG, sires of lactating ewes and the number of sires represented in each CG across production years were calculated (Figure 1).

Table 1. Evolution of the effective size of the Sarda herd book over the last 20 production years

Production year	BF	RF	RF1	MCR	UMR	KSR	AIR	BS	PTS
1997	1,011	93,409	24,984			82.1	13.8	1,397	507
1998	1,133	106,566	29,324			77.6	14.1	1,464	569
1999	1,132	115,658	30,032		42.2	78.9	13.8	1,557	622
2000	1,168	123,737	31,758	47.2	41.5	76.7	14.2	1,594	575
2001	1,176	126,111	33,255	48.0	40.6	77.2	14.9	1,625	482
2002	1,167	129,290	37,343	54.6	33.8	72.4	13.2	1,603	497
2003	1,157	135,858	35,781	51.0	30.8	70.5	11.8	1,617	552
2004	1,141	147,883	41,807	51.7	33.7	69.4	13.0	1,688	519
2005	1,091	142,939	35,326	48.7	22.7	70.8	9.4	1,711	501
2006	1,062	143,581	34,609	50.2	27.4	68.8	8.8	1,613	560
2007	1,075	141,320	31,197	51.7	26.5	66.3	10.0	1,449	461
2008	1,079	135,060	35,216	48.4	25.6	60.8	8.2	1,407	405
2009	1,042	127,231	31,083	49.9	23.9	61.2	7.8	1,371	390
2010	1,035	130,776	31,744	53.6	28.9	60.3	8.0	1,260	416
2011	1,025	126,920	30,491	48.3	30.4	61.2	7.2	1,204	365
2012	972	119,746	28,555	50.6	34.6	62.3	6.9	1,136	350
2013	953	117,404	26,372	48.2	41.7	61.5	4.9	1,093	368
2014	969	112,024	25,172	43.7	26.2	57.5	3.8	1,046	328
2015	943	114,877	27,482	47.3	28.9	57.9	3.7	1,048	307
2016	936	120,604	32,627	40.3	17.2	56.3	3.4	1,142	344

Number of breeding flocks (BF); number of milk recorded females within 4 years old (RF); number of ewes in first lactation (RF1); percentage of RF1 recorded for milk composition (MCR); percentage of RF1 recorded for udder morphology (UMR); percentage of RF1 with known sire (KSR); percentage of RF1 from AI sire (AIS); number of RF1 breeding sires (BS); number of BS progeny tested (PTS).

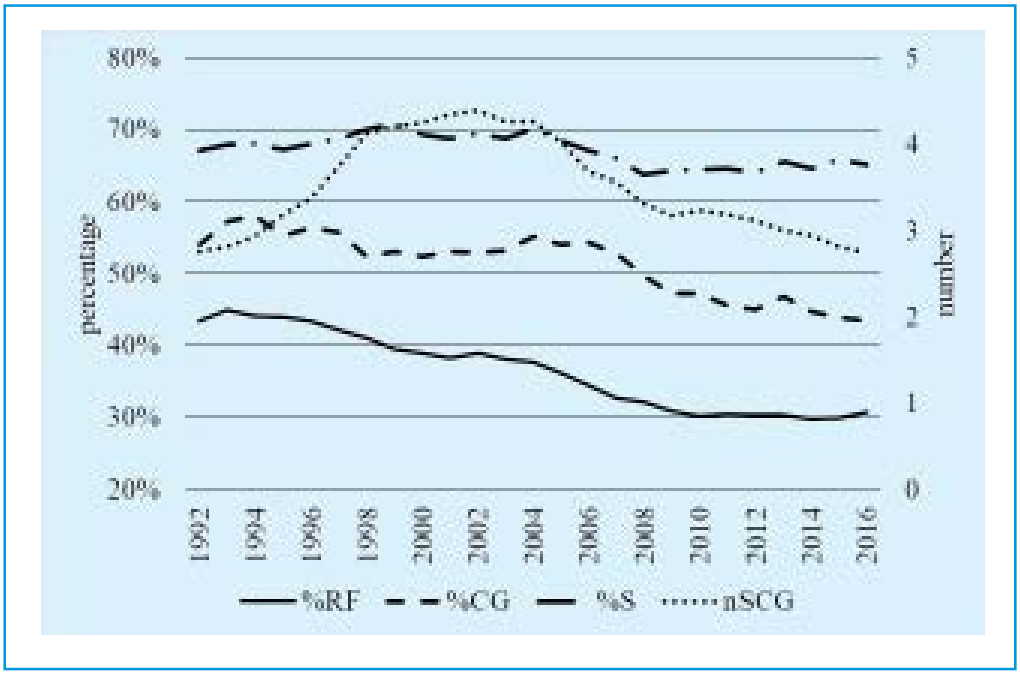

Figure 1. Rates of the number of recorded ewes (%RF), contemporary groups (%CG) and sires of lactating ewes (%S) respect to the official dataset and number of sires represented in the contemporary groups (nSCG) across production years in the genetically connected population.

Moreover, data of the genetically connected population were further split in three subsets according to the degree of application of the main selection tools (from 1983 to 2002, from 1998 to 2011 and from 2006 to 2016) to estimate milk yield heritability and to observe its evolution. A repeatability BLUP animal model was applied on milk yield adjusted for milking length and age-parity-lambing month interaction (Carta *et al.*, 1998) with the interactions "flock-year-class of age-class of lambing season" and "year-class of age-parity-class of lambing month" as fixed effects.

The percentage of the effective size of population remained stable until 2006 whereas successively a progressive decline was observed (Figure 1). Moreover, the number of rams per CG increased before 2000, remained stable until 2006 and then decreased, reflecting the trend of AI rate (Table 1).

The estimates of heritability of milk yield were 0.22, 0.18 and 0.17 in the three periods likely reflecting the reduction of the effective size of the population and of the connectedness.

Between 2000 and 2006, milk composition and udder morphology were recorded on more than 50% and 40% of ewes recorded for milk yield and then the percentages fell to around 40% and less than 20% respectively (Table 1).

Concerning milk composition, the low number of records per lactating yearling (on average 2.3) and the small size of the recorded population (Table 1) led to a lower heritability (unpublished results) compared to literature (Barillet, 2007; El Saied *et al.*, 1999) in particular for fat content. Differently from udder morphology traits, Sarda breeders do not exhibit great interest for milk composition because no payment system based on the chemical quality of milk has been implemented in Sardinia. The amount of rams indexed for udder morphology (Casu *et al.*, 2006) is limited by the low number of scored ewes which can not be extended due to organizational constraints.

As far as scrapie resistance is concerned, the ARR frequency in males moved from 42% to 80% between 2005 and 2015 birth year and since 2010 only homozygous resistant rams are allowed for reproduction.

Potential impact of the implementation of genomic tools

The progressive decline of the selection scheme of the Sarda breed described above combined with the difficulty to measure on large scale innovative traits related to production quality, health and sustainability led breeders to assess the feasibility of two genomic application: the selection assisted by causal or linkage disequilibrium (LD) mutations (MAS) and the genomic selection (GS).

Generally the identification of useful mutations for selective purpose needs long trials based on large populations measured for a lot of traits. GS in dairy sheep should be adapted to the specific preexisting breeding schemes taking carefully costs and benefits into account. The dairy cattle approach (Boichard *et al.*, 2015) has been successfully applied in some breeds such as the French Lacaune where the preexisting selection scheme with a large use of AI and accurate recording schemes for functional and health traits, beside the classical milk yield and composition, have made the GS of breeding males profitable (Baloche *et al.*, 2014).

Considering the above mentioned limitations of the Sarda HB, ASSONAPA and AGRIS created a female reference population (FRP) either for detecting QTLs or predicting genomic effects to be used for GS. In the following sections, we discuss the usefulness of FRP to identify causal or LD mutations and the feasibility of genomic predictions of Sarda rams based on records of FRP only.

Female reference population

FRP yearly consists of approximately 1,000 milking ewes with a replacement of about 25% generated by mating adult ewes with Sarda HB rams. Ewes are raised in an experimental farm that has a typical Sardinian dairy sheep farming system. The original aim of FRP was to detect QTL segregating in the Sarda breed. Thus, the number of daughters per ram had been 40 ewes on average until 2009. It was reduced to 9 after 2010 with the aim of increasing the number of breeding rams with daughters in FRP and, consequently, the number of represented *bloodlines* from HB. So far, 3,949 ewes have been generated by 161 rams. Ewes are routinely measured for several traits: production traits (milk yield, fat, protein and lactose content, body weight and body condition score), milkability and udder morphology type traits, health traits (somatic cell count, clinical mastitis, faecal egg count, ELISA test for paratuberculosis, ELISA test for visna-maedi, histo-pathological examination for paratuberculosis), fatty acids content and reproduction traits (fertility and prolificacy).

Genotyping and whole genome resequencing

To date, all FRP ewes and their sires have been genotyped with the Illumina Inc. OvineSNP50 Beadchip (50K). Moreover, whole genome resequencing of target animals, chosen on the basis of their genetic impact on FRP or because having high probability to be homozygous at causal mutations is in progress. Presently, the genome re-sequencing with 12X coverage has already been done for 24 animals and further individuals will soon be resequenced with a higher coverage.

The whole genome sequences of target animals jointly to genotypes from DNA arrays allow the imputation of large genome blocks to many individuals of FRP.

Detection of causal or LD mutations

Indeed, QTL detection analysis based on 50K data and a combined Linkage and LD mapping method using a principal component analysis to synthesize identity-by-descent matrix information have already been applied to the first generations of FRP (Usai *et al.*, 2014; Casu *et al.*, 2014b; Carta *et al.*, 2016; Casu *et al.*, 2018). Several significant locations affecting most of the measured traits have been identified (Table 2).

Among those, the most significant ones are chosen for further investigations with the aim of identify causal or LD mutations to be used for selection purposes. Target regions are first of all screened to verify whether they harbour evident candidate genes for the traits of interest, as it was the case for the casein genes cluster interval (Usai *et al.*, 2014) associated to milk protein content; FASN, AACS and SCD genes (Casu *et al.*, 2014b) associated to fat acids content and ratio in milk yield; MUC15 gene (Carta *et al.*, 2016) associated to gastro-intestinal nematode resistance; different regions of the Major Histocompatibility Complex (Carta *et al.*, 2016) associated to gastro-intestinal nematode and paratubercolsis resistance.

In a second step, candidate polymorphisms identified in the region of interest by exploiting re-sequences or imputed genotypes, can be annotated to the reference genome to search for variants potentially affecting gene expression (untranslated regions, splicing sites, CpG island, and promoter regions) or SNPs in coding regions that have nonsynonymous consequences. This approach was applied to explore a region on OAR6 significantly associated with milk protein contents and mapping close to the cluster of caseins genes (Casu *et al.*, 2014a). Genetic variants resulting alternatively homozygous in two sequenced animals alleged to carry the positive or negative alleles were annotated on Oar_v3.1 reference genome and classified using the gene annotation database from Ensembl release 74 with the Variant Effect Predictor

Table 1 - QTL regions significant at 5% genome-wide threshold for measured traits.

Measured Trait	Chromosome (number of regions in the chromosome)
Production traits[1]	
Milk Yield	2(2), 3(1), 5(1), 10(1), 11(1), 12(1),13(1), 16(1), 20(1), 22(1), 25(1)
Fat Yield	2(1), 10(1), 11(1), 15(1), 20(1), 22(1), 25(1)
Protein Yield	2(1), 3(1), 10(1), 11(1), 12(1), 15(1), 20(1), 25(1)
Fat Content	1(2), 2(2), 3(1), 4(1), 5(1), 6(1), 9(1), 10(1), 12(2), 13(1), 14(1), 16(1), 17(1), 18(1), 19(1), 20(1), 25(1)
Protein Content	1(2), 2(2), 3(2), 4(1), 5(1), 6(1), 8(1), 9(1), 10(1), 11(1), 12(1), 13(1), 15(1), 16(1), 17(1), 18(1), 26(1)
Udder morphology[2]	
Udder depth	9(1), 24(1)
Teat position	3(3), 6(1), 7(1), 8(1), 9(1), 10(1), 16(2), 17(1), 20(1)
Degree of separation of the two halves	2(1), 5(1)
Degree of suspension of the udder	1(1), 2(1), 4(1), 5(2), 7(1), 8(1), 9(3), 10(1), 14(1), 16(1), 20(1), 25(1)
Milk quality traits[3]	
C4:0	17(1)
C10:0	11(1)
C14:0	11(1)
C14:1	7(1), 22(1)
C14:1/C14:0	7(1), 22(1)
C15:0	16(1)
C16:0	26
C16:1	10(1), 22(1)
C16:1/C16:0	22(1)
C18:0	8(1)
C18:1/C18:0	19(1), 22(1)
CLA/vaccenic acid	22(1)
Health Traits[4]	
Somatic Cell Count	2(1), 3(2), 4(1)
Faecal Egg Count	1(1), 3(1), 4(1), 8(1), 12(1), 15(2), 17(1), 19(1), 20(1), 21(1)
ELISA test for paratuberculosis	20(1)
Histo-pathological examination for paratuberculosis	20(1)

[1]Usai *et al.*, 2014; [2]Casu *et al.*, 2018; [3]Casu *et al.*, 2014b; [4]Carta *et al.*, 2016.

Web tool (*http://www.ensembl.org/tools.html*). A manual scanning of the regions corresponding to the 3'UTR of caseins genes was also performed in order to identify potential miRNA target sites, which are known for modulating gene expression. This study allowed to identify, among others, two genetic variants in the CSN1S2 gene strongly candidates to be responsible for the protein content variation in sheep milk: one in positions 85190123 (rs411463377), which defines a splicing region, and another in position 85196954 responsible of the modification of a mi-RNA target site. However, since a new annotation version of Ovis Aries genome (*http://www.ncbi.nlm.nih.gov/genome/annotation_euk/Ovis_aries/102*) has been released a further variant classification analysis is needed in order to take in to account possible updates genes' information also considering that significant discordance remains in variant annotation among the tools, public resources, and literature (Yen *et al.*, 2017). Indeed, as pointed out by McCarthy *et al.* (2014), "Variant annotation is not yet a solved problem. Choice of transcript set can have a large effect on the ultimate variant annotations obtained in a whole-genome sequencing study. Choice of annotation software can also have a

substantial effect. The annotation step in the analysis of a genome sequencing study must therefore be considered carefully, and a conscious choice made as to which transcript set and software are used for annotation".

As an alternative to causative mutation, informative LD SNPs will be identified using the sequences of haplotypes of interest.

Causal or LD mutations associated with complex traits will be used to design low-density SNP panel with high predictive performance for the traits of interest and including SNP selected for parentage assignment.

Genomic selection

A first assessment of the feasibility of genomic predictions for milk yield with the aim of identifying useful criteria to optimize the size and the structure of FRP and modulate the flow of breeding animals from and toward the HB has been performed (Usai *et al.*, 2018).

A validation sample consisting of 537 HB rams was chosen representing all categories of breeding males: 144 AI rams with daughters in FRP, 105 AI rams without daughters in FRP and 288 rams used by SSM in HB with no daughters in FRP. For all these rams official EBV for milk yield were available.

The FRP lactation records were used to predict genomic breeding values (GBV) of the validation sample by exploiting the genomic relationship matrix (**G**). The theoretical accuracies of GBVs were calculated: $rGBV_i = \{1 - [SE_i^2/(\mathbf{G}_{ii}*\sigma_g^2)]\}^{1/2}$. The traditional GBV accuracy obtained through the correlation between GBV and EBV or deregressed proofs of a set of validation individuals with EBV's accuracy so high to be considered a gold standard (i.e. TBV), is not suitable in the Sarda population. In fact, in this population the rate of AI and simplified recording schemes do not allow to select a validation sample. As an example, less than 50% of genotyped HB rams had a number of daughters higher than 50.

Moreover, the impact of the information on relatives in FPR on the accuracy of GBV (rGBV) of rams of the validation sample was verified. Usai *et al.* (2018) proposed the diagonal element (diA) corresponding to the ram of the inverted section of the relationship matrix (**A**) that included all FRP ewes and the ram itself as indicator of the relationship of HB rams with FRP.

The authors demonstrated that diA is a good predictor of rGBV. This parameter could be used to select the new sires of FRP in order to maximize the number of HB rams with a sufficient rGBV and, thus, the number of rams that will be accurately evaluated by GBLUP after genotyping.

In fact, the average rGBV of validation sample was 0.58. Average rGBV of rams high related with FRP (diA $\geq$ 2.5) approached the average EBV accuracy of rams progeny tested with 30 daughters in HB (0.82); average rGBV of rams medium related with FRP (diA $\geq$ 1.25 and $\leq$ 2.5) approached the EBV accuracy of rams progeny tested with 2 daughters in HB (0.61); average rGBV of rams low related to FRP (diA < 1.25) was below the average accuracy of parent average of rams entering progeny test in HB (0.47). The average correlation of GBV with official EBV was 0.24. It ranged from 0.18 to 0.47 according to relationship with FRP.

In order to provide practical criteria to modulate the flow of animals from and toward FRP and allow farmers to reach a sufficient estimated rGBV for their rams, an estimate of the expected rGBV on the base of the number of relatives in FRP of a ram was carried out. A linear model (R^2= 0.84) on rGBV was performed using as covariates the

number of relatives in FRP with a relationship coefficient of 0.50 and the number of relatives in FRP with a relationship coefficient of 0.25. Rams having 5 daughters showed a predicted rGBV around 0.58. Sons of rams with 5 daughters (i.e 5 half sisters in FRP) showed 0.54. In the practical management, only young rams entering progeny test will be genotyped and part of those will be used as sires in FRP allowing genomic evaluation of them and their sons. This implies that, considering a replacement rate in the of 250 ewes, at least 50 new rams and their sons will be genotyped and GBV calculated with an accuracy higher than that of young rams in progeny test in the HB (parent average accuracy of 0.52).

Perspectives

Results of QTL detection for routinely and innovative traits and accuracies of HB rams genomic predictions for milk yield realized on the basis of the female reference population showed that it is a realistic option to increase the effectiveness of the current selection program of the Sarda breed. The female reference population was crucial to trigger the application of genomic tools to the breeding scheme. For traits costly to measure on large scale, the female reference population will allow to produce genomic predictions for adult and young rams with sufficient accuracies. On the other hand, for traits routinely measured in HB, the progressive pile up of males genotypes jointly to the application of ssGBLUP methodologies (Mistzal *et al.*, 2009) will allow the increase of the accuracies of genomic enhanced breeding values. Moreover, the use of customer DNA chips including LD or causal SNPs may help to accelerate the genetic progress and to make more feasible the pedigree recording. The impact of the female nucleus and its cost effectiveness may be increased by an organization of the HB in levels of flocks according to the rate of application of the selection tools *i.e* the incidence of pedigree known and the engagement in AI program.

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Effect of fat – protein ratio on somatic cell count in milk

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Summary

International genetic trend study in Brown Swiss bulls has revealed the largest changes in genetic trend direction for somatic cell count (SCC) (Gorjanc *et. al.*, 2011). Somatic cells in milk are represented by epithelial cells and leukocytes (immune cells), where the latter predominate and in case of udder inflammation (mastitis) increase to extreme extent. The relation between increase of SCC and immune response is confirmed by several studies (e.g. Concha, 1986; Burvenich *et al.,* 1994). Due to the low economic value of milk fat in the last decades, high selection pressure on the protein content (PC) has been applied. At the same time no selection pressure, or in some populations even negative one, has been applied on fat content (FC). Taking into account also the fact that precursors of milk components enter the mammary system from the blood, the hypothesis is that milk composition changes expressed as narrow fat - protein ratio (FPR) affect cow's immune response and result in higher SCC. Hypothesis was tested on Slovenian Brown Swiss dairy cattle population included in national milk recording scheme. Test day records (TD) from years 2004 to 2017 were used. Data set included 862,780 TD of 44,821 cows. Distribution of raw SCC values was right skewed hence the data were transformed using binary logarithm and the resulting values were almost normally distributed afterwards. To estimate variance and covariance parameters animal TD models were used. Two-trait model (model 1) included SCC and FPR while three-trait model (model 2) included SCC, FC and PC. Statistical model was the same as the model in the routine national genetic evaluation. Results of described models were compared with results of variance component estimates from routine single-trait evaluation for SCC, FC and PC. Heritability estimates for SCC were almost the same for all three evaluations (0.36). Heritability for FC increased for 0.01 in model 2 in comparison to national evaluation whereas heritability for PC decreased for 0.01. Estimated heritability was lower for FPR than for the other traits (0.17). Estimated phenotypic correlation between SCC and FPR in model 1 was negative and very low (-0.001) while genetic correlation among these traits was higher though still negative (-0.100). In model 2, phenotypic correlations were low for all three trait combinations (0.062 - 0.065) but genetic correlations showed to be quite different. Genetic correlation between FC and PC was moderate and positive (0.541), between SCC and PC was low and positive (0.064) whereas genetic correlation between SCC and FC was low and negative (-0.043). Moderate and positive genetic correlation between FC and PC indicates opposite orientation compared to the estimated genetic correlations between SCC and each of these two traits. This difference is supported

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by negative genetic correlation between SCC and FPR. The results show the complexity of the relations between considered traits. Especially traits with low and negative genetic correlations could on long term result in unexpected negative (unwanted) consequences. These results will also be tested on the other two Slovenian dairy cattle breeds. More conservative approach will have to be taken when making selection pressure changes on individual traits in breeding programs if the hypothesis is confirmed.

Keywords: Brown Swiss cattle, somatic cell count, fat-protein ratio, immune response.

Introduction

Study on Brown Swiss cattle made by Gorjanc *et al.* (2011) shows a positive genetic trend for milk's protein yield for a last couple of decades, and also for somatic cell count (SCC) since 1995. Interestingly, while protein yield seems to be raising continuously, trend for SCC has reverted from negative to a raising positive one in a span of a decade (between years 1990 and 2000). Moderate positive correlation was estimated between far content (FC) and protein content (PC) (r= 0.53), whereas weaker but also positive correlation was estimated for SCC to PC and FC (r= 0.24 and 0.13, respectively) (Rajcevic *et al.*, 2003). Cinar *et al.* (2015) also presented positive correlation estimates between SCC and PC (r= 0.291) and between SCC and FC (0.103), confirming the results of Rajcevic *et al.* (2003). Moreover, fat - protein ratio (FPR), which is a trait used for determining cow's energy balance and health status, has shown to be a heritable trait correlated to SCC and clinical mastitis (Negussie *et al.*, 2013).

Genetic trends that reflect selection pressure in dairy cattle can be explained by human dietary guidelines' and diet changes in the past decades. Nutritional recommendations issued by health organisations have been changing, following the progress in discoveries of studies researching connections among macronutrient intake and health. For instance, a strong positive correlation between dietary, saturated fat intake and increased risk factors associated with the occurrence of coronary heart disease was estimated (e.g. Knox, 1977; Menotti *et al.*, 1999). Findings such as these further influenced diet recommendations (e.g. Department of Agriculture, 1980) toward reduced dietary fat intake (with emphasis on cholesterol and saturated fat) and simultaneously toward protein promotion. Rising awareness of health importance has led to substituting the origin of fat; animal for vegetable, and thus lessened proportion of fat consumed in form of milk fat (Weir, 1974; Munro, 1974). Increased demand for lower-fat milk and dairy products and declined milk consumption resulted in a decrease of milk fat's economic value. Since milk price is mainly determined by demand and supply, but also by the value of the products made from it, milk pricing started to take into account also more highly regarded part of milk, PC, which is important for cheese production (Graf, 1974; Sims, 1998). As a response to the milk market changes, high selection pressure on PC and no or negative selection pressure on FC started to be applied (e.g. Welper, 1991).

SCC is another economically important trait. It is considered a marker of milk quality control and milk hygiene in ruminants, but also an indicator of mammary health (subclinical mastitis). Somatic cells in the udder and milk are predominantly represented by epithelial cells and leucocytes (polymorphonuclear neutrophils, macrophages, and lymphocytes). The latter cell type is associated with the immune response of the mammary gland, thus reflecting the health of the udder (Concha, 1986; Burvenich *et al.*, 1994). As leucocytes provide the first defence response in case of udder inflammation, their elevated number is associated with occurrence of mastitis; an inflammatory reaction within the mammary gland. The primary threshold for udder

inflammation in cows is SCC ≥ 200,000 cells/ml milk, and the threshold for significant udder infection is SCC ≥ 300,000 cells/ml milk (Burvenich *et al.*, 1994; Li *et al.*, 2014; AHDB Dairy, 2018). Raw cow's milk in the European Union is considered to be fit for human consumption and further processing if the criteria SCC ≤ 400,000 cells/ml milk (three month average) is met (Regulation (EC) No 853/2004).

Milk is composed for optimal nutrition of calves. Aspiring to moderate positively correlated milk component proportions such as FC and PC in the opposite direction seems not only difficult to attain but also not completely free of negative consequences on the animal's health. Animals have a biologically limited capability to physiologically adapt to the selection pressure on production traits. If animal's production is being emphasized genetically, one or several other biological traits are going to be genetically restricted (Oltenacu and Broom, 2010). Since elements of milk components stem from blood (Strucken *et al.*, 2015), genetic milk component alteration could mean genetic blood element alteration, which could have an effect on immune response and reflect as changed (elevated) SCC.

Based on the described premises, it is hypothesized that milk composition changes, expressed as narrow FPR affect cow's immune response and result in higher SCC. This study gives an insight of genetic consequences caused by low negative genetic correlation in Slovenian Brown Swiss cattle; a result of short-sighted selection decisions. It estimates the heritabilities for SCC, FPR, FC and PC, and genetic as well as phenotypic correlations between them in two- and three-trait model, comparing them to the estimates from the routine national single-trait variance component evaluation.

Material and methods

The data records of Slovenian Brown Swiss dairy cattle population included in national milk recording scheme were used. The data set consisted of 862,780 test day records (TD) stemmed from 44,821 cows, gathered in years 2004 to 2017. The data were obtained from the National dairy milk recording database of Agricultural Institute of Slovenia.

Traits considered in the analysis were FC (%), PC (%), SCC (x 1,000 cells/ml), and FPR. Distribution of raw SCC values was right skewed hence the data were transformed using binary logarithm ($\log_2$). The resulting SCC values were almost normally distributed.

To estimate variance and covariance components by software package VCE-6 (version 6.0.3-dev; 95% Bayesian credibility region) animal TD models were used. Two-trait model (model 1) included SCC and FPR while three-trait model (model 2) included SCC, FC and PC. Statistical models were the same as for the routine national genetic evaluation:

$$y_{ijklm} = \mu + C_i + b_{Ij} + b_{IIj}\,(t/305)^2 + b_{IIIj}\ln(t/305) + b_{IVJ}\ln^2(t/305) + P_j + hy_k + pe_{jl} + a_{ijkl} + e_{ijklm} \quad (1)$$

In the given model (1) y_{ijklm} represented trait of interest (FC, PC, SCC and/or FPR; standard lactation), and μ population mean. Fixed effects were C_i as a calving season (calving year x month interaction; from 2003 to 2017); b_{Ij} (t/305) + $b_{IIj}(t/305)^2$ + b_{IIIj} ln(t/305) + b_{IVj} $\ln^2(t/305)$ as state of lactation with lactation curve shaped by

Ali-Schaffer, nested within parity; and P_j as parity (j= 1, 2,…5). Random effects in the model were hy_k as herd-year; pe_{jl} as permanent environmental effect; and a_{ijkl} as additive genetic effect; e_{ijklm} represented residual in the model.

Results and discussion

Estimates of variance components from routine national single-trait evaluation for SCC, FC and PC are given in Table 1, and results of models 1 and 2 are shown in Table 2. Heritability estimates for SCC were very similar for all three evaluations (0.36). Heritability for FC from national evaluation (0.22) was lower but similar to heritability from model 2 (0.23). Heritability for PC was on the contrary little lower in model 2 (0.39) compared to the one from the national evaluation (0.40). Although converse, the change of heritability for FC and PC comparing the routine evaluation and evaluation made for this study was the same (± 0.01). Estimated heritability for FPR (0.17; Table 2) was lower than for any other trait. SCC heritability estimates in this study were almost 4 times higher than the highest estimate of Negussie *et al.* (2013), who reported heritability for SCC in Nordic Red cattle from 0.05 to 0.10. But the estimate of heritability for FPR seems to be in agreement with theirs which was between 0.13 and 0.25. Welper (1991) also reported lower but similar heritability for SCC (0.16) in Holstein breed comparing by our estimate.

Missanjo *et al.* (2013) estimated heritabilities of 0.08, 0.42 and 0.44, genetic variances of 0.0252, 0.0587 and 0.0105, and error variances of 0.25, 0.071 and 0.0117 for SCC, FC and PC, respectively in Jersey breed, while Bouver *et al.* estimated similarly low heritability (0.07) for SCC in South African Dairy Swiss population. Their heritabilities for SCC were quite lower than ours, but their heritability for FC was almost double compared to ours. Heritabilities for PC were similar, though ours were lower.

In Canadian Holstein population, Jamrozik *et al.* (2011) also estimated low heritability for SCC (0.17) but a higher one (0.71) for FPR. Compared to ours, the latter is substantially higher.

Estimated phenotypic correlation between SCC and FPR in model 1 was negative and very low (-0.001) and genetic correlation among these traits was also negative although higher (-0.100). Phenotypic correlations in model 2 were low and similar for all trait combinations (0.062 – 0.065) (Table 3).

As shown in Table 3, genetic correlation between SCC and PC (0.064) and also between FC and PC (0.541) were low to moderate and positive. Genetic correlation between SCC and FC was low and negative (-0.043) which is consistent with estimates reported by Negussie *et al.* (2013) that ranged from -0.01 to 0.20. They also estimated genetic correlation between FPR and clinical mastitis, which was positive and ranged from 0.12 to 0.21, confirming the FPR being an ‘indicative trait’ for udder health status.

Negative weak genetic (-0.01) and phenotypic (-0.01) correlations between SCC and PC for Jersey breed estimated by Missanjo *et al.* (2013) were lower and oppositely oriented than ours. The only data that we found to compare SCC-FPR genetic correlation with was from Jamrozik *et al.* (2011) for Holstein cows. Their estimation of genetic correlation was weak and positive (0.04) and thereby not in agreement with ours.

Table 1. Variance component estimates from routine single-trait evaluation.

Trait	[a]h^2	[b]δ^2_a	[c]δ^2_e
SCC	0.36	1.35	1.53
FC	0.22	0.10	0.33
PC	0.40	0.04	0.05

[a]h^2:heritability
[b]δ^2_a: genetic (animal) variance
[c]δ^2_e : error variance

Table 2. Variance component estimates from model 1 and 2.

Model	Trait	[a]h^2	[b]δ^2_a	[c]δ^2_e
1	SCC	0.36	1.35	1.53
	FPR	0.17	0.01	0.03
2	SCC	0.36	1.35	1.53
	FC	0.23	0.11	0.33
	PC	0.39	0.04	0.05

[a]h^2: heritability
[b]δ^2_a: genetic (animal) variance
[c]δ^2_e: error variance

Table 3. Genetic and phenotypic correlations of SCC, FPR, FC and PC.

Model	Trait	r^1_g	r^2_p
1	SCC-FPR	-0.100	-0.001
2	SCC-FC	-0.043	0.065
	SCC-PC	0.064	0.062
	FC-PC	0.541	0.065

r^1_g : genetic correlation;
r^2_p: phenotypic correlation

Higher positive genetic correlation between FC and PC, when compared to the estimated genetic correlations between SCC and each of these two traits, indicates pronounced orientation in the opposite direction. Negative genetic correlation between SCC and FPR furthers the contrast. Similar phenotypic correlation estimates between SCC and FC or PC lead to conclusion that regardless the differences in genetic correlation estimates, alteration of milk components in form of selection on FC or PC increases the SCC with similar intensity. However, estimates of (co)variance components for these traits, especially for FPR are scarce and the results are hard to compare.

Nevertheless, relations between considered traits are more complicated than it seems at first sight. Results show the importance of considering the impact of traits with current low negative genetic correlations. These traits could, on long term, result in unexpected negative (unwanted) outcome.

Conclusion

Somatic cell count (SCC) is a highly heritable udder health trait; its elevation usually marks an inflammatory reaction such as mastitis. Besides being used as a health indicator, it is also a milk quality indicator upon which the dairy can reduce the payment or reject the milk. Fat – protein ratio (FPR) is another heritable trait used to determine cow's health status, and shows to be positively correlated to clinical mastitis.

Due to the high selection pressure on protein content (PC), and concurrent non-existent or even negative selection pressure on fat content (FC), FPR has doubled in favour of the PC. As previously reported, PC and FC are positively correlated, whereas results of this study show the negative genetic correlation between FPR and SCC pointing out the possibility of cow's health alteration by unbalancing milk's composition.

These results are also going to be tested on the other two Slovenian dairy cattle breeds. Current indications stress the importance of a conservative but comprehensive approach in decision making when the selection pressure changes on individual traits are in question. Considering the results, we recommend a cautious approach even with traits that show weak genetic correlation. Changes in these traits, as a consequence of the indirect impact of high selection pressure on other traits, can only be seen in the long term, when they may be difficult to reverse. Further confirmation of the hypothesis will back up the significance of a cautious approach.

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Voice activated mating data capture

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Introduction

Improving fertility is one of the priorities of the dairy industry and is a key driver of farm profit. Currently, there is no easy way to capture cow-side mating information and most of this is written on dockets and hand entered later, if it is captured at all. There is a need to automate this so that this information becomes available to help farmers better manage the fertility of their herds.

The solution which DataGene developed with the help of Monash University students, Anthony Nguyen, Charul Manglani and Sam McCarthy is a cow-side data capture SmartPhone App which can capture the cow-side mating data, using voice detected commands right at the time of artificial insemination. The meta data includes, the Cow ID, Bull Common Name, Date of Artificial Insemination, Farm ID and the Technician ID. Given that the process of artificial insemination requires both hands of the technician, it was important to develop a solution which is hands free. A blue-tooth headset is the chosen method for the user to interact with the App. There are three use cases outlined below. It is important to note that in all use cases the farm may or may not have access to internet.

Use Case 1

The technician knows the cows that will be mated before reaching the farm, and the bulls they are to be mated to. In this situation, the farmer has used a separate Farmer App to upload the numbers of the cows, and the bulls to be used, or pre-planned his matings at the start of the season. In this case the AI information can be prepopulated, and the Technician App can alert the technician which bull to use upon him reciting the cow number.

Use Case 2:

The technician knows the number of cows that will be mated before reaching the farm, but not which bull they are to be mated to. In this situation the technician will recite the number of the cow, and the name of the bull which will be recorded by the App.

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Use Case 3:

The farmer has a stock of straws on his own farm. So the technician is unaware about the cows and bulls before he reaches the farm. In this case the farmer and technician will decide prior to, or during the AI process which cow is to be mated to which bull. The technician will then recite the cow number and bull name when completing the mating.

The application

The application has certain key words such as Start, Bull and Cow which act as triggers to recognise that the next word spoken is the start of mating run, bull name and cow number respectively. The remaining data such as Mating Date, farm Number and Technician Name are automatically collected by the application and stored on the local (phone) SQLite database SQLite and later synced with the server database, on availability of internet.

Nordic Cattle Data eXchange – a shared standard for data transfer

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Summary

As a common project of the five Nordic countries, a shared interface has been created for data exchange between farm management software and the national databases. Joining organisations only have to build the exchange between their own software and the interface. Main principles of the system are presented.

Keywords: milk recording, data capture, data processing

Introduction

With ever-increasing herd size and the rise of advanced dairy technology, especially automated on-farm data capture, nearly all milk recording organisations have noticed the need to create easy and reliable ways to transfer data directly from farm management systems to milk recording. This is especially true for automatic milking where more complex data sets are collected, and with the Nordic countries where the share of cows in automatic milking systems is already around 30 to 40% of the national herds.

Building a data transfer channel between a milk recording organisation and a farm management software programme may sound easy. Considering that there are many different kinds of farm management software each with their own data standards, and a lot of milk recording organisations each with their own specific needs, it is not surprising that some software providers have become reluctant to do much, especially with the smaller milk recording organisations.

Nordic cooperation

Having made each their own approaches at this problem, the milk recording organisations from Denmark, Sweden, Norway and Finland decided in 2013 to start working towards a common system where the demands to farm management software providers would be uniform. The development phase was finalised in 2015, and it

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was decided to build a common interface that would be able to feed all national databases from all farm software whose providers are interested to join. Mtech Digital Solutions from Finland was selected by the partners to build the interface.

Present phase of the NCDX

The interface is now ready and working in its primary form where it transfers cow data, recording and milk analysis data, and production related events. The kind of data transferred can be broadened in the future to include whatever the milk recording organisations and farm software providers need and agree to deliver. It is also possible to expand to new farm software providers and milk recording organisations later.

The NCDX working principle includes the idea that the NCDX Provider (Mtech) makes all agreements with both milk recording organisations and farm software providers (Figure 1). In this way, the milk recording organisation only has to take care of its own connection to the interface, and the same applies to the farm software provider. Both parties forgo lengthy discussions and projects with a number of partner organisations. The local milk recording organisation will still have to make user agreements and authorise its own customer farmers, however.

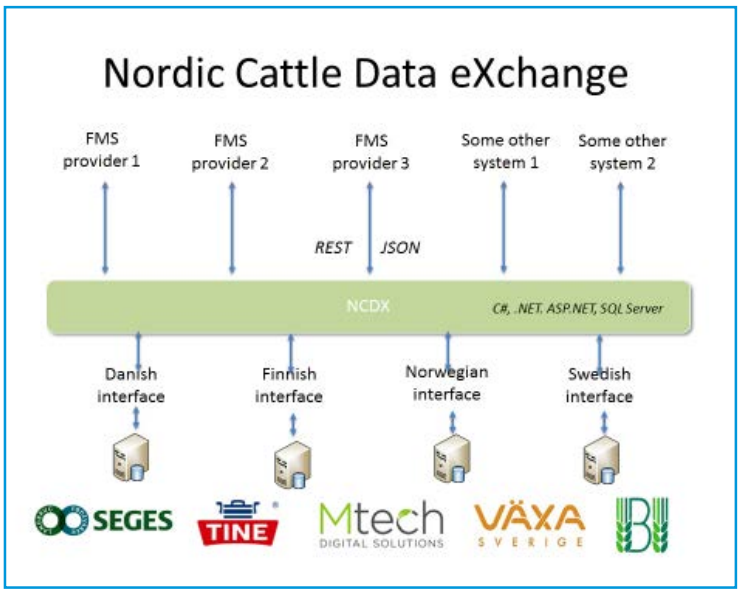

Figure 1. The working principle of the NCDX.

Conclusions

The NCDX has proved to be an easy tool for automatic data transfer both to the milk recording organisation and to the farm management software. It gives to each stakeholder the advantage of having only one connection and one agreement for data transfer. It can be expanded with new features, new farm software, and also new milk recording organisations.

Development and use of Mid Infrared Spectra to measure milk fatty acid parameters and estimated blood NEFA for farm management

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Summary

Partial least square (**PLS**) models were developed from Fourier transform mid-infrared (MIR) spectra, externally validated, and are being used commercially in the US for direct measurement of:

- Froups of milk fatty acids [i.e., de novo (**DN**), mixed origin (**MO**), and preformed fatty (**PF**) acids]
- Fatty acid (**FA**) chain length (expressed as carbon number).
- FA unsaturation (expressed as double bonds per FA).
- Estimated blood non-esterified FA (**NEFA**).

Six laboratories in different regions of the US are routinely using the models for bulk tank bovine milk analysis simultaneously with payment testing for individual farms on almost every milk pick up basis. Two research laboratories are testing both bulk tank and individual cow milk samples, while one is also testing sheep and goat milk. There is a high correlation in bulk tank milk of DN (C4 to C15) FA concentration (g/100 g milk) with increased bulk tank milk fat and milk protein percentage. The DN FA are made in the mammary cells from acetate and butyrate produced by the microbial fermentation of carbohydrates in the rumen. The changes in concentration of DN FA in milk reflect efficiency of rumen fermentation and the microbial biomass load (i.e., essential amino acid production) in the rumen. Seasonal variation in bulk tank milk fat and protein content are highly correlated with seasonal variation in milk DN FA. As milk FA chain length and double bonds per FA increase, milk fat decreases, and DN and MO FA synthesis and output per cow per day decreases. Farms with high bulk tank milk double bonds per FA, where the average days in milk of the herd is >120 d, have a much higher incidence of trans FA induced milk fat depression. These FA metrics in combination with milk fat and protein concentration, plus milk weight, MUN, and milk SCC have been used to make decisions to adjust feeding to increase production of grams of fat and protein per cow per day and net income from milk minus feed cost. The estimated blood NEFA and DN FA (expressed as DN as a percentage of total FA) are used in combination to monitor fresh cow metabolic

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status for early detection of individual cows that will develop clinical ketosis or displaced abomasum. These milk-based transition cow analytical tools provide an opportunity to intervene earlier thereby improving recovery while reducing the negative impact of these adverse metabolic health events on animal welfare and lactation performance.

Introduction

In 2014 (Barbano *et al.*, 2014), the application of mid-infrared (**MIR**) for rapid milk fatty acid (**FA**) analysis was introduced in a commercial laboratory and positive correlations of bulk tank milk fat test with a higher proportion and concentration of de novo FA in bulk tank milk were reported. The analytical aspects of reference milk FA analysis, PLS model development, and validation statistics were reported by Wojciechowski and Barbano (2016) and Woolpert *et al.* (2016). Briefly, partial least squares (**PLS**) chemometric prediction models for FA were developed from MIR spectra in the Cornell University laboratory using a Delta Instruments Lactoscope (Delta Instruments, Drachten, Netherlands). The form of the FA data from the MIR was structured to report fatty acid values for DM, MO, and PF fatty acids in g/100 g milk and calculated values as the relative proportions of de novo (C4 to C15), mixed origin (C16:0, C16:1, C17:0), and preformed (C18:0 and longer) FA in milk were also provided. The mean FA chain length (carbon number) and degree of unsaturation (double bonds/fatty acid) are chemical structure metrics, not concentration metrics. The ratio of SD of reference values for the modeling set divided by standard error of cross validation (RPD) for DN, MO, and PF are 10.4, 6.2, and 7.3, while the RPD FA chain length and degree of unsaturation are 2.1 and 3.3. Manley (2014) indicated that RPD values greater than 3 are useful for screening, values greater than 5 can be used for quality control, and values greater than 8 for any application. With field experience in testing bulk tank milk from commercial farms, we found that providing this FA information in units of grams per 100 grams of milk was more useful when making feeding and management decisions on whole herd or feeding group basis, while the relative percentages of DN, MO, and PF fatty acids are more useful for transition cow metabolic health diagnostics in combination with the results of PLS model for milk estimated blood non-esterified fatty acids (**NEFA**). This paper will focus on the use of the milk FA information for management of dairy cows at the bulk tank level and report the status of our work on individual cow data with respect to how these milk composition and production parameters change with stage of lactation for primiparous and multiparous cows.

Woolpert *et al.* (2016, 2017) reported the results of two studies to determine feeding and farm management factors influencing milk FA composition and their relationship to bulk tank milk fat and protein content and yield per cow per day. The first study (Woolpert *et al.*, 2016) used 44 commercial dairies that were identified as either predominantly Holstein or Jersey in Vermont and northeastern New York. The yields of milk fat, true protein, and de novo FA per cow per day were higher for high de novo (**HDN**) versus low de novo (**LDN**) farms. The HDN farms had lower free-stall stocking density (cows/stall) than LDN farms. Additionally, tie-stall feeding frequency was higher for HDN than LDN farms. No differences between HDN and LDN farms were detected for dietary dry matter, crude protein, neutral detergent fiber, starch, or percentage of forage in the diet. However, dietary ether extract was lower for HDN than LDN farms. Overall, overcrowded free-stalls, reduced feeding frequency, and greater dietary ether extract content were associated with lower de novo FA synthesis and reduced milk fat and true protein yields on commercial dairy farms in this study.

The difference in income per cow depends on the actual milk price at any point in time. The average fat and protein price for the USDA Federal Milk Order No. 1 for March and April 2014 was $2.10 and $4.62 per lb ($4.62 and $10.17 per kg), respectively. Therefore, at 55 lb (25 kg) of milk per cow per day, the average HDN

farm earned a gross of $5.50 and $7.72 per cow for fat and protein, respectively. The average LDN farm at 55 lb (25 kg) milk per cow per day earned a gross of $5.26 and $7.29 per cow for fat and protein, respectively. These differences for fat and protein between HDN and LDN herds at 55 lb (25 kg) of milk per 100 cows per year would result in a gross income difference of $8,544 for fat and $15,695 for protein.

Woolpert *et al*. (2017) conducted a second study with 39 commercial Holstein herds in Vermont and northeastern NY. No differences in milk (about 70.5 lb (32 kg) /cow/d), fat (2.73 lb (1.24 kg)/cow/d), and true protein (2.2 lb (1.0 kg)/cow/d) yields were detected between HDN and LDN farms, but the percentage of milk fat (3.98 vs 3.78%) and true protein (3.19 vs 3.08%) were both higher on HDN farms. The HDN farms had higher de novo FA, a trend for higher mixed origin FA, and no difference in preformed milk FA daily yield per cow per day. This positive relationship between de novo FA and milk fat and true protein percentage agrees with previous results of Barbano *et al*. (2014) on bulk tank milk composition from 400 commercial dairy farms. The average fat and protein price for USDA Federal Milk Order No. 1 for February through April 2015 (US Department of Agriculture, 2015) was $1.90 and $2.61 per lb ($4.19 and $5.74 per kg), respectively. Therefore, at 66.1 lb (30 kg) of milk per cow per day, the average HDN farm earned a gross of $5.00 and $5.49 per cow for fat and protein, respectively. The average LDN farm at 30 kg of milk per cow per day earned a gross of $4.75 and $5.30 per cow for fat and protein, respectively. These differences for fat and true protein between HDN and LDN herds at 66.1lb (30 kg) of milk would result in gross income differences of $9,125 for fat and $6,935 for true protein per 100 milking cows per year. Management (i.e., frequent feed delivery and increased feed bunk space per cow) and dietary (i.e., adequate physically effective fiber and lower ether extract) factors that differed between these HDN and LDN farms have been shown in earlier studies to affect ruminal function.

Based on data from these studies the following graphs (Figures 1 to 4) for Holstein farms were developed to help farms understand the relationships between bulk tank milk FA composition and bulk tank fat and protein tests.

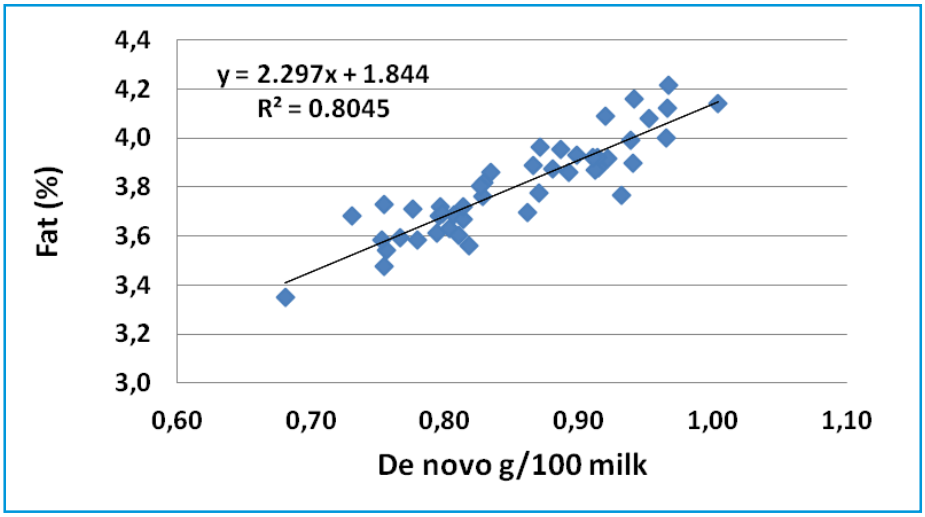

Figure 1. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of de novo FA in milk. In general, a farm needs to have a concentration of de novo FA higher than 0.85 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

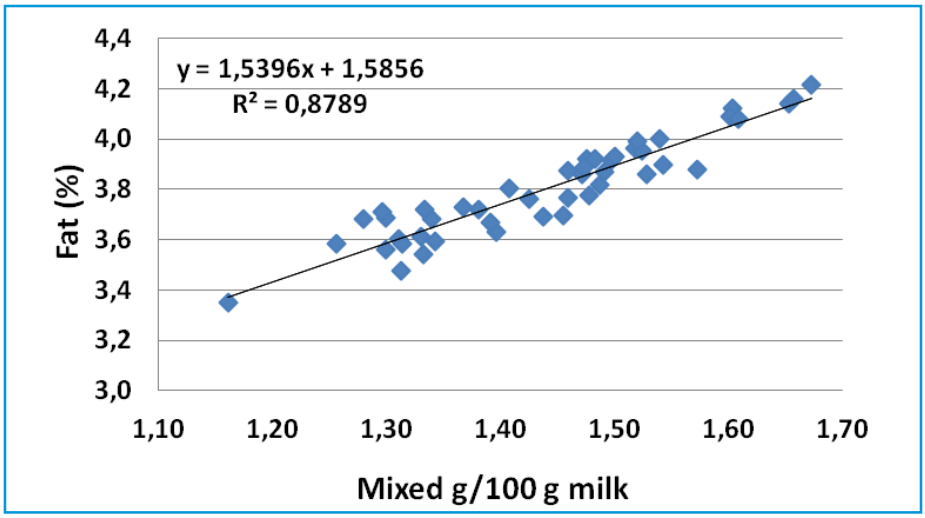

Figure 2. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of mixed origin FA in milk. In general, a farm needs to have a concentration of mixed origin FA higher than 1.40 g/100 g milk to achieve a bulk tank fat test higher than 3.75%.

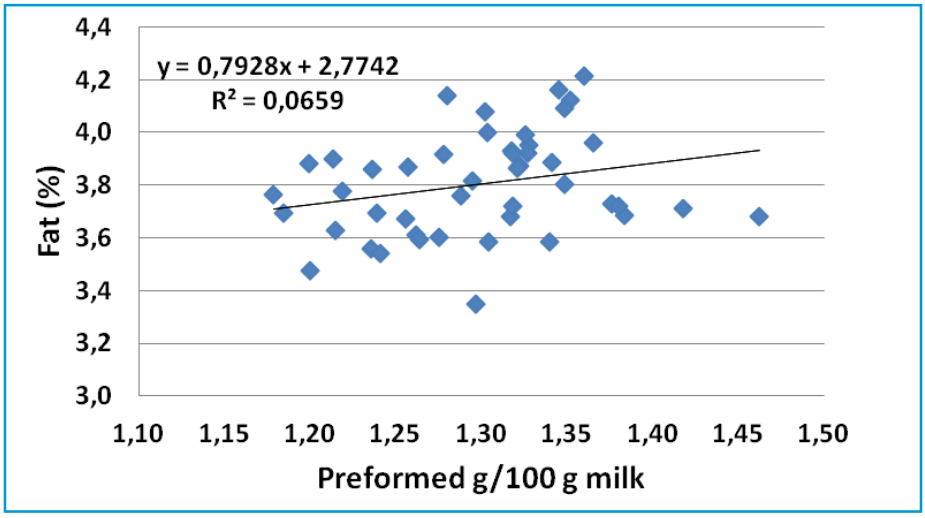

Figure 3. Relationship of bulk tank milk fat test to concentration (g/100 g milk) of preformed FA in milk. In general, the variation in preformed FA concentration in Holstein herds is less than de novo and mixed origin FA and is not well correlated with bulk tank milk fat test.

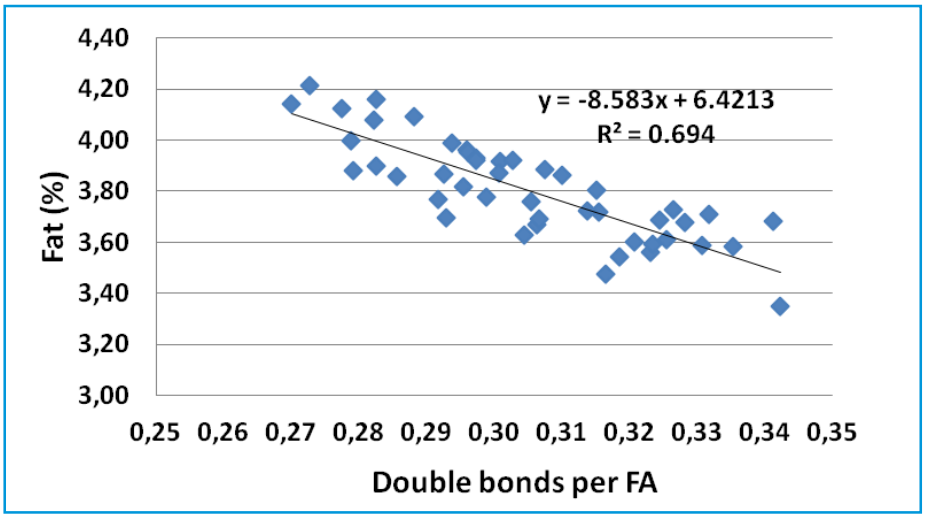

Figure 4. Relationship of bulk tank milk fat FA unsaturation with bulk tank milk fat test. As double bonds per FA increases the bulk tank milk fat test decreases. To achieve a 3.75 % fat test a farm needs to have a double bond per FA of less than 0.31.

Field adoption of routine milk fatty acid analysis

Starting in February of 2016, information on FA composition of bulk tank milk was provided to the individual producers of the St Albans Cooperative (Vermont) along with their payment test data on the same milk samples and in the summer of 2017 Agrimark Cooperative (Springfield, MA) and Cayuga Milk Ingredients (Auburn, NY) have started providing similar data to their producers on the official bulk tank milk samples that are used for milk payment testing. For producers that are not members of those cooperatives, milk samples can be analyzed at commercial laboratory (i.e., Minnesota DHIA) and our research laboratories at Cornell University and Miner Institute. The MIR milk analysis models used for measuring de novo, mixed origin and performed FA and fatty acid chain length (i.e., carbon number) and unsaturation (double bonds per fatty acid) are specific PLS models designed to measure these milk parameters using the MIR equipment produced by Delta Instruments. The values cannot be accurately calculated from other measured fatty acid parameters. The procedures used for development of these herd management MIR PLS models, external validation of the PLS models, and performance statistics for the models have been published (Wojciechowski and Barbano, 2016; Woolpert *et al.*, 2016). Other MIR milk analysis equipment manufacturers may develop similar PLS models to measure these parameters, validate the models and make them available to their customers in the future.

Seasonality of bulk tank milk

Over the past 3 to 4 years we have followed the pattern of seasonality of milk fat and protein in relation to milk FA composition on a group of 40 farms with the St. Albans Cooperative. The data January 2014 through July 2017 are from the routine testing results using MIR-analysis in the St. Albans Cooperative on fresh bulk tank milk samples used for payment testing (Figures 5 to 8).

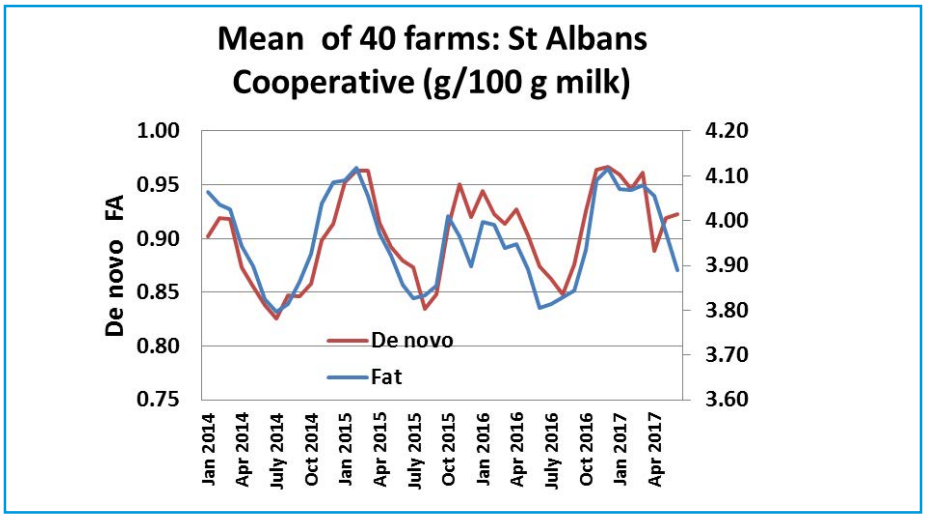

Figure 5. Seasonality of milk fat and de novo FA in milk.

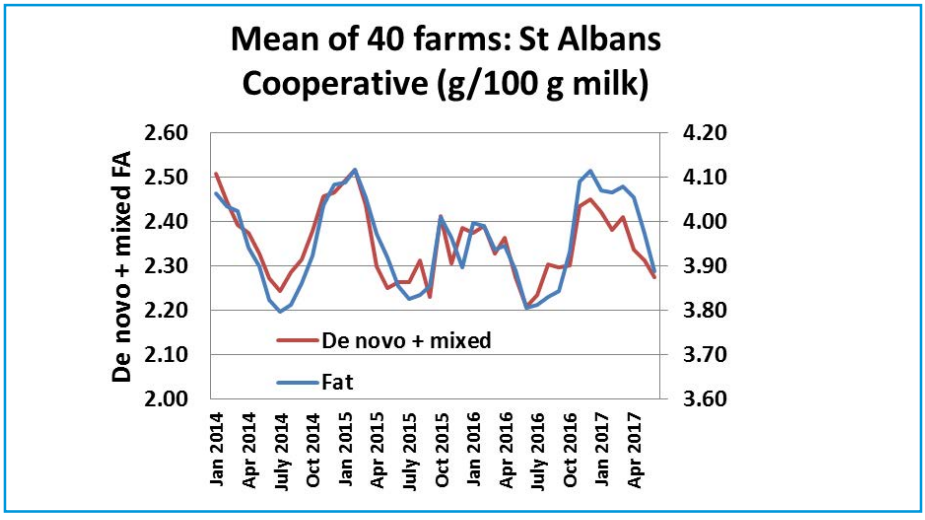

Figure 6. Seasonality of milk fat and de novo + mixed origin FA in milk

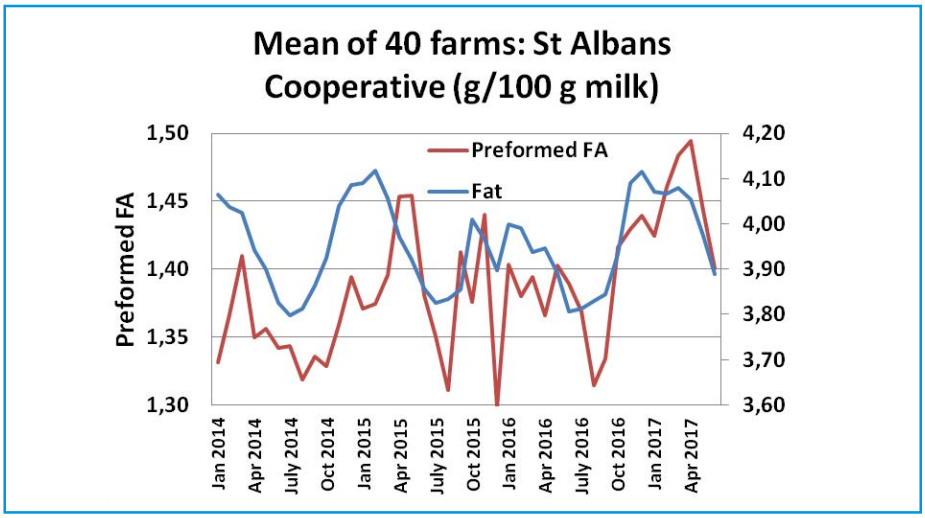

Figure 7. Seasonality of milk fat and preformed FA in milk.

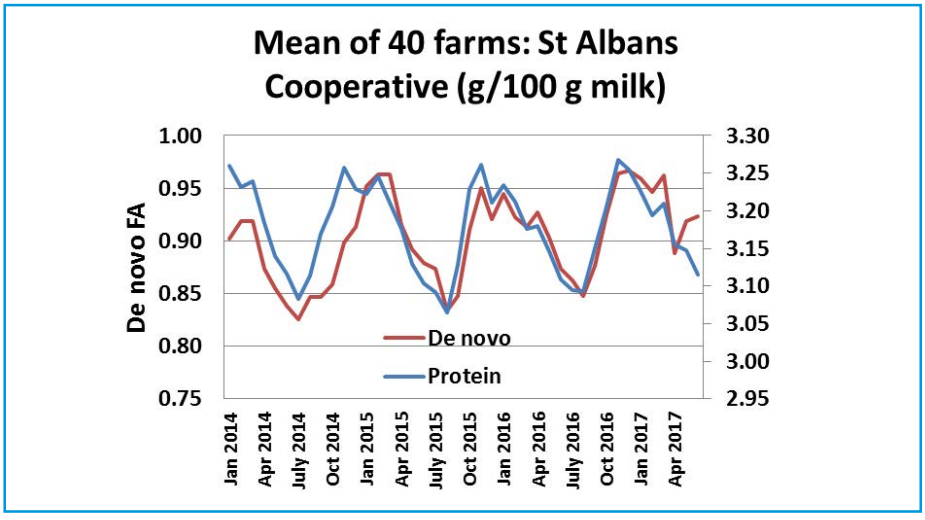

Figure 8. Seasonality of milk protein and de novo FA in milk.

The seasonality of de novo and mixed origin milk FA concentration follows the seasonal pattern of milk fat (Figure 5) and protein (Figure 6) variation while variation in preformed fatty FA in milk does not (Figure 7). Much of the variation in the mixed origin FA concentration is probably due to variation in the portion of the mixed origin FA produced by de novo synthesis from acetate and butyrate from forage digestion in the rumen. These seasonal changes may be related to time and temperature induced changes in the fermentation of corn silage, starch degradability, forage quality and heat stress on the cows.

Herd to herd variation in milk composition in North America

Over the past year, bulk tank milk sampling has been done on a wide range of farms from various regions of the US to confirm if the same milk fat, protein and milk FA composition relationships are observed in bulk tank milks from different regions of the US. These samples were collected daily for 5 to 7 days on each farm, preserved and refrigerated. At the end of the collection period, the milk samples were shipped on ice to Cornell University for MIR analysis and spot-checking FA composition with GLC analysis, particularly to obtain more detail about milk trans FA levels at each farm. There were some grazing herds, organic herds, and very large conventional herds in the population with a wide range of milk production per cow and milk composition.

The findings from these 167 farms were reported at the 2017 Cornell Nutrition Conference (Barbano et. al., 2017). The behavior of bulk tank milk fatty acid composition as it related to bulk tank fat and protein test is shown in Figures 9 and 10 for herds managed and fed over a wider range of types of feeds and management systems than we encountered in our studies of farms in the Northeast US. The relationship between de novo and de novo plus mixed origin observed in bulk tanks milk produced by farms from across the US were similar those found for Holstein herds in the Northeast. A level of about 0.85 g de novo FA per 100 g of milk will achieve about a 3.75% fat test (as seen by comparison of Figure 1 versus Figure 9).This indicated that the milk fatty acid metrics (de novo, mixed, preformed, fatty acid chain length, and

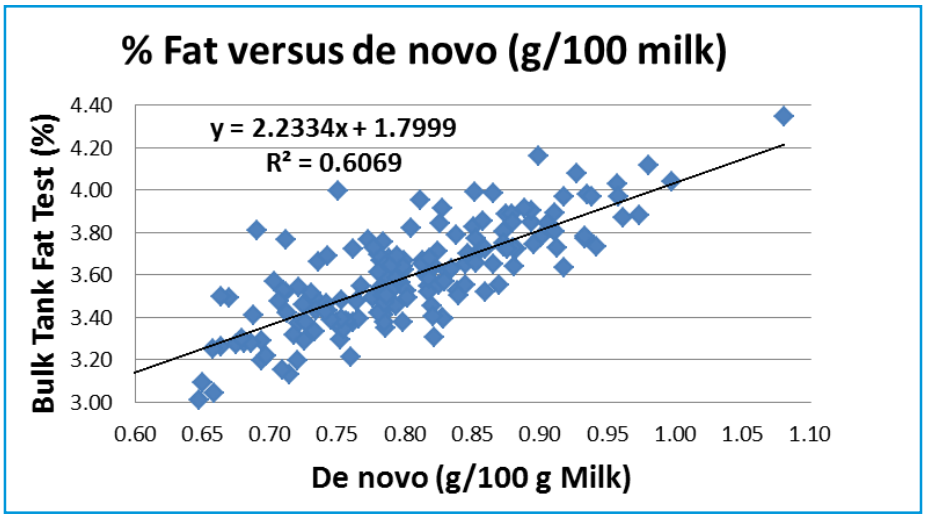

Figure 9. Correlation between bulk tank fat and de novo FA concentration (167 farms).

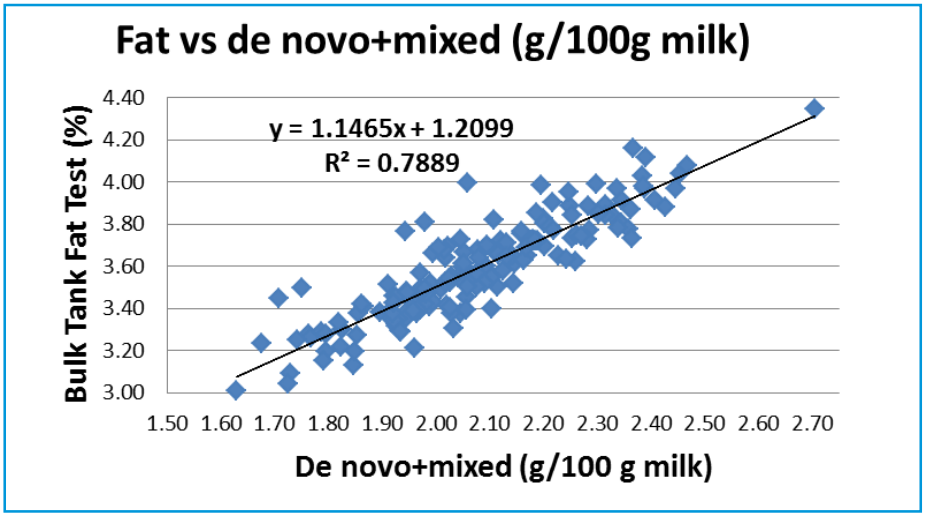

Figure 10. Correlation between bulk tank fat and de novo + mixed origin FA (167 farms).

double bonds per fatty acid) are robust indices for use for herd management and apply over the wide range of condition found across North America. A discussion of interpretation of bulk tank milk fatty acid composition was reported previously (Barbano *et al.*, 2017).

Milk fat and protein output per cow per day are also strongly correlated with total weight of milk produced per day. Those relationships for the 167 farms from across North America are shown below in Figures 11 and 12.

Overall, dairy cows have the potential to produce more grams of fat and protein per day if they produce more milk. The synthesis of lactose and increasing the grams per day output of lactose is needed to produce more pounds of milk per day. Lactose production is highly dependent on glucose metabolism in the cow. To produce more milk per cow, a cow will need to produce more lactose per day, as shown in Figure 13 below. The correlation is very strong. To achieve 90 to 100 lb (40.9 to 45.4 kg) of milk, the cows need to be producing between 1900 and 2100 grams of lactose per day. A major factor that would compete for use of glucose that could be used in support of milk synthesis is an immune response by the animal because of some adverse health event (e.g., leaky gut, mastitis, lower GI viral infection, etc.). Thus, in our field work we are encountering situations where the fatty acid profile and a high bulk tank fat and protein test indicate that rumen function is good, but the weight of milk per day is low (i.e., low lactose output per cow per day) and as a result total weight output per cow per day of fat and protein are not as high as they should be. This is a sign that there is some non-feed/non-rumen fermentation problem that is limiting milk production.

As this new milk testing technology becomes more widely available in the dairy industry it is likely to be used as a herd management tool to test milk from different feeding groups of cows that may have a very different number of days of milk (**DIM**) from one group to another or have a different parity status from one group to another. Both DIM and parity influence milk and milk FA composition. There are large changes in milk FA composition with stage of lactation, particularly during the transition period. When looking at milk composition and FA composition, differences in parity or stage of lactation needs to be taken into account when interpreting data. As a result, we have been collecting data at the Miner Institute to produce lactation curves on all of these milk parameters.

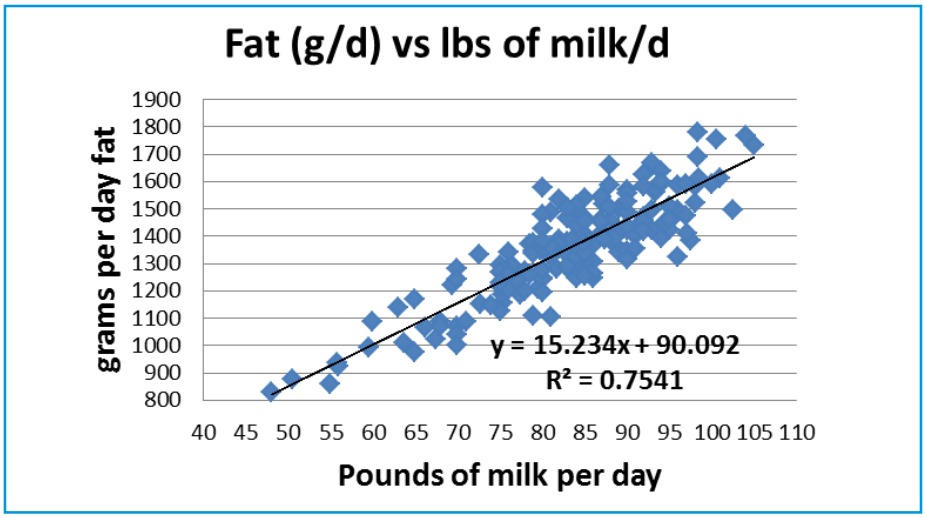

Figure 11. Grams of fat per cow per day and milk production (167 farms).

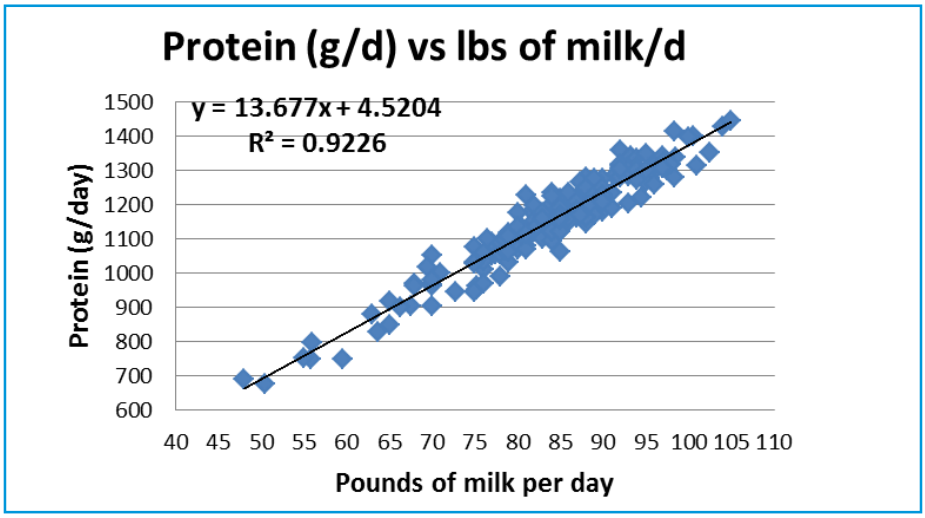

Figure 12. Grams of protein per cow per day and milk production (167 farms).

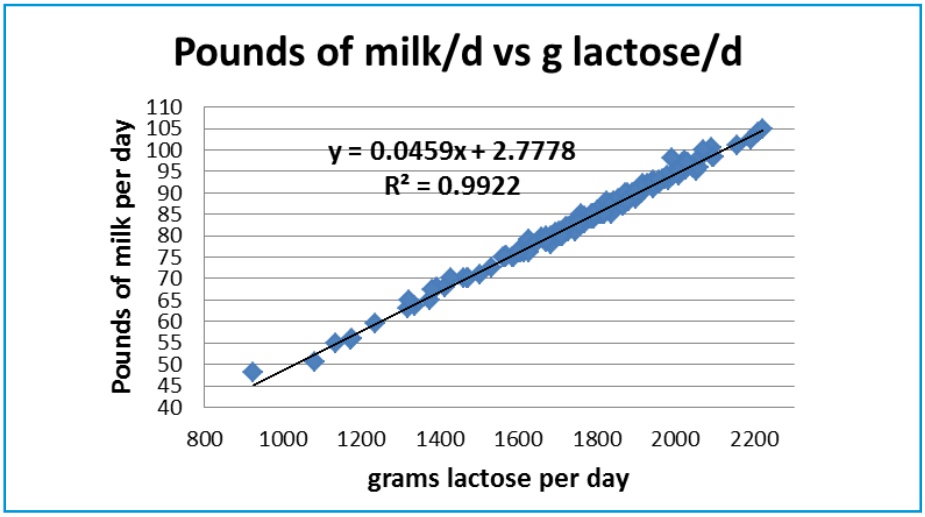

Figure 13. Grams of lactose produced per cow per day and milk production (167 farms).

Stage of lactation affects milk fatty acid composition

For the past 2 to 3 years, we have been conducting an intensive study at Miner Institute with individual cow milk analysis to better understand changes in milk FA across the lactation cycle. The goal is to build stage of lactation curves for all the new milk analysis parameters on both a concentration basis and a daily output per cow basis. Milk is collected from the entire herd (~400 cows) weekly from 3 milking shifts within the same day and analyzed on-site with a high-speed MIR milk analysis system.

As expected, the concentrations of FA in milk changes with DIM. The changes are particularly large in early lactation (i.e., the transition period) when the cow is in negative energy balance. During this period it is normal for the preformed FA to be high and the mixed and de novo FA to be low. However as dry matter intake increases after calving, the milk FA composition should change quickly if the cow's blood NEFA concentration decreases normally. If milk sampling and testing for FA is being done on different groups of cows within a herd, then these stage of lactation changes need to be considered to properly interpret that data. The graphs below (Figures 14 to 17) are stage of lactation data collected from Holstein cows over a period of 2 to 3 years that were milked 3 times per day, had a rolling herd average of ~30,000 lb (12,636 kg) and were fed total mixed rations based on stage of lactation (i.e., fresh, 1st lactation, high and low groups). In general, the diets were typically 50 to 60% forage with at least 2/3 of forage coming from corn silage. Grain mixes typically contained corn grain, soybean meal, commercial soy/canola products, byproducts, rumen inert fat, plus mineral and vitamin supplements. Diets were balanced for lysine and methionine. The change in g/100 g milk of de novo, mixed, and preformed FA with week of lactation is shown in Figure 14 and the relative percentages are shown in Figure 15.

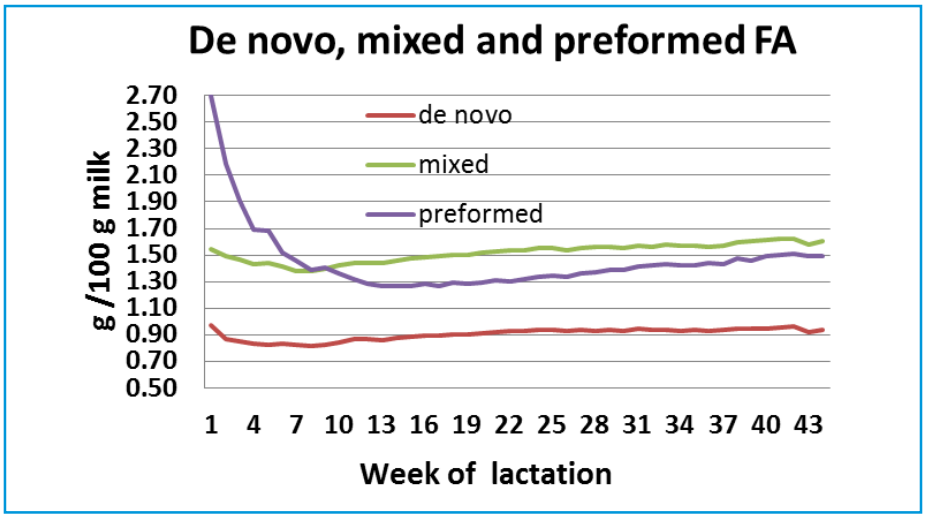

Figure 14. De novo, mixed, and preformed FA (g/100 g milk) over lactation for all cows.

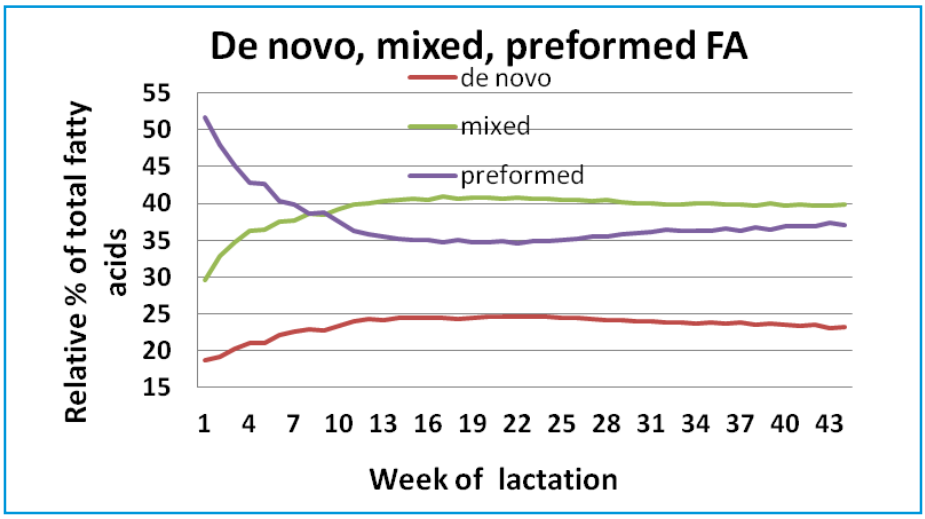

Figure 15. De novo, mixed, and preformed FA (relative %) over lactation for all cows.

There are large changes in milk FA composition during the first 10 weeks of lactation on both a g/100 g milk and relative percentage basis with the preformed FA being high at the beginning of lactation and decreasing to relatively stable levels by about 10 weeks of lactation. When testing milk on larger farms from groups of cows that differ in stage of lactation, these changes in milk FA composition with stage of lactation need to be considered when interpreting data along with information on milk production per cow per day, cow health, milk SCC, feed composition, and dry matter intake.

Interpretation of results from a management point of view becomes even more interesting when the data are converted to grams per day per cow output. Figure 14 represents the average of all cows in the herd, but the stage of lactation graph for

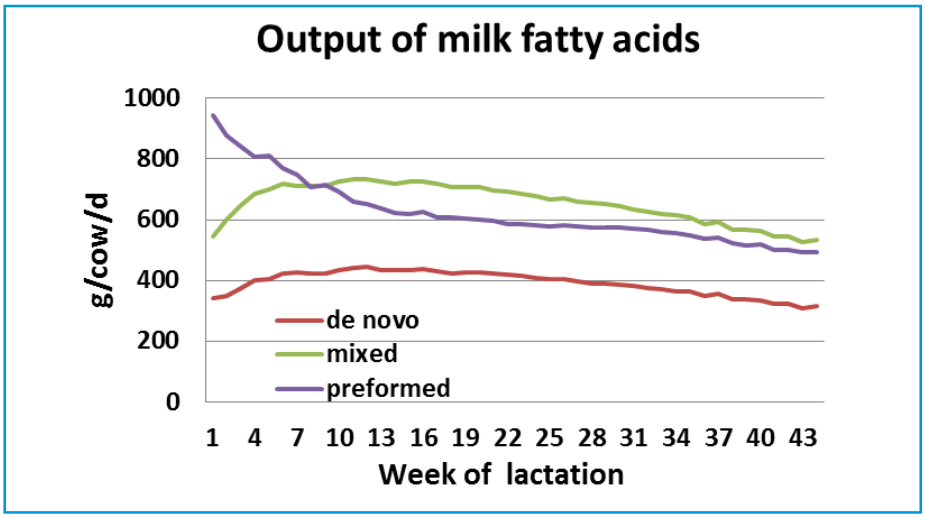

Figure 16. Stage of lactation production graph for all cows (g/cow/day).

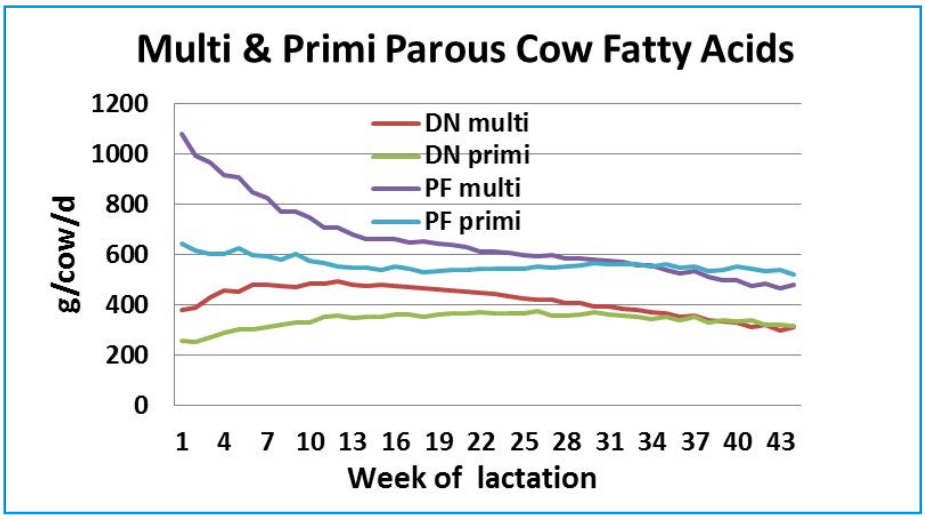

Figure 17. Stage of lactation: de novo (DN) and preformed (PF) fatty acids for primiparous and multiparous cows.

grams per cow per day is very different for first parity versus older cows. When evaluating performance of older versus younger cows, this factor needs to be considered. The difference between multi and primiparous cows for output of de novo and preformed FA per cow per day is shown in Figure 17. The output of all groups of FA in grams per cow per day is much more stable over time for primiparous cows versus older cows. The older cows have much higher preformed FA output per cow per day in early lactation due to body fat mobilization than primiparous cows.

Milk fatty acid data and milk estimated blood NEFA: tools for management of metabolic health during the transition period

To apply milk analysis to transition cow health management the frequency of individual cow milk testing needs to be much higher than what is currently being done with monthly DHIA milk testing. Our research results indicate that there may be an excellent farm management and individual cow health management opportunity with a higher frequency of milk analysis. As shown above, milk fatty acid composition and yield per cow per day changes rapidly especially during the first 10 weeks of lactation when cows are transitioning from negative to positive energy balance. Separately if blood samples are collected and blood non-esterified fatty acid concentration is measured, cows with a high probability of metabolic related disorders (e.g., ketosis, displaced abomasum, etc.) can be identified. Barbano *et al.* (2015) reported a method for estimation of blood NEFA (µEq/L) directly from the MIR milk spectra, not by calculation from fatty acid data. Use of milk estimated blood NEFA is faster, less expensive, and timelier than blood analysis. Are there differences in milk fatty acid profile and milk estimated blood NEFA that can be used to better manage transition cow health? To address this question, we did high frequency (1 milking per day) testing of milk from individual fresh cows at Miner Institute to compare data from healthy cows and cows that had clinical diagnoses of DA and/or ketosis. In general, milk was collected before fresh cow exam/check. The milk information was pared with health data collected and stored in a herd management software program.

Typically, blood samples are collected from early lactation cows that may have a high risk of a metabolic disorder. However, when blood NEFA is estimated from the MIR spectra of milk, it becomes relatively easy to produce a milk estimated blood NEFA lactation curve. The change in milk estimated blood NEFA for primiparous and multiparous cows throughout lactation is shown in Figure 18. In early lactation when cows are in negative energy balance, blood NEFA is high as the cows mobilize body fat to help meet energy requirements of milk production in very early lactation when their dry matter intake is increasing. In general, multiparous cows are mobilizing more body fat in the first few weeks of lactation than primiparous cows.

Displaced Abomasum (DA)

In general the milk estimated blood NEFA was much higher (about 1400 µEq/L) for cows that were clinically diagnosed with a DA than healthy cows (about 800 µEq/L) (Figure 19) and milk de novo fatty acids (Figure 20) were lower for cows with a DA (ca. 13 vs 19% g/100 g fatty acids). Post DA surgery, milk estimated blood NEFA decreased and milk de novo fatty acids increased but did not equal the values for

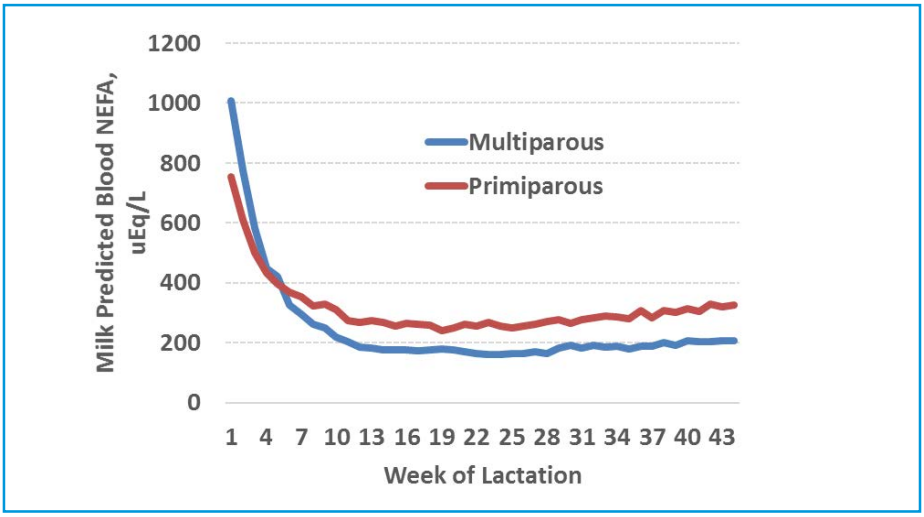

Figure 18. Stage of lactation: milk predicted blood NEFA (µEq/L) for primiparous and multiparous cows.

healthy cows at the same days in milk, indicating that there will probably be some longer term negative effect of the DA event on milk production for that cow as lactation continues.

Ketosis

In general the milk estimated blood NEFA was much higher (about 1400 µEq/L) for cows that were clinically diagnosed with ketosis than healthy cows (about 800 µEq/L) (Figure 21) and milk de novo fatty acids (Figure 22) were lower for cows with ketosis (ca. 13 versus 19% g/100 g fatty acids). Post-ketosis treatment with propylene glycol, milk estimated blood NEFA decreased and milk de novo fatty acids increased but did

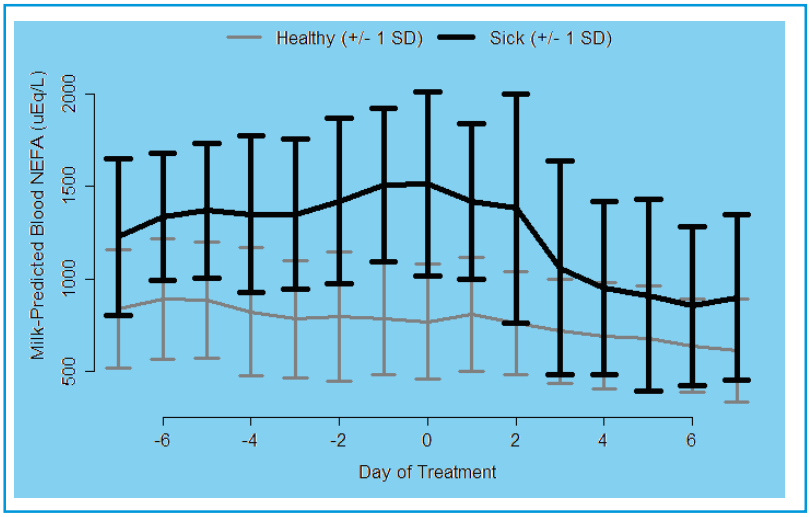

Figure 19. Milk predicted blood NEFA for multiparous cows (n = 47) with a DA versus healthy cows (n = 191) with day zero being the day of treatment. Day 0 = 9.4 days in milk. Healthy cows were matched with DA cows based on days in milk and calving date.

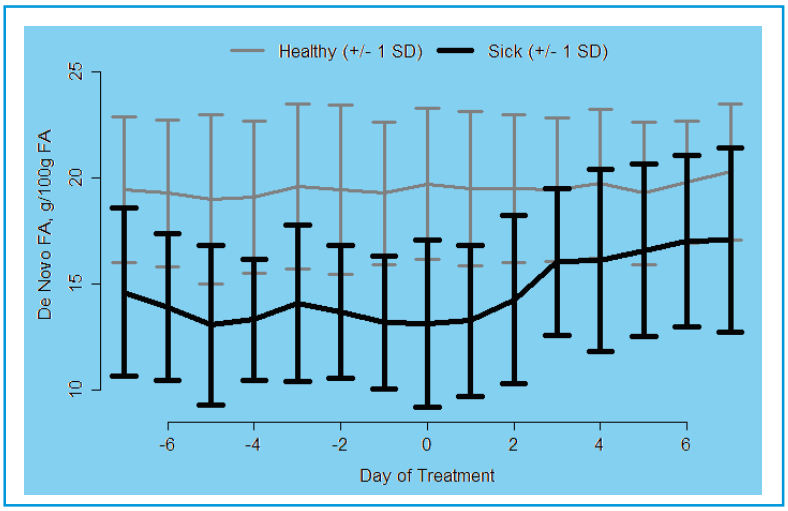

Figure 20. Milk de novo fatty acids relative % (g/100 g fatty acids) for multiparous cows (n = 47) with a DA versus healthy cows (n = 191) with day zero being the day of treatment. Day 0 = 9.4 days in milk. Healthy cows were matched with DA cows based on days in milk and calving date.

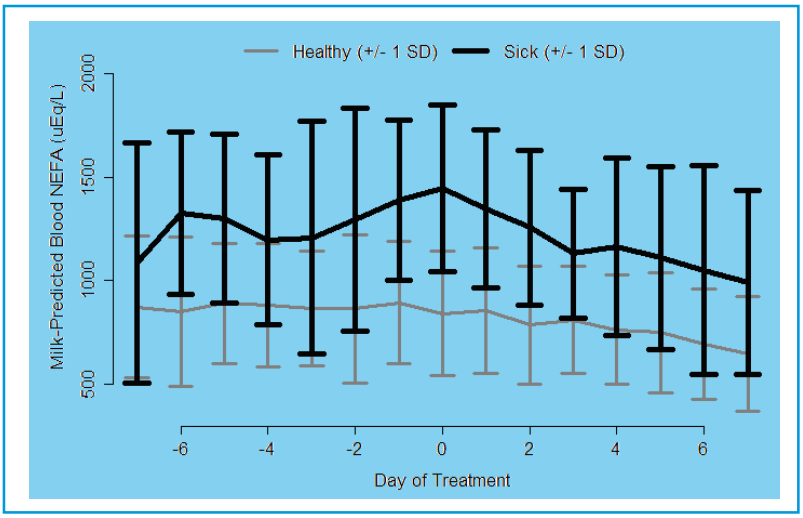

Figure 21. Milk estimated blood NEFA for cows (n = 87) with ketosis versus healthy cows (n = 239) with day zero being the day of treatment. Day 0 = 7.3 days in milk. Healthy cows were matched with ketotic cows based on days in milk and calving date.

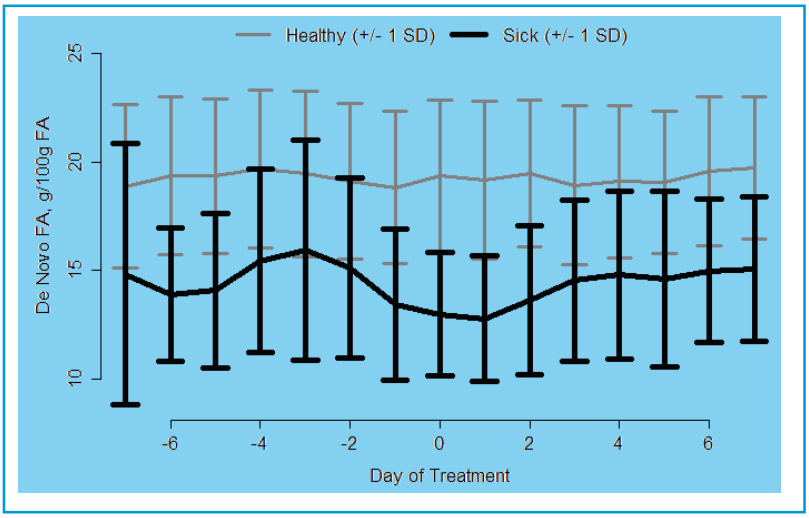

Figure 22. Milk de novo fatty acids relative % (g/100 g fatty acids) for multiparous for cows (n = 87) with ketosis versus healthy cows (n = 239) with day zero being the day of treatment. Day 0 = 7.3 days in milk. Healthy cows were matched with ketotic cows based on days in milk and calving date.

not equal the values for healthy cows at the same days in milk, indicating that there will probably be some longer term negative effect of the ketosis event on milk production for that cow as lactation continues.

Conclusions

Data from routine high frequency (i.e., daily) bulk tank milk component, SCC, and milk FA testing combined with milk weight per cow for whole herd diagnostic analysis of overall nutritional and management status of dairy herds. The testing was done using MIR as part of the routine milk payment testing. The advantage of this approach is that no additional sampling collection cost is required, the instrument that does the milk FA analysis can be the same instrument that produces the milk fat and protein test result, and it does not take any longer to test each milk sample. There would be additional cost to purchase reference milk samples for calibration of the FA parameters for the MIR milk analyzer. The positive correlation between increased de novo fatty acid synthesis and bulk tank milk fat and protein concentration can be used as an indicator of the quality and balance and the rumen fermentation of carbohydrates and if changes in feeding and management are impacting de novo synthesis of milk fat. Seasonal variation in whole herd milk fat and protein concentration was highly correlated with seasonal variation in de novo FA synthesis. Milk FA composition changes with both DIM and differs between primiparous and multiparous cows. Milk fatty acid testing and this diagnostic approach could be applied to testing milk from large feeding groups of cows within the same farm, if representative feeding group milk samples can be collected and tested and the milk produced per cow is known. For feeding group or individual cow milk testing care must be taken to consider the milk weight per cow per day, diet composition, dry matter intake, DIM and parity into the interpretation of the milk composition data.

Data from high frequency MIR milk testing of individual cow milks, particularly during the transition period can be used to identify quickly cows at high risk for displaced abomasum and ketosis before a clinical diagnosis is made oftentimes. The concentration of milk estimated-blood NEFA (µEq/L) was higher and milk de novo fatty acids as percent of total fatty acids (g/100 g fatty acids) was lower than healthy for cows for cows diagnosed with clinical ketosis or displaced abomasum. At the present time based on the current milk analysis tools, we were not able to differentiate whether a cow was going to have ketosis or a displaced abomasum in advance, but further research being done to develop milk analysis tools to differentiate these health events in advance of clinical diagnosis. This may allow development of earlier intervention strategies to reduce the severity of these metabolic disorders and their negative impact on milk production. Mid-infrared analysis of milk from transition cows may be an alternative to blood sampling and testing for management of transition cow health.

Acknowledgments

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The new CombiFoss™ 7 DC – An update on differential Somatic Cell Count and other advancements in milk testing

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Summary

The new CombiFoss™ 7 DC is the latest generation milk analyser from FOSS. The instrument allows to test raw milk for up to 19 parameters, including the new Differential Somatic Cell Count (DSCC) parameter, simultaneously at a speed of up to 600 samples per hour. DSCC represents the proportion of specific immune cells (neutrophils (PMN) and lymphocytes vs. macrophages) and thus provides more information about the actual udder health status of dairy cows. This, in turn, opens up the possibility to develop new tools for improved management of mastitis, the most costly disease in dairy farming. Numerous activities validating the practical application of DSCC as a new supporting tool for mastitis management through DHI (dairy herd improvement) testing are ongoing. The main objective of this paper is to provide an update on the status and first outcomes of these activities.

In a first study, the development of the two parameters SCC and DSCC before, during, and after artificially induced mastitis under controlled conditions was investigated. Briefly, both SCC and DSCC increased evidently after the induction of mastitis. Interestingly, DSCC increased significantly even when the observed SCC increase was only moderate. The results of the study indicated that the combination of SCC and DSCC opens up the possibility to determine the stage of mastitis (i.e., early vs. late stage).

Another study aimed for the investigation of the development of SCC and DSCC in fresh lactating cows over a period of 10 days. While SCC revealed to vary evidently, the DSCC parameter was consistently high in infected cows. Hence, the combination of SCC and DSCC could be used for enhanced mastitis monitoring (e.g., low SCC but high DSCC results as an indicator for mastitis).

Ketosis, a metabolic disorder of high-yielding dairy cows, is a prevalent challenge on dairy farms nowadays. The possibility of offering ketosis screening in the frame of DHI testing is utilised successfully in many countries around the world. This service has been described as simple, practical, rapid, inexpensive, and valuable.

Up to 19 different parameters can be determined using the CombiFoss 7 DC. This, in turn, allows milk-testing laboratories to offer a variety of services to their clients. The new DSCC parameter provides more detailed information on the actual inflammatory status of a cow's udder. In this context, various practical applications for enhanced

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mastitis management based on DSCC are currently under validation. Based on the results of a first study, the combination of SCC and DSCC could be used to differentiate between the early and late stage of mastitis. However, further work is needed to mature this application. Other newer value-adding milk testing services such as ketosis screening and fatty acid analysis are successfully used in many countries around the world.

Keywords: DSCC, mastitis, ketosis.

Introduction

FOSS has launched the 7th generation of the CombiFoss milk analyser, which allows testing for up to 19 parameters including the new Differential Somatic Cell Count (DSCC), in 2016. DSCC indicates the combined proportion of polymorphonuclear neutrophils (PMN) and lymphocytes in percent and is described in detail (e.g., method, technology) elsewhere (Damm *et al.*, 2017; Schwarz, 2017a). The DSCC parameter provides more detailed information on the actual udder health status of dairy cows and thus opens up the possibility for enhancements in mastitis management. Mastitis, the inflammation of a cow's udder, is still causing tremendous losses of •32 billion to the dairy industry worldwide and thus the most costly disease in milk production (Seegers *et al.*, 2003).

Ketosis, a metabolic disorder in high yielding dairy cows, where energy demands exceed energy intake is another issue causing significant economic losses on dairy farms nowadays. The incidence of ketosis has been estimated to be 25-60% in dairy herds with costs of 260 Euro per case (Mc Art *et al.*, 2013, 2015; Mahrt *et al.*, 2015). The possibility of using DHI samples and FTIR technology for herd level screening with good values for sensitivity and specificity has been demonstrated (de Roos *et al.*, 2007; Denis-Robichaud *et al.*, 2014).

The main objective of this work is to provide an update on the status as well as first outcomes of the different activities regarding development of practical applications of DSCC. Beyond that, a short update on the latest developments in terms of ketosis screening and fatty acid analysis will be provided.

The concept and technology behind differential Somatic Cell Count

The concept and technology behind the DSCC parameter have been described previously (Damm *et al.*, 2017; Schwarz, 2017a). Briefly, the new Fossomatic™ 7 DC allows to measure 2 parameters, SCC and DSCC, simultaneously at a speed of up to 600 samples per hour. The key elements of the new milk analyser are a new chemistry, a new incubation unit, and a new measuring unit and were described in detail elsewhere (Schwarz, 2017a). DSCC represents the combined proportion of PMN and lymphocytes in percent (Damm *et al.*, 2017; Schwarz, 2017a). While DSCC is low in healthy mammary glands, DSCC increases evidently in mastitic milk. Overall, DSCC provides more detailed information on the actual inflammatory response of the mammary gland (Damm *et al.*, 2017; Schwarz, 2017a; Schwarz, 2018).

Various activities in terms of the development of the practical application of DSCC were initiated. While 2 studies were completed and first results are available most of the other activities are still in progress.

Update on differential SCC

Development of DSCC and SCC during controlled mastitis

A study where mastitis was induced under controlled conditions was performed (Wall *et al.*, 2018). Briefly, 8 healthy dairy cows were recruited and treated with cell wall components, either lipopolysaccharide (LPS) or lipoteichoic acid (LTA), to induce a defined inflammatory response. With LPS from *Escherichia (E.) coli,* a bacterium usually associated with acute, clinical mastitis and LTA from *Staphylococcus (S.) aureus,* a bacterium often associated with chronic mastitis, bacterial cell wall components representing two very common mastitis-causing pathogens (Sampimon *et al.*, 2009; Schwarz *et al.*, 2010) were chosen. Milk samples were collected at the following time points:

1. Baseline (i.e., days -3, -2, -1).
2. Right before treatment (i.e. day 0).
3. Five hours after treatment (i.e., day 0.2).
4. Early cure phase (i.e., days 1 and 2).
5. Late cure phase (i.e., days 3, 4, 5, 6, 7, and 14).

The SCC increased significantly at time point 3 compared to time points 1 and 2 in cows treated with LTA from *S. aureus* (Figure 1). DSCC increased significantly, too. Both SCC and DSCC returned to pre-infection levels at time point 5. The development of both parameters was very similar in case of LPS (*E. coli*) treatments. The control quarters showed a minor but not significant SCC and DSCC increase each after treatments. Interestingly, the DSCC parameter even increased evidently in cases where SCC only increased moderately (to levels of <200,000 cells/ml). The results of the study are described in detail elsewhere (Wall *et al.*, 2018).

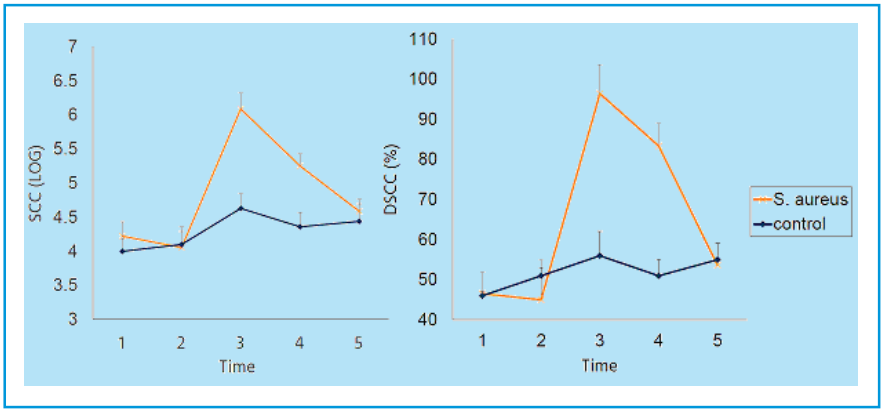

Figure 1. Development of SCC (logarithmic values) and DSCC in S. aureus treated (orange) and control (blue) quarter during the experiment. Time/time point:
1. Baseline (i.e., days -3, -2, -1);
2. right before treatment (i.e. day 0);
3. 5 h after treatment (i.e., day 0.2);
4. early cure phase (i.e., days 1 and 2);
5. late cure phase (i.e., days 3, 4, 5, 6, 7, and 14).

In summary, the study clearly showed that the SCC parameter increased significantly as a result of the induction of mastitis, as expected. DSCC increased significantly, too, thus clearly indicating the shift of cell populations from mainly macrophages to predominantly PMN. The results of the study support that the combination of SCC and DSCC opens up the possibility of determining the stage of mastitis. Specifically, high SCC (>200,000 cells/mL) and high DSCC values (>86%) could be used as an indicator for the early stage of mastitis. High SCC (>200,000 cells/mL) in combination with low DSCC values (<86%), however, could be an indication for the late stage of mastitis.

Development of DSCC and SCC over course of time

A study with the objective of investigating the development of DSCC and SCC in 20 fresh lactating dairy cows over course of time was performed. Cows were milked twice per day and milk samples (i.e., quarter foremilk and cow-composite) were collected at each milking for a period of 10 days (i.e., 18 sampling points). DSCC, SCC, and bacteriological analyses were carried out.

Healthy cows (i.e., no detection of mastitis pathogens) showed consistently low SCC (<50,000 cells/ml). DSCC values in samples with such low SCC are unreliable due to too low numbers of cells available for determination of DSCC (Damm *et al*., 2017). Cows infected with *S. aureus* indicated huge SCC variations, as expected (Harmon *et al*., 1994), whereas DSCC values were consistently high (exemplary: Figure 2).

In summary, the study showed that SCC varied quite evidently while DSCC results were rather at consistently high levels in infected cows. Thus, the combination of SCC and DSCC could be used for enhanced mastitis monitoring given that even cows showing unsuspiciously low SCC results would appear suspicious based on clearly elevated DSCC results.

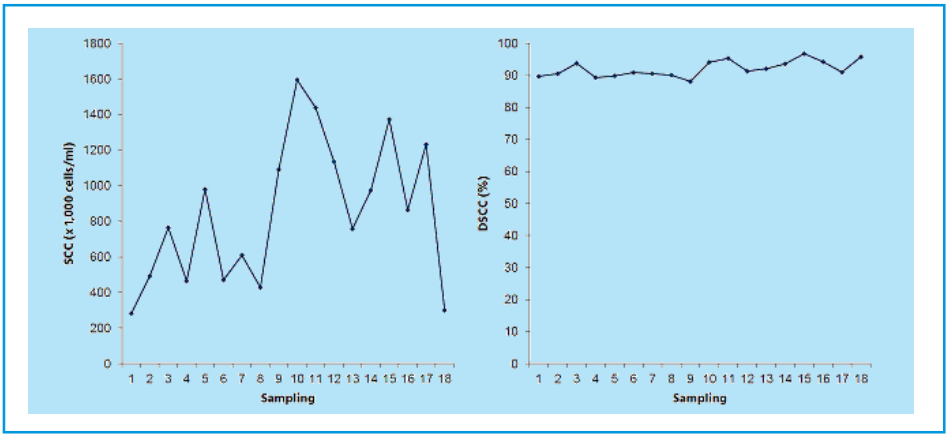

Figure 2. Example for the development of SCC and DSCC in cow-composite samples of one S. aureus-infected cow over a period of 10 days (2 milkings per day).

On-going DSCC projects

Numerous activities aiming for the development of DSCC applications in the frame of DHI testing are currently in progress. Specifically, two large-scale projects are running in Denmark and Germany. In the Danish project the data generation phase was just completed recently. 500+ dairy cows were tested (DSCC, SCC, bacteriology) once per month for a period of one year. In a next step, the data analysis will be performed. In the German project the Fossomatic 7 DC has been validated and implemented in routine operation. The next working package of the project is focused on the development of practical applications of DSCC.

Ketosis screening

Numerous applications for FOSS's ketosis screening calibrations were developed and are widely utilised by dairy farmers around the world, e.g. Ketodetect, CLASEL, France; Ketolab, Valacta, Canada; Ketomonitor, AgSource, US; Ketoscreen, CanWest DHI, Canada; ketosis screening by CRV and Qlip, the Netherlands, today. The service has been described as simple, practical, rapid, and inexpensive as well as highly valuable to milk recording clients as it elevates awareness of an otherwise undetected problem (Schwarz *et al.*, 2015). The service has actually helped to reduce the incidence of ketosis by 10% in Canada and France, as presented previously (Schwarz *et al.*, 2015). It was further seen that the keys to success in establishing ketosis screening as a service were the use of a quality assurance programme as well as proper and clear communication of test results to dairy farmers (Schwarz, 2017b).

Recently published articles describe milk BHB and effects of ketosis on the performance of dairy cows (Santschi *et al.*, 2016), risk factors associated with ketosis (Tatone *et al.*, 2017), and logistic and multiple linear regression models for prediction of ketosis (Chandler *et al.*, 2018) in detail.

Fatty acid analysis

Fatty acids can be categorised according to chain length and/or degree of saturation as well as the major fatty acids can be determined using FTIR technology. Fatty acid information are used for different purposes such as, e.g., optimisation of feeding of dairy cows (e.g., Visiolait application used in Germany and France) or production of value-added dairy products (i.e. products containing enhanced concentrations of unsaturated fatty acids – application in UK).

Conclusions

The CombiFoss 7 DC allows laboratories to measure up to 19 parameters in milk samples at low cost and at high accuracy, speed, reliability, repeatability, and robustness. This, in turn, allows milk-testing laboratories to offer a variety of services to their clients. Mastitis is still the most costly disease on dairy farms nowadays. The new DSCC parameter provides more detailed information on the actual inflammatory status of a cow's udder. In this context, various practical applications are currently under validation in several countries. Based on first results, the combination of SCC and DSCC could be used to differentiate between the early (high SCC, high DSCC) and late (high SCC, low DSCC) stage of mastitis. However, further work is needed to mature this application. Other newer value-adding milk testing services such as ketosis screening and fatty acid analysis are successfully used in many countries around the world.

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A landscape of the heritability of single-band Fourier-Transform Infrared spectra data in Canadian Holstein

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Summary

Fourier-transform mid-infrared (FTIR) milk spectra data is routinely collected within milk recording programs of different countries. This information can be used both for assessing milk composition and for genetic evaluations. Establishing an optimal strategy for the use of spectra data in genetic evaluations require knowledge of the heritability of individual bands. Therefore, in this study we used data from about 1.8 million test-day records of Canadian Holstein cows to produce a landscape of the heritability of FTIR data by band (1,060 evenly-spaced bands), parity (from first to third) and month of the lactation (from 1st to 4th). Several regions of the spectrum that have been reported to be associated to important milk components (e.g., lactose, fat and protein) showed moderate-to-high heritability estimates (0.40-0.50). We confirm many of the heritability patterns reported in previous studies and report novel findings related to differences in the heritability of FTIR spectra across parities and month of the lactation.

Keywords: milk spectra, FTIR, spectrometry, high-throughput phenotyping, Bayesian, BGLR.

Introduction

Fourier-transform infrared spectroscopy (FTIR) can be used to describe the molecular structure of many different materials. In the dairy industry, FTIR data derived from electromagnetic wave absorbance within the mid infrared (MIR) spectrum, is routinely used to assess milk composition for payment, quality control, herd management purposes as well as for selective breeding. Indeed, FTIR-derived predictions of protein (PROT%) and fat percentage (FAT%) from individual samples of milking cows are routinely stored in national recording systems and used as inputs for the genetic

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evaluations (ICAR, 2012). The clear majority of research efforts and industry applications using FTIR data has focused on developing prediction equations for economically important phenotypes such as milk composition (Rutten *et al.*, 2010; 2011) and technological properties (Cecchinato *et al.*, 2009). FTIR data has also been shown to be a valuable tool for assessing health and reproductive phenotypes (Heuer *et al.*, 2001; Belay *et al.*, 2017) as well as feed efficiency and methane emission (Shetty *et al.*, 2017a; b).

Spectra-derived predictions of phenotypes (e.g., FAT%, PROT%) are routinely used as "traits" in genetic evaluations. However, the best predictor of a phenotype is not necessarily the best selection index for the same trait. Developing an optimal selection index requires knowledge of genetic and environmental variance and co-variance components (Hazel, 1943). A few authors have estimated and reported genetic parameters of FTIR data (e.g., Soyeurt *et al.* 2010; Bittante and Cecchinato, 2013; Wang *et al.* 2016). These studies have shown that absorbance (and transmittance) at certain regions of the spectrum can be moderately heritable.

However, the studies published so far were based on limited sample size (<2,000 cows) and did not considered the possibility that the heritability of FTIR data may vary across parities and over the course of lactation. Therefore, our goal was to produce a landscape of the heritability of FTIR spectra data across parities and over the course of the lactation. To this end we analyzed a very large Canadian dataset comprising ~1.8 million records of FTIR data from Holstein milking cows linked to covariates and pedigree data.

Materials and methods

Data (n=1:781,005 test-day records, from 560,131 Holstein cows, milked in 6,368 herds distributed in seven Canadian provinces) were from the Canadian Dairy Network (Guelph, Ontario) collected between January 2013 to June 2016. Milk samples were processed using two FTIR MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark) spectrophotometers at the Canadian DHI organizations CanWest DHI (Guelph, Ontario) and Valacta (Sainte-Anne-de-Bellevue, Québec). Milk spectra consisted of FTIR absorbance data at 1,060 bands located between wavenumbers 5,011 cm^{-1} and 925 cm^{-1}. In addition to FTIR spectra data, milk yield (MY) and spectra-derived predictions of PROT% and FAT% were available for each test-day milk sample. Additional information included the herd, year and test-date at which the samples were collected and a pedigree that traced back three generations from each of the individuals with phenotypic records.

Stratification. We divided data into disjoint groups defined by 3 parities (1, 2 and 3) and 4 stages of lactation as defined by 30 days in milk (DIM) intervals between calving and 120 DIM. Thus, there were a total of 12 strata. The number of records per stratum ranged from 39,164 to 82,367 (*Table 1*).

Models

We estimated the heritability of single-band absorbance spectra data, single test-day MY, PROT% and FAT% using a standard mixed model of the form:

$$y_{ij} = \mu + hys_i + a_j + e_{ij},$$

where y_i ($i=1,\ldots,n$) represents a phenotype (e.g., absorbance data on one of the bands), μ represent the overall mean, hys_i is the random effect of i^{th} herd-year-season level (four seasons were considered: January-March, April-June, July-September and

Table 1. Average (SD) of milk yield (kg), and [number of records] by parity and stage of lactation.

Parity	Stage of lactation			
	1-30 DIM	31-60 DIM	61-90 DIM	91-120 DIM
1	28.7 (6.3) [71,140]	32.8 (6.2) [82,367]	32.8 (6.1) [81,608]	31.9 (6.1) [80,795]
2	38.4 (8.2) [55,954]	42.1 (8.1) [63,956]	40.5 (7.8) [62,902]	38.4 (7.6) [62,396]
3	40.3 (8.9) [39,164]	44.7 (8.8) [44,169]	43.4 (8.4) [42,985]	41.0 (8.2) [42,343]

October-December, 33,119 *hys* levels), a_j is the additive genetic effect of j^{th} cow and ε_{ij} is an error term. The vector of herd-year-season (hys) and additive effects (a) and the vector of error terms (ε) were assumed to follow multivariate normal distributions of the form: hys ~ MVN $(0, I\sigma^2_{hys})$, a ~ MVN $(0, A\sigma^2_a)$ and ε ~ MVN $(0, I\sigma^2_\varepsilon)$, respectively. Here $\boldsymbol{I}$ is an identity matrix, $\boldsymbol{A}$ is the pedigree relationship matrix, σ^2_{hys} is the variance of the random combined effect of the herd-year-season factor, σ^2_a is the additive genetic variance, and σ^2_ε is the error variance. We implemented statistical analyses within a fully Bayesian framework.

Software

All the analyses were carried out in the R-environment (R Core Team, 2016). The pedigree was processed using the pedigreeR R-package (Vazquez and Bates, 2017). Models were fitted using the BGLR software (Pérez and de los Campos, 2014). For each analysis, we computed posterior means, posterior standard deviations and 95% credibility regions for each of the variance parameters and for the heritability (h^2) defined as:

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{hys} + \sigma^2_\varepsilon}$$

where σ^2_a, σ^2_{hys}, and σ^2_ε are the variance terms defined before.

Results

The estimated heritabilities of MY, PROT% and FAT% increased from the first to the second month of the lactation (Figure 2) and then remained stable (or varied with no clear pattern) towards the third and fourth month of the lactation. Among those three traits, PROT% had the highest estimated heritability (~0.4 in the 2nd, 3rd and 4th month of the lactation), followed by FAT% (~0.25-0.30 in the same months) and MY (~0.15-0.20 for months 2-4). There were no clear differences in heritability estimates across lactations.

Figure 1 shows a landscape of the estimated h^2 of the 1,060 bands of milk spectra along the four stages of lactation for each parity. The estimated heritability curves per strata, and including 95% confidence regions are given in Figure 3. Heritability estimates ranged between values close to zero to values about 0.55. Estimated heritability curves were rather smooth, in that heritability estimates of adjacent bands for the same strata

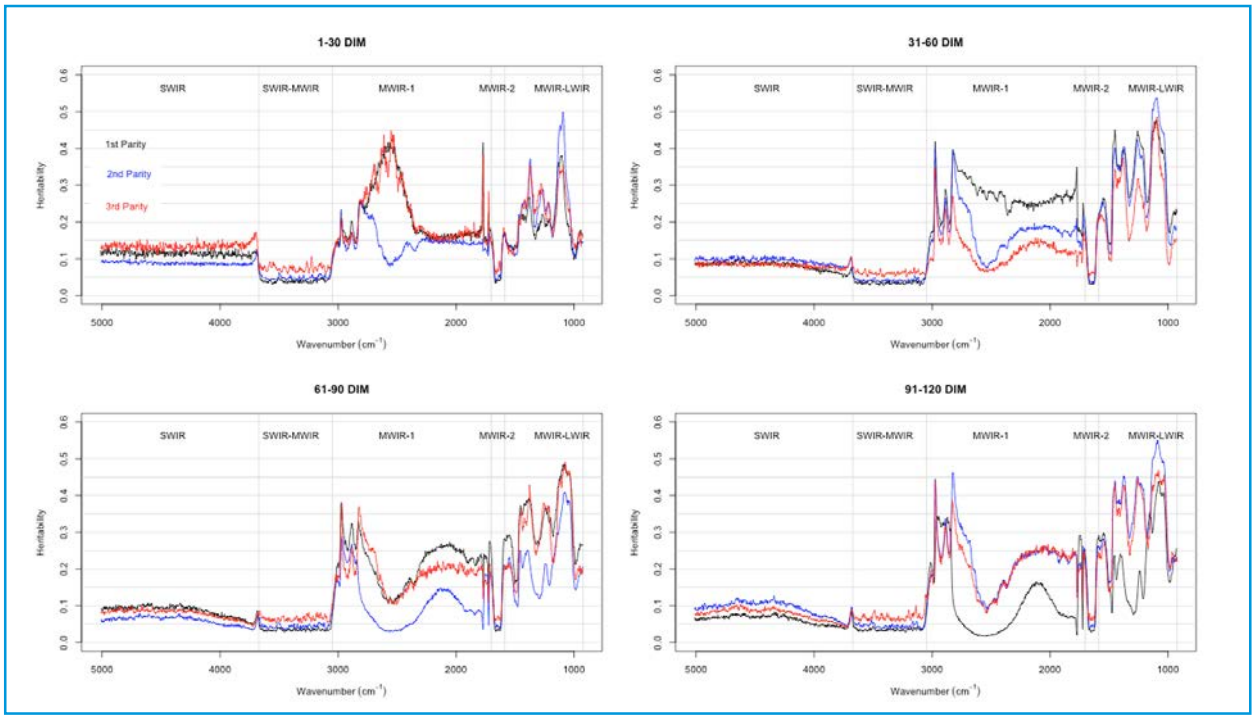

Figure 1. Heritability estimates by band (horizontal axis), parity (each color correspond to one parity) and stage of lactation (DIM).

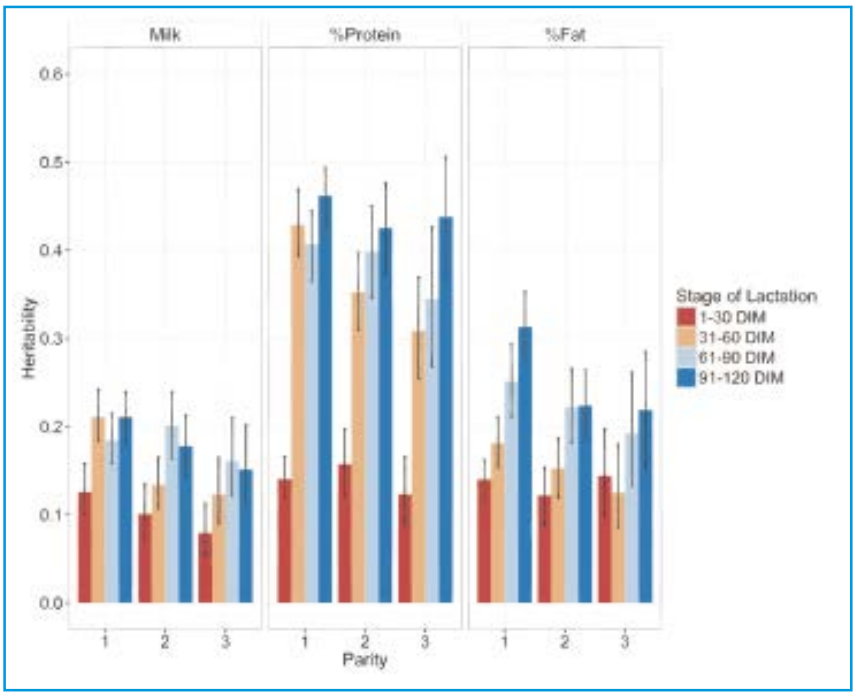

Figure 2. Heritability estimates for test-day milk yield, percentage of protein and percentage of fat by parity and stage of lactation.

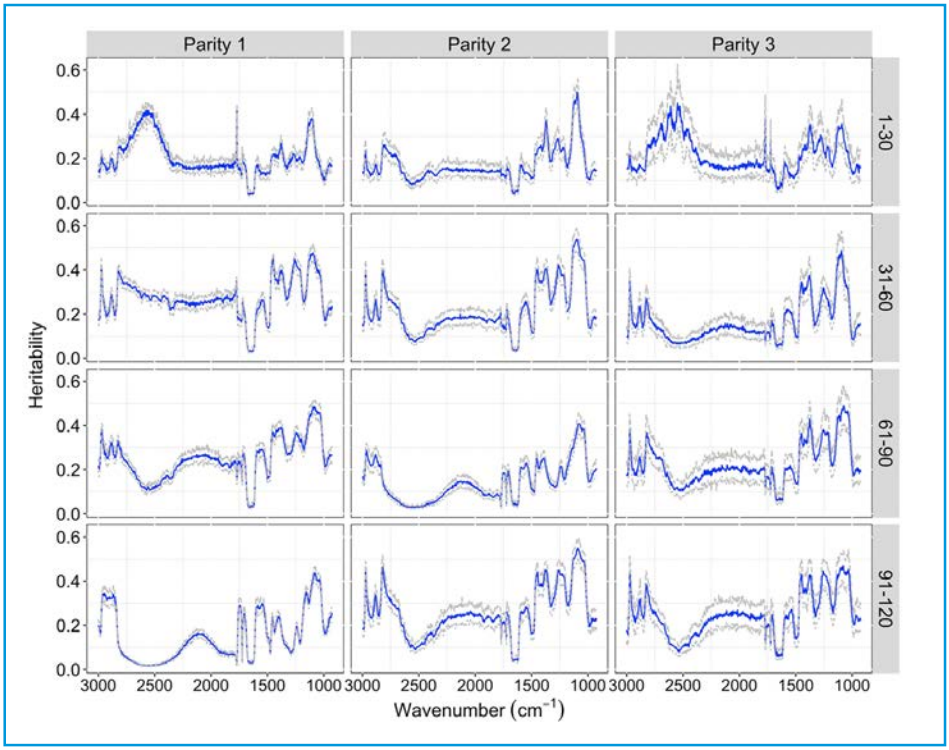

Figure 3. Estimated heritability (95% Bayesian credibility region) of individual bands in the regions between wavelengths 3,000 cm-1 and wavelengths 925 cm-1, by parity and stage of the lactation.

were very similar. There was not a clear trend that indicate higher h^2 for a specific parity. However, we recognized some patterns across regions of the spectra consistent across the various strata.

Firstly, the short-wave-length infrared (**SWIR**) region of spectra (spanning between wave numbers 5,066 cm^{-1} to 3,672 cm^{-1}) and the transition region between SWIR and mid-wave-length infrared (**MWIR**, spanning from wavenumbers 3,672 cm^{-1} to 3,050 cm^{-1}) were the regions with lowest h^2 estimates, with values ranging from values near zero to 0.13. These patterns were very similar across parities and month-in-milk (Figure 1).

Secondly, the region of **MWIR** (spanning between wavenumbers 3,050 cm^{-1} to 1701 cm^{-1}) showed substantial variability in h^2 estimates across months of the lactation and parity. Here estimates ranged from near-zero to moderately high (values near 0.44). In this sector, there appeared to be two different patterns across lactation stages. For 61-90 DIM and 91-120 DIM there were two peaks of heritability: one from wavenumbers 3,050 cm^{-1} to ~ 2,800 cm^{-1}, and one with a peak of heritability estimate in the neighborhood of wavenumber 2,200 cm^{-1}. Between these two peaks is a “valley” of low-heritability bands. These patterns are clear in 61-90 DIM and 91-120 DIM for all parities (Figure 1). However, the first month of lactation (1-31 DIM) demonstrated a different pattern; here, in the first and third lactations there was a clear peak of heritability estimates around wavelength 2,700 cm^{-1} which was not present in later stages of the lactation. The results for the second month of lactation show a somehow transitional pattern between that of the first month and those observed in the third and fourth month of lactation.

Finally, the last sector of the spectra, which spans between wavenumbers 1,586 cm^{-1} and 925 cm^{-1} and comprises the **MWIR** and **long-wavelength infrared bands** (**LWIR**) showed heritability estimates ranging from 0.14 to 0.55. In this region, there were three consecutive peaks of high-heritability estimates with valleys in between; the pattern appeared with different intensities in all the strata analyzed.

Discussion

Our heritability estimates for test-day milk yield, FAT% and PROT% are in line with previously reported estimates for the same population (Loker *et al*., 2009; Nixon *et al*., 2009; Koeck *et al*., 2013). For individual bands, we obtained heritability estimates ranging from values near zero to values of about 0.55. As noted by Bittante and Cecchinato (2013), there are multiple regions of the spectra showing higher heritability than that of yield traits commonly used in genetic evaluations. We confirmed this in the Canadian-Holstein population, using a significantly larger sample size.

The patterns of heritability estimates by regions of the spectra reported here confirm those reported by previous authors for other populations (Bittante and Cecchinato, 2013; Soyeurt *et al*., 2010). However, we found substantially higher heritability estimates in many regions of the spectra. Several factors may explain this. Beside the differences in the breed-lines and production systems considered, it is worth noting that our analyses were stratified by parity and month of the lactation and that we consider only the first 120 DIM; therefore, we did not treat spectra from different stages of the lactation curve as the same trait.

The comparison of our results with those previously reported for different regions of the spectra leads to some important conclusions. Firstly, similar to what was reported by Bittante and Cecchinato (2013) we found that the SWIR, SWIR-MWIR and MWIR-2 are regions characterized by low-heritability estimates. Secondly, the MWIR-LWIR regions have some of the wavelengths with highest heritability. The patterns of heritability found in these regions (three waves of high heritability estimates) are very consistent across parities and lactation stage; however, these patterns are not as well defined in the first month of lactation. This region is known to be related to important milk components (e.g., lactose and some proteins). Finally, in the MWIR region the patterns of heritability estimates of the 3rd and 4th month of lactation were different than those of the 1st month of the lactation. Interestingly, in the first month of lactation the patterns of heritability estimates for 2nd parity was different to those of the first parity. We do not have a clear explanation of what may explain these differences in heritability between early and advance stages of lactations; however, considering the molecules that have been link to the bands on this region (e.g. N-H ammonium ions) it is possible that the different patterns observed may be linked to energy-balance-related aspects, this hypothesis needs to be further examined.

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Electronic and visual identification for sheep and goats in Brazil

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Summary

Intra-ruminal devices and plastic ear tags were evaluated in sheep and goats (01-08 years old) in different systems of production in Brazil, where there is no mandatory legislation on traceability for small ruminants. Four electronic devices and two commonly used ear tags were studied in 283 animals distributed in five experiments, in the medium and long terms. The time required for application, the retention rate and the readability of the devices were determined. In Experiment 1, we assessed the long-term retention of plastic ear tags (4.25 g) and mini-bolus (20.0 g) applied on 35 Suffolk male lambs. The retention rate of both devices was 100% after 6 months. At 12 months, the boluses presented 100% retention and the ear tag was 96.9%. The readability for both devices was 100%. In Experiment 2, 57 Suffolk ewes were used. Three intra-ruminal devices (mini-bolus of 21.65 g, n=21; small bolus of 29.52 g, n=18 and standard bolus of 74.4, n=18) were evaluated for ease of application, readability and retention rate. The time of application varied ($P<0.05$) depending on the devices. The standard bolus showed longer time for application (32.8 ± 6.9 s) compared to the mini-bolus (9.5 ± 2.7 s) and small bolus (8.27 ± 2.0 s), which did not differ ($P>0.05$). After 6 months, retention rate and readability for all devices was 100%. In Experiment 3, 127 Ile de France ewes reared in semi-intensive systems were used to evaluate three intra-ruminal boluses (mini-bolus of 21.65 g, n=43; intermediate bolus 40.23 g, n=42 and standard bolus of 74.4, n=42). Standard and intermediate boluses showed 100% readability after 6 months. The readability of the mini-boluses was 97.1%. In Experiment 4, 42 Ile de France ewe lambs were used to evaluate the performance of two intra-ruminal bolus (mini-bolus, 21.65 g, n=23; small bolus 29.52 g, n=19) and an ear tag (5.2 g, n=42). The time required for the application, the readability and retention rate of all devices were determined after 6 months. The time of application for the devices ($P<0.05$) depended on the type of device, and was higher ($P<0.05$) for the mini-bolus (6.34 ± 2.36 s) compared to small bolus (4.45 ± 1.83 s). The intra-ruminal boluses showed 100% of retention and the ear tag, 94.5%. The estimated readability did not change ($P>0.05$) according to the type of device. In Experiment 5, standard bolus (74.4 g), small ear tag, 50 mm x 15 mm (width x height) and big ear tag (42 mm x 48 mm) were evaluated in 22 crossbreed

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Boer female goats for six months, in pastures. The mean time required for the application was 21s and did not differed (P>0,05) among the devices. The loss of one big ear tag was registered, and the retention rate was 95.5%. The readability was 100%. All electronic devices have proven to be highly efficient (100% RR) and could be recommended for sheep and goats. Readability failures and losses of visual devices were registered and it should be often highlighted to the producers. Also, an economic analysis performed along with the electronic devices industry can contribute to the farmers' choice.

Keywords: animal identification, intra-ruminal bolus, ear tag, Brazil, sheep, goats.

Introduction

The need for reliability and accuracy in identifying animals in production systems for recording animal performance (e.g., body weight, inventory, weight gain, births) is one of the key points for traceability (Cappai *et al.*, 2014).

The conventional identification systems of small ruminants like plastic ear tags, necklaces and tattoos are not reliable due to the possibility of violation, exchange and loss of devices. In addition, difficulties in readability of ear tags are observed (Pina *et al.*, 2005).

In Brazil, the use of electronic identification in small ruminants is not yet required by the national legislation. Nevertheless, the farmers have been improving animal control (Hentz *et al.*, 2014), in order to receive the subsidy offered by the meat co-ops, which appreciate better control of herd information.

The objective of this study was to evaluate four electronic ruminal boluses and two commonly used plastic ear tags in five experiments, in medium and long terms, in semi-intensive systems in Brazilian sheep and goat herds.

Material and methods

283 animals were evaluated - 261 Ile de France (n=169) and Suffolk (n=92) sheep and 22 crossbreed Boer goats - in five experiments in the medium and long terms (Hentz *et al.*, 2014; Kowalski *et al.*, 2014). The Suffolk sheep belonged to Sheep and Goat Production and Research Center (Federal University of Paraná, Pinhais, PR, Brazil), Ile de France ones belonged to a private farm (Tangará Ranch, Reserva, PR, Brazil) and the Boer goats to Cabanha do Espanhol private farm (Colombo, PR, Brazil).

A metal applicator with size adjustment (Gesimpex®, Barcelona, Spain) according to the dimensions of the boluses was used for administration in all the experiments. Bolus were manufactured by Certag® Saint Gobain - Ceramics and Plastics - and plastic ear tags by Allflex®. A completely randomized design was used and the animals were the experimental units.

In experiment 1, 35 weaned Suffolk male lambs (22.4 ± 2.6 kg body weight (BW) and 77.5 ± 12.9 days) were used. Mini-boluses of 20.0 g (n = 35), inserted at the weaning, and ear tag of 4.25 g (n=35) inserted on the first day of life, were evaluated. Readings to determine the retention rate of the devices were performed weekly until 6 months and monthly until 1 year.

In the experiment 2, 57 Suffolk ewes (6.0 years and 85.07 kg BW) were monitored for six months in a semi-intensive production system. The animals were raised in Tifton-85 (*Cynodon* spp.) and ryegrass (*Lolium multiflorum* Lam.) and concentrate supplementation was fed daily. The mini bolus of 21.65 g (n=21), small bolus of 29.52 g (n=18) and standard bolus of 74.4 g (n=18) were evaluated.

In experiment 3, 127 Ile de France ewes (3.43 years old and 62.7 kg BW) were monitored for six months in a semi-intensively grazing system of Tifton-85 (*Cynodon* spp.) and Aruana (*M. maximum* cv. Aruana). The mini bolus of 21.65 g (n=43), small bolus of 40.23 g (n=42) and standard bolus 74.4 g (n=42) were evaluated.

In experiment 4, 42 Ile de France ewe lambs (24.2 kg BW) were evaluated in pasture of Aruana grass *(M. maximum* cv Aruana) and ryegrass (*L. multiflorum* Lam.). The devices evaluated were a 5.2 g (n=42) ear tags placed on the first day of life, a mini bolus of 21.65 g (n=23) and a small bolus of 29.52 g (n=19), applied at 82 days of age. The time required to apply the devices was determined. Readings were made between 1-7 days and monthly to determine device retention and readability for 6 months.

In the experiment 5, 22 crossbred Boer goats (4 years old and 52.6 kg BW) were evaluated in a semi-intensive system in limpograss (*H. altissima* cv. Florida) pastures. Standard bolus (74.4 g), small ear tag, 50 mm x 15 mm (width x height) and big ear tag (42 mm x 48 mm) were evaluated since the first week to six months. The time spent for administration / application, readability and retention rate were evaluated.

The readability (Re) and the retention rate (RR) were calculated as described by Caja *et al.* (1999). Data on time for device administration/application were analyzed by ANOVA using the general linear model (GLM), considering the randomized effect associated with the animals. Device retention rate data were submitted to survival analysis by the chi-square test. Statistical analyses were performed using the R Project for Statistical Computing version 2.10.1.

Results and discussion

In experiment 1, no electronic faults were observed and the boluses presented 100% reading capacity. There were also no difficulties in reading the number on the ear tags that could compromise reading ability. Carné *et al.* (2009) suggested that the loss of ear devices could be associated with the detachment of the male and female parts since they observed differences in the coupling diameter between parts of the devices. The retention rate at the end of the evaluation was 97.6% and the mini-bolus presented a 100% retention rate. Thus, after 12 months of evaluation, only the intra-ruminal bolus met the requirements of the ICAR ($\geq$ 98% of TR at 12 months) (ICAR, 2007).

For experiment 2, after six months, 98.2% of the animals initially identified were monitored. Effective retention (100%) at 1 day and 1 week after application is indicative of adequate device design, and after 1 month of evaluation no device was lost. The mean retention rate was 100%. Also, no electronic faults were observed or another problem that compromised the identification of the number of transponders, and, therefore, the estimated reading capacity between the devices was 100%. During the study approximately 5% of the animals lost the ear tag. At the 6-month evaluation, all the devices had 100% retention and thus met the requirements of ICAR ($\geq$ 99% TR at 6 months). The estimated reading capacity for the different boluses was 100%. Because no device losses or failures were observed, statistical differences could not be established for the variables.

At the end of experiment 3, 95.2% were still being monitored. No early losses (1st day of the 1st week) were observed for any of the devices used. This reinforces the idea that their characteristics were adequate and that their retention rates could be maintained along the time. The percentage of electronic failures observed in this study was 0.78%. During the evaluation period, approximately 13 animals (10.23%) lost their ear tags. Most animals that lost the ear tags were older than 2 years, suggesting that visual devices do not allow identification of the animal throughout its life. The results obtained for the mini-bolus and standard bolus are according to the reported in adult sheep (Ghirardi *et al*., 2007).

In experiment 4, after six months, 36 lambs (94.2%) remained monitored. For the mini-bolus, the mean time of application was 6.34 ± 2.36s, being higher (P <0.05) than the time for the application of the small bolus (4.57 ± 1.83s). Ghirardi *et al.* (2007) suggested that the size of the bolus, especially its diameter, are determinant for the swallowing of the animal at the application. The estimated reading capacity of all devices was 100% and the boluses had 100% retention rate, and did not change (P >0.05) according to the device. After two losses in the second month, the retention rate for ear tags was 94.5%.

Finally, in experiment 5, the time spent in the application of all the devices was, in average, 21 s (P = 0.7084) and corroborates the time reported by Carné *et al.* (2010). One big ear tag was lost after one month, reducing the retention rate to 95.5%, and it did not meet the requirements of ICAR (2007). The small auricular ear tags and intra-ruminal boluses had retention rate of 100%, but no difference (P = 0.3170) among the devices was noted.

In general, we may consider that the physical characteristics of the devices were adequate for the effective pre-stomach retention of sheep and goats and the time for application was acceptable. Readability failures and losses of visual devices were registered and it should be often highlighted to the farmers. Also, an economic analysis performed along with the electronic devices industry can contribute to the Brazilian farmers' choice.

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Application of 11 STR markers for the evaluation of genetic variation in sheep

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Summary

It is important to study and monitor changes in genetic structure of small old sheep breeds. Microsatellite markers are widely used for estimating genetic diversity within and differentiation among populations. For the study we used the following set of 11 polymorphic STR markers: CSRD247, ETH152, INRA005, INRA006, INRA063, INRA172, MAF065, MAF214, McM042, McM527, OarFCB20 and AMEL *locus*.

The objective of the research was to study the genetic structure of 3 breeds included in the sheep genetic resources conservation programme in Poland (Wielkopolska – 100, Old Type Polish Merino – 93 and Olkuska – 88 animals) and to determine genetic differentiation between them based on polymorphism of 11 STR markers.

Genetic analyses were performed in an ABI 3130xl sequencer and the results were analysed using GeneMapper Software 4.0. The identified alleles were used to estimate observed (Ho) and expected heterozygosity (H_E), polymorphic information content (PIC) and Nei's genetic distance D_N (1987).

There were identified 101 alleles in 11 microsatellites loci with a mean of 6.87 alleles per *locus*. The range of H_O and H_E was from 0.2903 to 0.8710 and 0.3307 to 0.8370, respectively. All the markers were highly polymorphic. PIC values for each marker were high and exceeded 0.5 except for INRA172 locus in Merino (PIC=0.3171) and ETH152 locus in Olkuska sheep (PIC=0.4781). The highest polymorphism was observed in INRA63 of Merino sheep where PIC and Ho were 0.8710 and 0.8181, respectively. The estimated coefficient of genetic distance, calculated based on all markers, was low and ranged from D_N=0.0836 between Merino and Wielkopolska to D_N=0.2187 between Merino and Olkuska sheep. It can be concluded that each breed became genetically distinct.

Introduction

Farm animal genetic resources should be conserved due to their current and potential economic, scientific and cultural significance. Native Polish sheep breeds are very well adapted to local environmental conditions, have low feeding requirements, and are highly resistant to disease and poor living conditions. In order to save these breeds from extinction and perpetuate valuable characteristics in the population, they have been included in the genetic resources conservation programme (Kawecka and Krupinski, 2014).

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Sheep parentage verification based on blood groups, which was carried out in Poland since 1973, was replaced with analysis of DNA microsatellite polymorphism in 2016. The application of microsatellite markers has increased from their discovery in the 1980s. Today they are widely used for individual identification, parentage testing, biodiversity assessment, and in forensics (Rychlik *et al.* 2003; Radko *et al.* 2006).

In order to standardize sheep parentage testing based on DNA microsatellite sequences, the International Society of Animal Genetics (ISAG) has recommended a panel of 13 markers: AMEL, CSRD247, ETH152, INRA005, INRA006, INRA023, INRA063, INRA172, MAF065, MAF214, McM042, McM527, OarFCB20.

The objective of the study was to evaluate the polymorphism of selected loci and their usefulness for testing genetic structure, and to determine differences between the populations of three sheep breeds covered by the genetic resources conservation programme.

Material and methods

The material of the study comprised genomic DNA isolated from whole blood of 281 sheep: Old-Type Polish Merino (93 head), Olkuska (88 head) and Wielkopolska (100 head).

Extraction of genomic DNA from blood was carried out using the Sherlock AX kit (A&A Biotechnology) according to the manufacturer's directions.

For analysis of DNA polymorphism, 12 microsatellite markers recommended by ISAG were chosen and two multiplex sets were determined:

- Multiplex I: AME, INRA63, INRA006, MAF214, McM042, CSRD247, INRA172;
- Multiplex II: MAF065, McM527, OarFCB20, ETH152, INRA005.

For the analysis of microsatellite sequences, PCR reaction was performed in a Master Mix reaction mixture (Qiagen). The amplification conditions consisted of initial denaturation at 95°C for 5 min, 28 cycles: 95°C for 30 s, 61°C (multiplex I) / 58°C (multiplex II) for 3 min, 72°C for 30 s, and elongation at 72°C for 45 min. The primers used for the amplification were fluorescently labelled with four different dyes (FAM, VIC, NED, PET), which allowed for the simultaneous detection of twelve microsatellite markers in one gel lane. The PCR products obtained were electrophoresed on denaturing 7% polyacrylamide gel in the presence of 500 LIZ size standard, using the 3130xl Genetic Analyzer (Applied Biosystems). The size of separated DNA fragments and the genotypes were determined with GeneMapper® Software 4.0 (Applied Biosystems). Statistical calculations were performed using IMGBOVSTAT – IZOO PIB software.

Results and discussion

In the analysed material collected from 281 sheep, 101 alleles at 11 microsatellite loci were detected. The breeds differed in the size range and frequency of identified alleles (Table 1).

The mean number of alleles per locus varied from 5.6 in Olkuska sheep to 7.8 in Wielkopolska sheep (Table 1). The greatest number of alleles (12) was obtained at locus INRA005 in the Wielkopolska breed, and the smallest at loci ETH 152 – 4 alleles in each breed under study and CSRD247 – 4 alleles in Olkuska sheep (Table 2). For

Table 1. Allele size ranges (bp) and number of alleles per locus (for 11 markers) in the studied breeds.

Locus	Breed					
	Merino		Olkuska		Wielkopolska	
	Range	Number	Range	Number	Range	Number
CSRD247	211-239	8	213-229	4	211-239	9
ETH152	186-192	4	186-192	4	186-192	4
INRA005	125-149	10	125-145	6	125-151	12
INRA006	110-134	7	110-134	6	110-134	6
INRA172	126-164	6	126-162	5	126-162	7
INRA63	169-197	10	169-197	7	169-201	11
MAF065	125-137	7	123-135	5	125-139	7
MAF214	187-263	7	187-261	7	187-261	8
McM042	81-97	5	81-107	5	81-103	7
McM527	164-174	6	164-174	5	164-176	7
OarFCB20	87-113	9	87-113	8	87-107	8
Mean		7.2		5.6		7.8

Table 2. Degree of observed (Ho) and expected heterozygosity (He), inbreeding coefficient (Fis), and polymorphic information content (PIC).

Locus	Breed											
	Merino				Olkuska				Wielkopolska			
	HO	HE	FIS	PIC	HO	HE	FIS	PIC	HO	HE	FIS	PIC
CSRD247	0.5161	0.5365	0.0379	0.5020	0.5795	0.5799	0.0006	0.5167	0.6800	0.6173	-0.1016	0.5924
ETH152	0.5591	0.6719	0.1679	0.6109	0.6591	0.5387	-0.2235	0.4781	0.5400	0.6034	0.1051	0.5428
INRA005	0.7742	0.8184	0.0540	0.7938	0.8523	0.7480	-0.1394	0.7146	0.7400	0.8244	0.1024	0.8035
INRA006	0.7419	0.7113	-0.0431	0.6747	0.3750	0.5378	0.3028	0.5132	0.7400	0.7087	-0.0442	0.6725
INRA172	0.2903	0.3307	0.1220	0.3171	0.6136	0.5874	-0.0447	0.5174	0.6700	0.6655	-0.0068	0.6308
INRA63	0.8710	0.8370	-0.0406	0.8181	0.7273	0.6708	-0.0841	0.6264	0.7300	0.7892	0.0750	0.7585
MAF065	0.7957	0.7183	-0.1078	0.6687	0.8409	0.7510	-0.1197	0.7050	0.7900	0.7613	-0.0378	0.7196
MAF214	0.6344	0.6586	0.0367	0.5937	0.7045	0.7159	0.0159	0.6818	0.7400	0.7565	0.0217	0.7168
McM042	0.4731	0.5808	0.1853	0.5192	0.6705	0.5768	-0.1624	0.5265	0.7100	0.7504	0.0538	0.7123
McM527	0.6344	0.7211	0.1202	0.6762	0.7273	0.6642	-0.0950	0.6073	0.7300	0.7548	0.0329	0.7170
OarFCB20	0.6774	0.7090	0.0446	0.6804	0.8295	0.7829	-0.0596	0.7532	0.7400	0.7554	0.0204	0.7225

Table 3. Nei's genetic distance for the sheep groups under study.

Breed	Merino	Olkuska	Wielkopolska
Merino	0.0000	0.2187	0.0836
Olkuska	0.2187	0.0000	0.1990
Wielkopolska	0.0836	0.1990	0.0000

the ETH152 marker in Balochi sheep, Wajid et al. (2014) obtained 3 alleles in the 168-219 bp range. Greater differences in the number of alleles were reported by Yilmaz *et al.* (2015), Radha *et al.* (2011) and Rendo *et al.* (2011), who obtained from 7 to 23 alleles in the 209-261 bp range for locus CSRD247 in sheep.

To evaluate the polymorphism of the analysed markers, the observed (H_O) and expected heterozygosity (H_E) as well as the coefficient of inbreeding (Fis) were calculated (Table 2). The H_O and H_E values for all the studied breeds were high (above 0.5161) except for INRA172 in Merino sheep (H_O=0.2903, H_E=0.3307) and INRA006 in Olkuska sheep (H_O=0.3750). The highest values were observed for INRA63 in the Merino breed (H_O=0.8710, H_E=0.8370). Similar levels of heterozygosity for this marker were obtained by Kawecka and Piórkowska (2011) in Podhale Zackel (H_O=0.774, H_E=0.824) and by Radha (2011) in Kilakarsal sheep (H_O=0.740, H_E=0.790), and lower levels by Kawecka and Piórkowska (2011) in Swiniarka sheep (H_O=0.472, H_E=0.544).

The coefficients of inbreeding obtained for the studied breeds were similar. Average positive F_{IS} values were observed in Wielkopolska (F_{IS}=0.0201) and Merino sheep (F_{IS}=0.0525), and an average negative value was found in Olkuska sheep (F_{IS}=-0.0554), which may be due to selection. In the case of some *loci*, H_O differed from H_E. The greatest differences were observed in Olkuska sheep; they ranged from -0.2235 at ETH152 to 0.3028 at INRA006. F_{IS} estimates obtained by other authors showed varying levels of inbreeding: F_{IS}=0.09 in Colombian sheep (Ocampo *et al.*, 2016), F_{IS}=0.137 in Turkish breeds (Yilmaz *et al.*, 2015), and F_{IS}=0.2954 in Indian Vembur sheep (Pramod *et al.*, 2009).

An important parameter showing whether markers are suitable for population studies and for parentage verification is polymorphic information content (PIC). The most extreme values were observed in Merino. The lowest PIC (0.3171) was noted for INRA172, and the highest (0.8181) for INRA63 (Table 2). A comparably high PIC value of 0.891 for locus INRA63 was reported by Radha *et al.* (2011).

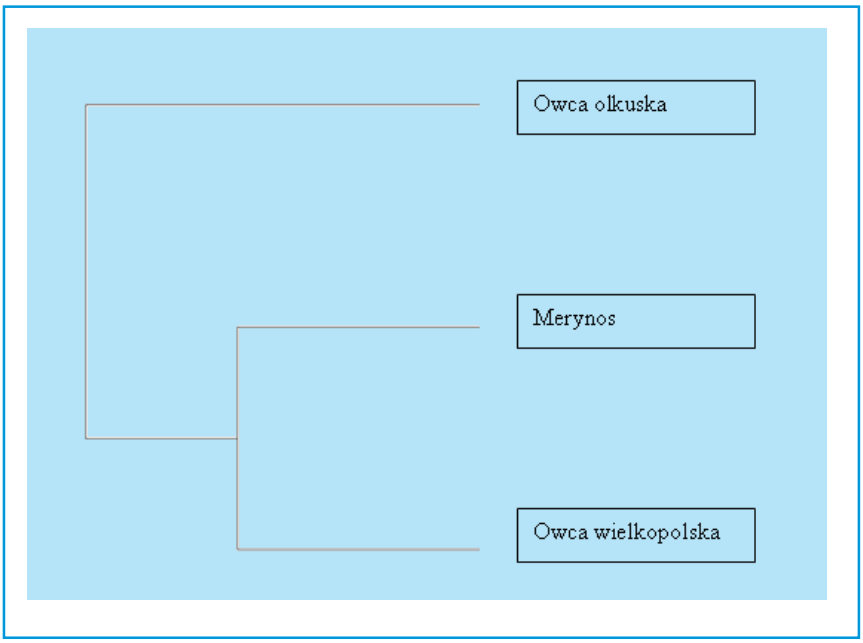

Figure 1. Dendrogram of genetic distance between the studied breeds.

Differences between the studied breeds were determined based on the values of Nei's genetic distance. They ranged from 0.0836 to 0.2187 (Table 3). Based on the genetic distances, unweighted pair group mean analysis (UPGMA) was performed to generate a dendrogram, which is a graphic representation of similarities between the studied breeds. Wielkopolska sheep was closely clustered with Merino and distantly clustered from Olkuska (Figure 1). This is supported by the historical origin of the Wielkopolska breed, because Merino sheep were used in composite crossing.

The results of the present study indicate that the tested set of 11 microsatellite markers can be used for evaluation of genetic structure and parentage testing in sheep.

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Designing a support system in decision making for better management of livestock production

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Summary

Animal identification and record keeping are building blocks for enhancing livestock productivity and effective decision making for genetic improvement programme. The aim of the present situation was to develop online national data repository for goat production system to be utilized by researchers, government, policy makers and developmental agencies. The database will help to manage complex livestock performance data set and will provide input for livestock management decision for better profitability. Goat Production Management Information System (GMIS) has a centralized structure which is user-friendly, E-based information management interactive system which can only be accessed by authorized users. It has been developed using My-SQL, PHP, Java script, HTML, CSS and platform independent. The GIMS is designed to address the following six key issues in management of livestock performance data – Uniform and standardization of data definition – data retrieval of standard performance traits for statistical analysis and report preparation. In order to address these various aspects of livestock data management, the GMIS software has a modular design, on different aspects of livestock production. The modules described for management of animal inventory, growth, milk yield, reproduction, health management, physiological response, buck/doe distribution, trainings conducted, farmers registered, number of cluster/villages covered, details of exposure visits made, awareness camp organized. Besides this, the database will enable us to manage resources, manpower, fund as well as other targets of the project. The website (http://pcgoatcirg.icar.gov.in/) of AICRP on Goat Improvement also provides “knowledge portal” for visitors. Through GMIS, we present a new and simple way of storing and retrieving data for managing livestock production by effective decision making. The database will be used for genetic evaluation of animals by retrieving information on different aspects of goat production.

Keywords: Goat Production System, Database, GMIS.

Introduction

The animal identification and performance recording of livestock species have been carried out by different developing countries. Animal identification and record keeping are building blocks for enhancing livestock productivity and effective decision making for genetic improvement programme. It is necessary to analyze the genetic worth of livestock and to evaluate the breeds for conservation purpose. It is not possible to analyze the data in time frame manner as they are scattered in different registers

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across the region. Therefore, it is necessary to develop the information management software to trace the pedigree as well as performance record. Goat farming with modern scientific inputs will bring social transformation by providing livelihood security to people in most disadvantage places, thereby fulfilling the objective of "Inclusive growth" in our society. Goat production management information system (GMIS) is an emerging field in the intersection of goat production informatics, farmers interaction and business to goat production services and information delivered or enhanced through the Internet and related technologies". The goat production information system is undergoing fundamental changes. Examples of such changes include inventory, growth, male/female reproduction, mortality record and the treatment of chronic diseases that actively involves the goat herd. The emergence of web-based GMIS portals is a natural result of such changes because such portals provide scientist, farmers and other stakeholders easy accesses to information no matter where they are. According to a recent survey, most scientist and farmers agree to access e-base information system via a web-based portal system. The database is primarily aimed to manage data on goats however; in future it can be modified to fulfill the need of other species too.

Main objective of GMIS is to provide users with a computerized tool that allows them to manage complex livestock performance data set, and to provide help in livestock management without relying on specialist computer and data management support.

Material and methods

Language, platform and modules

GMIS, an information system based on MySQL, PHP a server side scripting language along with HTML were used to prepare the information system which can be hosted either on Windows or on Linux platform. Provision has been made for each of the AICRP centers collaborating forms to open secure login and password and update data which can be manage through specific login and password there for each of the calibrating form can update their own records but can't manipulate others. However the administrator being head of the information and management system will have permission combine to collate the information in the manner required to create knowledge base and for statistical analysis. The computational requirement for this information system would be a server having IP address, DNS and dedicated internet gateway. The modules described for management of animal inventory, growth, milk yield, male & female reproduction, health management, physiological report, buck/doe distribution, capacity building programmes, farmers registered, number of cluster/villages covered and funds allocated and its utilization, details of exposure visits made, awareness camp organized and different publications is maintained in integrated databases using a relational database management system. Such a system will enable collecting appropriate data, including quality management and inspection controls i.e. for funds allocated and its utilization, farm/field visits made, number of farmers participated, income and revenue generation by selling of buck/doe etc. Data entry can be made through various modules however, standardized excel formats has been developed and provided for uploading large amount of data in database on one click. This will help to reduce the time consumed while uploading mass data also help in increasing the amount of data in creating the repository. Regular updates in details of budget allocated and utilization leads to prepare monthly reports at Project Coordinator Unit.

The reported data through GIMS can be fetch in M S excel format and direct printing facility to all approved users of AICRP on Goat Improvement. However the basic information regarding breed, publications, availability of elite germplasm, technology developed & its impact and good practices is available at "Knowledge portal" of the AICRP of goat improvement website (*http://pcgoatcirg.icar.gov.in*/) for visitors or general public.

Modules under GMIS database

Following formats were included in Information system.

1. Inventory.
2. Growth.
3. Milk yield.
4. Reproduction.
   - Male reproduction.
   - Female reproduction.
5. Health management.
6. Mortality.
7. Staffing pattern.
8. Finance.
9. GIS.
10. Capacity building.

Results and discussion

The design of GMIS database is, however, particularly challenging due to its unique functionality and security requirements. First, a traditional design of portal systems will encounter difficulties in integrating heterogeneous e-based information system for small remnants production and research and goat rearing techniques implemented with different technologies. The complexity of such integration will make it difficult to extend an existing system with new services. We address the above issues through the design and implementation of a secure web-based centralized information system. To meet the functional requirements, we adopt a knowledge-oriented approach to the design of our database. We then tackle various security issues involved in such a design. More specifically, we outline our solutions for authentication and authorization of users by providing secure login through username and password, for preserving centers privacy through preference negotiation and database technology. Formats for Pedigree and performance recording of goats under farm and field conditions were taken from livestock farms and AICRP units and were standardize to bring uniformity and make it universally applicable in most farming conditions and production system. Initially, the software was launched for 60 days as pilot project. GMIS has definitely quickened the entire of process of performance recording and record keeping. The traditional method of recording information involves individual manual entry; an arduous and a time consuming process. Also there were no uniformity and proper formats for record keeping under the project.

Research data

Inventory

This interface includes information on animal, sire, dam ID along with species, breed, location, sex, mode of entry (by birth or purchase), date of birth and case of disposal. Number of progenies produced by the scenario will automatically be taken from the pedigree record. All the data will be viewed by clicking on the “stock view”.

Growth

This module includes body weight and body measurement of the animal. Once animal ID is entered and click on load button then software automatically collects and show sire, dam ID and past growth records from the inventory and growth database. This will authenticate that correct growth information is entered in the field. It also collects information on date of birth, sex, parity of dam from the reproduction database and shows them for verification. Then body weight growth at birth, 3, 6, 9 and 12 months can be updated by entering the data in the field provided. The data on body measurement like BL (Body Length), BH (Body Length), HG (Heart Girth), can also be entered. The growth data at three months interval is shown from relevant databases. This module also generates average daily gains for different age periods.

Milk yield

In this module the information on weekly milk production data for each can be added. The software calculates partial milk yield for 90 days, 140 days, lactation milk yield, lactation period and average daily milk yield for the lactation period. It also automatically calculates the type of kidding, parity from reproduction database.

In case of Changthangi breed, pashmina production has been recorded. In this module data on pashmina production is maintained. The information on animal number, its parent, sex, parity, pashmina yield, date of pashmina yield, season and weight of animal at pashmina yield can be added. In this module information on pashmina produced from the pashmina goat can be viewed by clicking view yield button in summarized form.

Reproduction

This module includes reproduction performance of doe and buck goats. Like all modules, if animal ID is given, the information on sire and dam, date of birth will be retrieved from Inventory database. The date of service, weight of animal at service, buck identity, date of kidding, type of kidding can be added. After the information on an individual female is entered in the database the software automatically calculates age at first kidding, kidding interval, gestation, service period and parity.

Health management

Health management modules have three sub-module i.e. Health management operations, physiological response and mortality. This module includes the details of preventive health care. After each visit to the cluster / village information on vaccination and treatment related data can be added.

Buck distribution

In buck distribution module user can maintain the records of Buck Distribution of field and income generated by the center. Details of the name of cluster, tag no, name of village, name of farmer, mode of distribution, date of buck provided can be added . By clicking view button user can see all buck distribution record in summarized form.

Finance and accounts

Project Management database

In finance module information related to budget allotted to unit, fund utilization, head wise fund utilization and income generation can be maintained.

a) **Budget Estimate (BE):** Allotted budget of each unit for the financial year is shown in this section. Click on Finance and select Budget Estimate to view BE.

b) **Revised Estimate (RE):** The installment wise amount distributed by PC Unit to each unit is shown in this section. Click on Finance and select Revised Estimate to view RE.

c) **Fund Utilization:** In this module user can submit the details about head-wise monthly fund utilization. Overall budget details i.e. balance funds and expense details can be viewed by clicking on “fund detail

Farmer’s registration

This module maintains the information on registered farmers. In this module entry of adopted farmer related information viz. name of farmer, mobile number, number of goat, registration date, address of farmer can be added. By clicking view button user can view all farmers’ information in summary.

Overview

The overview module helps in monitoring and evaluating the performance of a particular unit by acknowledging flock strength, growth rate, milk production, kidding rate and interval, type of kidding age at first kidding and mortality etc. Such an approach is aimed to establish functional relationship between all AICRP Units and the PC Unit, also in this way the raw data is reportable and readable for experts.

Discussion

A generic data set is distributed along with the GMIS software, consisting of the definitions of essential and useful data files and variables. This generic data set can be individually configured by the user to suit personal requirements by changing existing data files and variables, as well as by adding new data files and variables. The possibility to individually configure the data structure allows users to accommodate a large variety of different goat breed related data. Before GMIS, it creates hazel in preparing sudden weekly and monthly reports and gathering information from eighteen centers of AICRP on goat improvement was a haphazard task. Also for referring past data, we have to find out past year’s annual reports of centers. But after introducing GMIS, it is very easy to search past year’s progress reports, data and publication at one place only.

All the modules in GMIS are prepared as per the manual paper formats which was used traditionally with little bit of modification. Broadly, GMIS is focusing on five main factors of record keeping

1. Inventory.
2. Growth.
3. Reproduction.
4. Health.
5. Capacity building.
6. Finance.

For each of these modules, there are various sub-modules of predefined data. Each module contains the specific layouts which are customized as per the specifications of a particular breed.

Conclusions

In order to address these various aspects of livestock data management, the GMIS software has a modular design, with different modules addressing each of the above mentioned issues. The GMIS database package has been designed to address the following key issues in management of livestock performance:

- Balance of flexibility and standardization of data definition.
- Documentation and definition of data sets.
- Assistance in deriving non-observed data (breed, parity, mating–parturition connection.
- Data validation and error correction
- Reporting for animal management
- Data extraction and calculation of standard performance traits for statistical analysis.
- Number of farmers registered.
- Registered farm overview (Total animals, number of elite bucks, Buck Distributed, Farmers Registered and Growth Rate etc.).

The database will also maintain backup of whole data breed wise/center-wise. GMIS will help to improve the quality and effectiveness of data recording for making better financial decisions. GMIS is user friendly and affordable. It have easiest way to document breed wise record keeping for various aspects like animal identification, growth, lactation/pashmina, reproduction, mortality, income and expense etc.. Simultaneously, GMIS provides complete to PC Unit to evaluate and monitor the overall performance of the entire units pan India quickly. The database provides an insight to research management, analyse production performance and taking appropriate measures for disease occurrence and deficiency. The database also provides an edge to farmers for different advisory with respect to various climatic hazards. Farmer can manage the flock in different agroclimatic zone by following specific package of practices and general practices. The database will serve as repository of availabiliy of improved animals for genetic improvement programme. The database provides the information

the skill requirement and skill acquired by stakeholders. The database will work as repository of available technologies in goat production and as a source motivation for model goat farming and profit making.

Acknowledgement

We acknowledge the help and support of the Director, CIRG and all unit in charges of different AICRP units in developing and implementing their valuable support. Also we would like to thank Dr. S. K. Singh for giving us this opportunity as well as this idea for creating management information.

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Challenging concept for tropical sire summary

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Summary

Genetic evaluation beyond common economic traits are necessary in tropical sire summary. Thailand Tropical Holstein dairy cattle has been development for improvement of milk yield (MY), milk composition (Fat, Protein, SNF), SCC, days open under tropical environment for decades. Nowadays, there are several challenges in dairy genetic evaluation under climate change, especially in production under hot-humid environment. In tropical countries, most of dairy cattle are crossbred (*Taurine* x *Zebuine*) with different genetic fractions of *Bos tuarus*. Therefore, the development of suitable model for genetic evaluation to improve EBV accuracy is necessary. The genetic evaluation under heat stress has been researched. The critical temperature-humidity index (THI) for heat stress under hot-humid production was estimated. Effect of heat stress on Thai Holstein crossbreds increased greatly with parity and for cows with high *Taurus* genetics. It was found that the moderate and severe heat stress occur when THI > 74 and THI > 80, respectively.

Nowadays, EBV under heat stress (heat tolerance index) could be estimated under THI-BLUP model (Boonkum *et al*., 2011). Milk fat to protein ratio was a convenient indicator for negative energy balance (NEB) and acidosis in dairy production. Our study showed that the optimum FPR was in the range of 0.9 to 2.1. Apply this critical range, the genetic evaluation for negative energy balance tolerance can be performed (Puangdee *et al*., 2017). Another challenging trait for sire evaluation under heat stress is conception rates (CR). Continuous improving milk yield has induced a decrease in reproductive performance. The effects of heat stress accelerate the severe effects. Data recording is one of the difficulty. Although the indirect trait such as days open is available, the conception rate was claimed to a direct fertility response trait. We developed random regression threshold models (RR-THM) with Legendre polynomials for genetic evaluation of conception rate in tropical sire summary (Buaban *et al*., 2016). According to low quality of tropical feed resources and low-technology input in production, the genetic potential could not be fulfilled. In addition, the culling parity has been decreased drastically.

Therefore, genetic improvement for robust and endurable/longevity traits has been developed and aimed to be included in tropical sire summary (Saowaphak *et al*., 2017). EBV index combining production, milk quality, and fertility under heat stress

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should be developed under multi-trait analysis. In modern sire summary, genetic evaluation (EBV/GEBV) for NEB tolerance, longevity, genetic markers from candidate or GWAS approaches should be included.

Keywords: sire evaluation, heat tolerance, NEB tolerance, robust, Thailand Tropical Holsteins.

Introduction

Genetic evaluation beyond common economic traits are necessary in tropical sire summary. Thailand Tropical Holstein dairy cattle has been development for improvement of milk yield (MY), milk composition (Fat, Protein, SNF), SCC, days open under tropical environment for decades. Nowadays, there are several challenges in dairy genetic evaluation under climate change, especially in production under hot-humid environment. In tropical countries, most of dairy cattle are crossbred (*Taurine x Zebuine*) with different genetic fractions of *Bos tuarus*, Therefore, the development of suitable model for genetic evaluation to improve EBV accuracy is necessary. Heat stress is an important problem for dairy production in many parts of the world because of its negative effects on productivity and profitability. Ravagnolo and Misztal (2002) proposed a method for the study of genetic response under heat stress. After calving, in extreme states of negative energy balance (NEB). The milk fat to protein ratio (FPR) is used primarily as a diagnostic tool to determine NEB. The optimum FPR has been previously established to be between 1.2 and 1.4 for healthy cows. Dairy cows become metabolically stressed and develop increased incidences of disease and metabolic disorders. There are several scientific reported about NEB effects to length of productive life (LPL) and days open (DO). Therefore, these economic traits are necessary in tropical sire summary of Thailand. EBV index combining production, fertility, NEB tolerance and longevity and under heat stress has been challenged. In modern sire summary, genetic evaluation (EBV/GEBV) for, genetic markers from candidate or GWAS approaches should be included.

Material and methods

In heat stress model, a temperature humidity index (THI) based upon the formula utilized by the National Oceanic and Atmospheric Administration (1976):

THI = (1.8 x temp + 32) - (0.55 - 0.0055 x RH) x (1.8 x temp - 26).

A repeatability test day model (REP) proposed by Ravagnolo and Misztal (2000) was utilized. Firstly, univariate analyses by parity were performed using the REMLF90 program (Misztal, 1999). The model was as follows:

$$y_{ijklmn} = hmy_{ij} + dim_{k}(bg_{jl}) + age_{jm} + a_{jn} + \alpha_{jn} \times f(THI) + p_{jn} + \pi_{jn} \times f(THI) + e_{ijklmn}$$

$f(THI)$ is a function of THI and was defined as:

$$f(THI) = \begin{cases} 0 & THI \leq THI_{threshold} \quad (\text{no heat stress}), \\ THI - THI_{threshold} & THI > THI_{threshold} \quad (\text{heat stress}) \end{cases}$$

In our study, the outcome of an insemination event (referred to as the conception rate, or CR) is defined as a binary trait when estimating parameters using RR-THM. The basic underlying idea consists of modeling the additive genetics and other random

effects in the model as a function of an observed dependent variable (DIM) through a set of random coefficients. The equation for analyzing conception rate was written as such:

$$l = X\beta + Z_h h + Z_a a + Z_p p + Z_s s + e$$

where ***l*** is a vector of unobserved liabilities for service records from a binary outcome of insemination events (1 = success or 0 = failure) and the other notation was described in Buaban *et al.* (2016).

Results and discussions

Evaluation of milk yield under heat stress

Figure 1 shows declining of test-day milk yield across all crossbreds and parities was found at two points, THI of 74 and 80 for moderate and severe heat stress, respectively. The threshold for effect of heat stress on test-day milk yield was set to a THI of 80. The effect of heat stress on Thai Holstein crossbreds increased greatly with parity and was especially large after a THI of 80 for cows with a high percentage of Holstein genes (≥93.7%). The THI threshold of 80 is higher than the thresholds of 72 to 76 reported for US Holsteins (Ravagnolo *et al.*, 2000). Effect of heat stress on Thai Holstein crossbreds increased greatly with parity and for cows with high *Taurus* genetics. Correlations between additive effects (EBV) and heat stress effects (a) was range -0.21 to -0.31 for all parities. The result showed that the increasing of genetics for milk yield caused more stress with negative effects to milk yield when THI above threshold point.

Evaluation of conception rate

The RR-THM was more precise in PE variance estimates, due to the adjustment of the environmental effects on the day of service. The average heritability estimates for CR across DIM was 0.058. The change over time in heritability of the CR show variation due to genetics at <60 day and >300 day of calving. In CR evaluation, the consequence

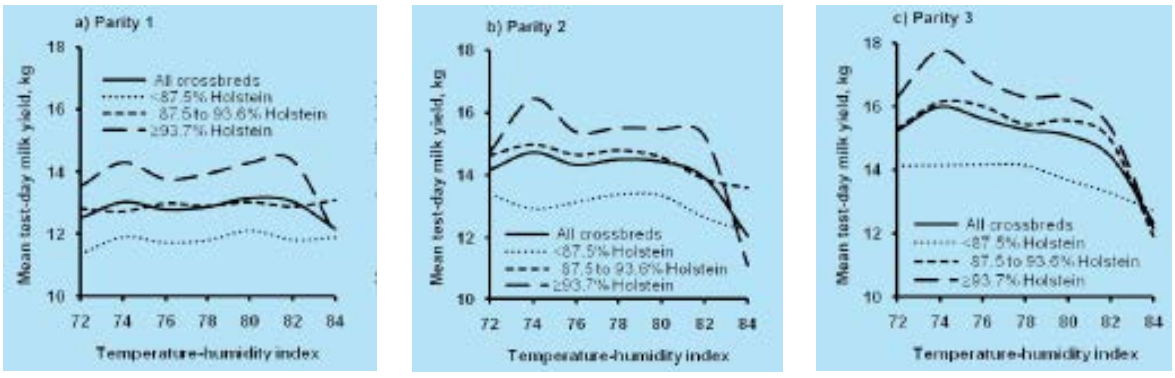

Figure 1. Mean test-day milk yield for Thai Holstein crossbreds across a temperature-humidity index by breed group based on percentage of Holstein genetics for a) parity 1, b) parity 2, and c) parity 3 (Boonkum et al., 2011).

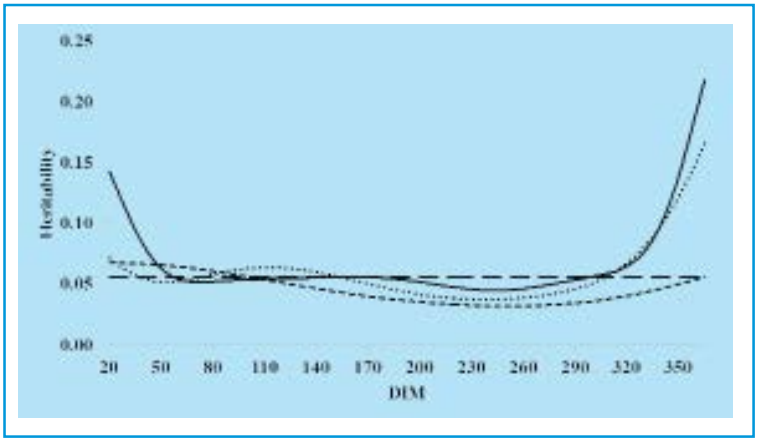

Figure 2 Heritability of conception rate using RR-THM as a function of time with the second- (———), third- (…….) and fourth- (——) order of Legendre polynomials. (Buaban et al., 2016)

of higher heritability and additive genetic variance at the end of lactation is somehow less significant. The CR genetics for each animal can be evaluated only within early to mid-stages of lactation after calving.

Determination of optimum FPR at genetic level

The estimated heritability of MY along the FPR ranged from 0.19 to 0.27. The heritability of MY in this study was in the range of previous reports for tropical Holsteins (Boonkum and Duangjinda, 2014). In addition, the oscillated of the heritability curve of MY revealed an imbalance of energy utilization leading to different individual genetic expressions of FPR effects outside the range of 0.9-2.1. The high heritability for MY reflected the genetic difference in cow sensitivity to NEB influence upon FPR, resulting in accuracy of genetic evaluation. Therefore, the results suggest that the threshold for optimum FPR corresponding to genetics-controlled energy balance for MY was 0.9-2.1. This appears acceptable with regard to high FPR corresponding to NEB, whereas the low FPR corresponded to ruminal acidosis (Toni *et al.* 2011).

Investigation of QTL using GWAS

The GWAS results for all traits in this study, with variances explained for 10-SNP windows to identify the putative QTL regions. The QTL regions was performing by a high proportion of variances (higher than 2.3E-04, which is an expected proportion of variance accounted for by one window). The diffuse peak spread mainly on chromosome 1, 4, 5, 8, 15, 26 and X. The result showing a total of 23 QTL regions were associated with all three traits, and gene that are within the QTL regions. We detected ten SNPs located in QTL regions were associated with LPL, which mostly located on BTA5 and BTAX. There are 4 genes within the regions on BTA5 (SYT1 gene) and BTAX (DOCK11, KLHL13 and IL13RA1 genes). Eight QTLs were associated with DO on BTA1, BTA4 and BTA26, which has only one gene within the region on BTA26 (*PRKG1* gene). Five QTLs were associated with FM305, which found within 2 genes on BTA8 (*GNA14* gene) and BTA15 (*LRRC4C* gene). In this study, not have

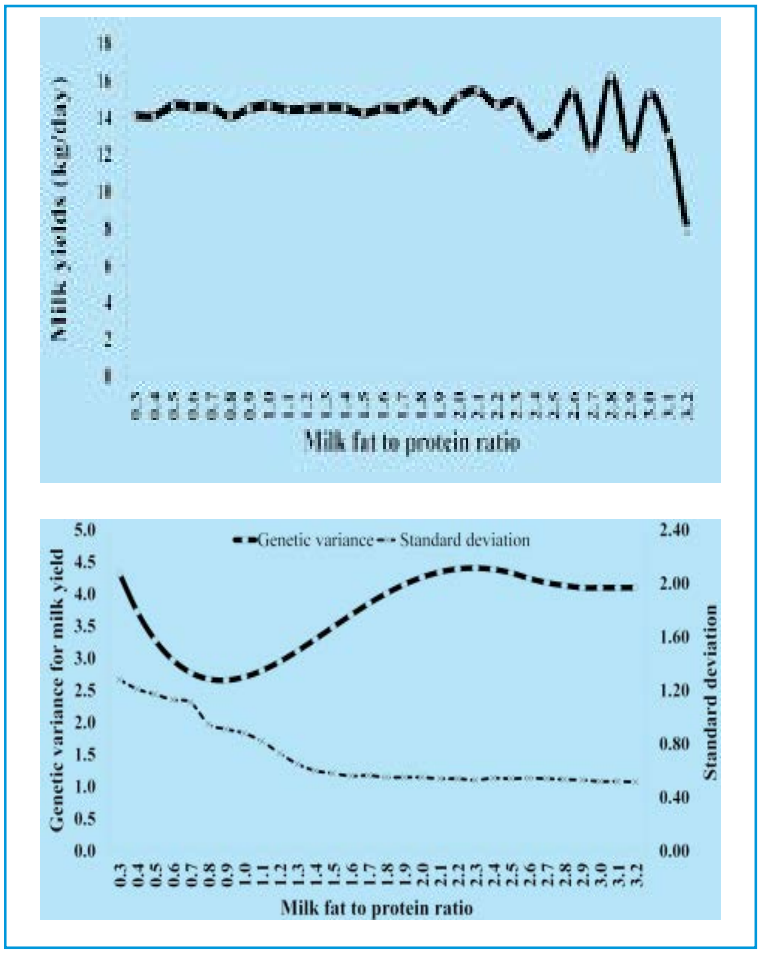

Figure 2 Average milk yield (a) genetic variance for milk yields at different milk fat to protein ratio (FPR) (Puangdee et al., 2017)

overlapping regions between LPL, DO, and FM305 traits. Previous study reported QTL for trait in this study containing 867 QTL for length of productive life found on every chromosome, 10 QTL for day open found on BTA2, 4, and 18. 305-days milk yield found 26 QTL on BTA3, 4, 5, 6, 7, 12, 13, 14, 16, 18, 19, 23 and 29 (*http://www.animalgenome.org/cgi-bin/QTLdb/BT/index*).

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Evaluation of infestation level of cattle by the tick *Rhipicephalus microplus* in New-Caledonia : Test of a new assessment grid

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Summary

The cattle ticks represent a particularly important danger for ruminant production, because of the losses they directly cause, or because of the infectious diseases they are associatedwith. In New Caledonia, as in other tropical regions, the tick *Rhipicephalus microplus* represents a real plague, leading to very important losses in cattle herds. Moreover, since a few years, the efficiency of acaricide treatments classically used mark time and resistance of the parasites becomes widespread. So, the development of alternative methods of control of the infestation by the ticks is nowadays essential. Among these, the identification of more resistant lineages of cattle to the ticks, or the culling of the most sensitive animals, are interesting tools to decrease the impact of the ticks in the herds. The evaluation of the level of individual infestation represents therefore a particularly interesting tool for the herd survey.

An assessment grid of the individual infestation by the ticks was worked out in New Caledonia, and applied periodically, in several herds. This semi quantitative grid allows a relatively fast and precise evaluation of the level of infestation, by taking into account the number of semi- and engorged females and the importance of the infestation by the immature stages. The number of semi- and engorged females on one side of the body is either counted, or estimated according to a classification in 7 classes (from 0 to more than 100 ticks), according to the ease of the observation of the ticks on the animals. For the immature stages, the observations are realized in three physical locations of preferential infestation by the ticks (tie of the tail, perineum, neck), following a classification in 5 classes in each location. Finally, these notes are combined in a score of degree of infestation, which varies in a continuous way from 0 to more than 100. This score follows a Poisson distribution, the most common statistical distribution in parasitism phenomena.

Various applications are in progress on the field in New Caledonia. First of all, the evaluation of the infestation by the breeders to identify the most infested individuals is a useful management tool. It allows, after several counting, to identify animals most regularly infested in order to cull them first. The identification of resistant lineages of cattle to the ticks requires a more important implication of the breeders with the realization of regular counting and the follow-up of the genealogies of animals. Such

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a study started in New Caledonia, on about 300 individuals of known filiation. Finally, this method allows to compare the degree of infestation of the animals of various breeds.

The method tested in New Caledonia is operational. Its application in the herds represents a usable additional tool in alternative strategies of fight against the ticks. It also interests the breeders in the tropical regions who face the danger of the tick *Rhipicephalus microplus*.

Keywords: cattle ticks, susceptibility, resistance, survey.

Introduction

The ticks of the cattle represent a danger particularly important for herbivores, because of the losses which they cause directly, or by the diseases with which they are associated. In New Caledonia, as in other tropical regions, the tick *Rhipicephalus microplus* causes very heavy direct losses in the cattle herds. Since a few years, the efficiency of the current acaricides decreases, and the phenomena of resistance of the parasites to the products become widespread. So, the implementation of alternative methods of control of the infestation by the ticks is today essential. Among these, culling of the most sensitive individuals and the identification of more resistant lineages of cattle are interesting tools to decrease the impact of the ticks. The evaluation of the individual level of infestation is then a tool of particularly useful follow-up. A notation grid of the individual infestation level of the cattle by the ticks was worked out, and is applied for more than three years, in three breeding herds in New Caledonia.

Material and methods

This semi quantitative grid allows a relatively precise evaluation of the infestation, by taking into account the number of engorged females, and the intensity of the infestation by the immature stages. The number of engorged females on one side of the body is either counted exactly, or estimated according to a classification in 7 classes: (0 ;]0 ;10] ;]10; 20] ;]20 ;30] ;]30 ;50] ;]50 ;100] ; >100, according to the ease of the observation of the ticks on the animals. A score of adult stage infestation is then assigned, corresponding to the number of adult ticks counted, or to the median value of the class of infestation. For the immature stages, the observations are realized in three physical locations of preferential infestation by the ticks (tie of the tail, perineum, neck), following a notation in 5 classes in every location (0 ; 1 ; 2 ; 3 ; 4). A score of infestation by the immature stages is assigned, which is the sum of the scores attributed at each of the three locations, multiplied by 10. In the end, average of both scores is calculated to establish an average score of degree of infestation, which varies in a continuous way from 0 to more than 100.

In total, we obtain 1145 scores of infestation, on 469 individuals (from 1 to 8 observations by animal), observed during 38 visits, between August, 2014 and January, 2018. These animals belong mainly to the breed Charolais (621 notations on 201 individuals) and Limousine (368 notations on 169 individuals), but also in other bovine breeds (156 notations on 99 individuals). Besides, we obtain the pedigree of each of Limousin and Charolais cattle.

The final scores underwent a log transformation, with the aim of the realization of variance analyses, by means of the software SAS®. To assess the repeatability of the measure, we used the MIXED procedure, with a model including the group of contemporary (defined by the herd and date of measure), To study the variability

within each main breed (Charolais and Limousin), the statistical models included the direct effects of the herd, the season, the sex, the age and the random effect of the father of the animal.

Main results

This evaluation grid may have various applications in New Caledonia. First of all, the evaluation of the infestation by the breeders themselves is a tool particularly interesting for the management of the herds, because it allows assessing if all or part of the herd has to be treated against the ticks. The realized notations show that the average score of infestation of a herd can vary from 3 to 92 (average 28 +/- 21). Furthermore, 21% of the individuals carry 52% of the total parasitic load (adults and immatures) and 80% of the adult female ticks, while 10% are not infested by any stage, and 32% carry only immature stages (Figure 1).

The application of this grid also allows identifying animals most regularly infested, in order to cull them first. Indeed, on all the measures, the repeatability is about 0.27, what shows that these measures could be a good indicator of the susceptibility of the animals. An Australian study already showed that the reform of the 15% most sensitive animals allowed reducing by 7 the global infestation of the herd in 15 years (Frisch *et al.*, 2000).

Besides, for Limousin and Charolais animals, the analyses intra breed showed that the random effect of the sire is very significant (p=0.018 in Limousin and $P<0.0001$ in Charolais). The average levels of infestation vary, between the extreme “families”, from 3 to 28 in Limousine breed, and from 13 to 49 in Charolais (Figure 2). These first results give encouraging perspectives for the selection of animals onto this criterion.

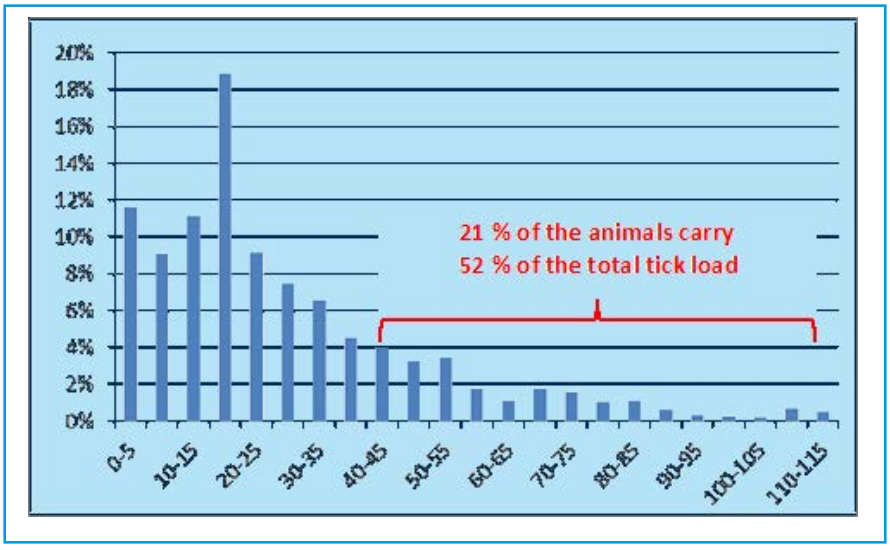

Figure 1. Distribution of tick load between animals.

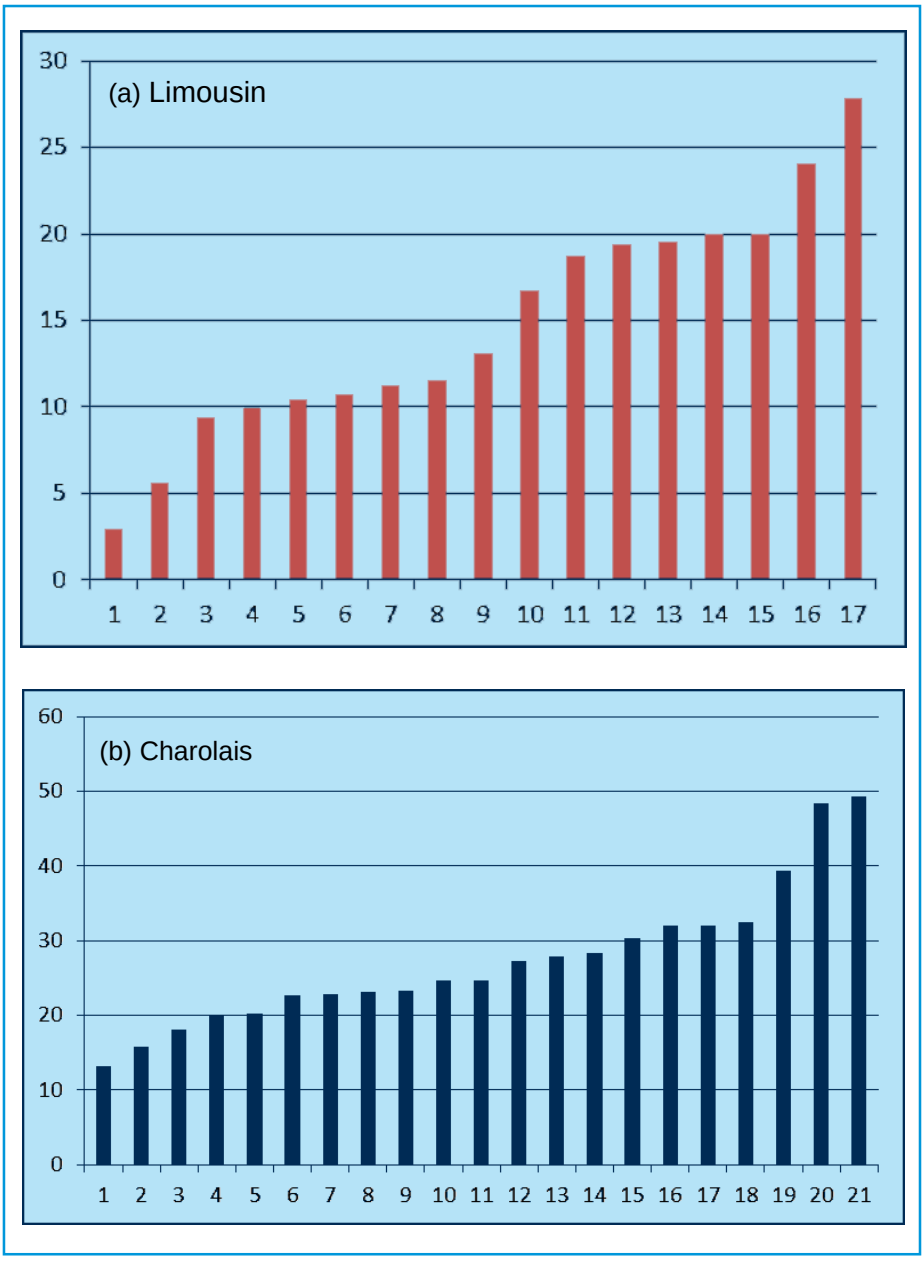

Figure 2. Ranking of the sires according to the infestation level of their offsprings, in Limousin (a) and Charolais (b) breed (for sires with more than 10 observations).

This method also allows to compare the degree of infestation of various breeds and to estimate their level of sensitivity or resistance against the ticks in a given context. On a small studied sample (161 animals of 8 breeds), the breeds Charolais and Limousine appear the most sensitive breeds, with levels of infestation respectively 10.6 times higher and 6.0 times higher than the Brahman breed, which is the most resistant. Droughtmaster, Senepol and Belmont Red breeds, and the crosses of Brahman with Charolais (Charbrais) or Limousin (Bramousin) present intermediate levels of infestations, between 1.4 times and 2.4 higher than Brahman (Table 1).

Table 1. Level of infestation according to the breed.

Breed	Ln(score+1)	Std Err	Score	OR	Diff.[1]
Brahman	1.05	0.22	2.9	1.0	a
Charbrais	1.37	0.45	3.9	1.4	ab
Belmont Red	1.58	0.25	4.9	1.7	b
Brahmousin	1.69	0.33	5.4	1.9	b
Senepol	1.75	0.25	5.7	2.0	b
Droughtmaster	1.93	0.31	6.9	2.4	b
Limousin	2.85	0.18	17.3	6.0	c
Charolais	3.41	0.24	30.3	10.6	d

[1] Different letters indicate a significant difference between breed (P<0.01).

Conclusions and perspectives

The method raised and tested in New Caledonia is operational. Its application must be pursued to validate the first results, in particular to estimate the genetic parameters of this criterion. It represents however an easily usable tool in cattle management, as an alternative strategy for the control of tick infestation, in tropical regions confronted with the tick *Rhipicephalus microplus*.

This rapid assessment grid can now be used to develop new research. The hair length is now recorded during tick counts in order to study a possible correlation between hair length and tick burden in Limousine and Charolais breeds. It can also be used as reference technique for the development of new way of research in tick resistance phenotypes, like skin test or analysis of blood parameters. At last, it will be an unavoidable tool in the search of tick resistance genes especially for these European *Bos taurus* breeds.

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Genetic and phenotypic parameters for feed and water efficiency in Senepol cattle

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Summary

The sustainability of beef production in the world demands the identification and selection of efficient animals that can produce more products with fewer inputs. Feed accounts for around 50-70% of variable costs of beef cattle systems, depending on the level of intensification adopted. Water has been traditionally considered an inexpensive, readily available, and renewable natural resource. However, growing concerns about the availability of drinkable water have increasingly pushed pressure on livestock production, especially cattle. Thus, genetic and phenotypic parameters were estimated for feed and water efficiency in Senepol cattle in order to evaluate their use as selection criteria. Records on 587 Senepol heifers, involved in performance tests, were used. Traits studied included residual feed intake (RFI), residual water intake (RWI), average daily feed intake (ADFI), average daily water intake (ADWI) and average daily gain (ADG). Individual daily feed and water intake records were collected over a 70-day period, using electronic feed and water bunks developed by Intergado Ltd. The ADG was calculated dividing the total weight gained during the test by its duration. A linear regression model of ADFI on metabolic weight (mean weight$^{0.75}$) and ADG was fitted, within each test edition. RFI was calculated as ADFI minus that predicted using the regression equation. The same was performed for calculating RWI by using ADWI instead of ADFI in the linear regression model. Genetic (co)variances were estimated using two-trait animal models and software AIREMLF90. Direct heritability estimates for RFI, RWI, ADFI, ADWI and ADG were 0.12 ± 0.10, 0.39 ± 0.12, 0.23 ± 0.11, 0.47 ± 0.12 and 0.15 ± 0.09 (averaged across all analyses), respectively. RFI was genetically (r_g = 0.50 ± 0.65) and phenotypically (r_p = 0.37 ± 0.04) correlated with RWI. Both RFI and RWI presented phenotypic correlations near to zero with ADG (r_p = -0.11 ± 0.05 and -0.09 ± 0.05, respectively). Genetically, RFI was not correlated (r_g = 0.06 ± 1.12) with ADG, whereas RWI was (r_g = 0.45 ± 0.79). The correlations between the pairs RFI-ADFI and RWI-ADWI were all positive (r_g = 0.68 ± 0.91, r_p = 0.78 ± 0.02; and r_g = 0.90 ± 0.11, r_p = 0.84 ± 0.01, respectively). ADFI was positive correlated with ADWI (r_g = 0.75 ± 0.41, r_p = 0.57 ± 0.03), and both traits presented similar correlations with ADG (r_g = 0.61 ± 0.77, r_p = 0.28 ± 0.04; and r_g = 0.70 ± 0.69, r_p = 0.29 ± 0.04, respectively).

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Genetic improvement for feed and water efficiency in Senepol cattle can be achieved through selection. Genetic progress for water efficiency is expected to be superior to the one for feed efficiency. Feed intake and efficiency can be genetically improved by selecting animals for water intake and efficiency.

Keywords: beef, correlation, heritability, residual feed intake, residual water intake, selection.

Introduction

Increasing food production for the growing human population of a constraining land base will require greater efficiency of production (Berry and Crowley, 2013). With limited resources available for production, there is a need to identify and select for efficient animals that can produce more product with fewer inputs (Ahlberg *et al.*, 2017).

Feed accounts for around 50-70% of variable costs of beef cattle systems, depending on the level of intensification adopted. Hence, feed efficiency, undoubtedly, has a major role to play in increasing production efficiency (Berry and Crowley, 2013).

Water has been traditionally considered an inexpensive, readily available, and renewable natural resource (Brew *et al.*, 2011). However, with the increasing demand for animal products in the coming decades, balancing animal productivity with water use will require a concerted effort among producers, scientists, agroindustries, and consumers to reduce the risks associated with animal water demand and scarcity (Palhares *et al.*, 2017). According to Nardone *et al.* (2010), the efficiency of water utilization will be the primary mission necessary to achieve sustainability of animal agriculture.

The Senepol breed was developed from the beginning of the twentieth century on the Virgin Island of Saint Croix as a tropically adapted taurine breed. Since the arrival of the first animals in Brazil in 2000, the population has increased its census considerably. Considering only taurine breeds with semen produced in Brazil in 2014, the Senepol breed was surpassed only by the Angus breed (ASBIA, 2015).

Thus, genetic and phenotypic parameters were estimated for feed and water efficiency in Senepol cattle in order to evaluate their use as selection criteria.

Material and methods

Records from 587 Senepol heifers (*Bos taurus taurus*), progenies of 61 sires and 264 dams, were used. The pedigree contained 1,965 animals. The data were obtained from a compilation of eight commercial performance tests performed on Grama Farm, Pirajuí, São Paulo, Brazil (21º 59' S; 49º 27' W), between 2014 and 2016. The animals started the tests with an average weight of 397 ± 52 kg and age of 520 ± 59 days.

Animals were housed in collective pens, where individual daily feed and water intake records were collected over, approximately, a 70-day period, using Intergado® System (Intergado® Ltd, Contagem, Minas Gerais, Brazil). For more information about Intergado® System, see Chizzotti *et al.* (2015) and Oliveira Jr *et al.* (2017). Prior to the tests, the animals were allowed to adapt to the diet and facilities for a minimum period of 14 days. The animals had ad libitum access to diet and water. The feed composition of the diet offered was modified over the tests, but was equivalent in energy and protein content, with 2.64 Mcal of metabolizable energy and 14% of crude protein in dry matter basis (DM).

The studied traits included average daily gain (ADG – kg d^{-1}), average daily feed intake (ADFI – kg DM d^{-1}), average daily water intake (ADWI – L d^{-1}), residual feed intake (RFI – kg DM d^{-1}) and residual water intake (RWI – L d^{-1}). The ADG was calculated dividing the total weight gained during the test by its duration. A linear regression model of ADFI on mid-test metabolic body weight (mid-test body weight$^{0.75}$) and ADG was fitted (Koch *et al.*, 1963), within each test edition. RFI was calculated as ADFI minus that predicted using the regression equation. The same was performed for calculating RWI by using ADWI instead of ADFI in the linear regression model.

The contemporary groups were defined as test edition and farm of origin of the heifer. Records outside the interval of ± 3.0 standard deviations from the mean of the contemporary group were eliminated. Only animals with valid records for all five traits studied were kept. Animals from contemporary groups with less than five individuals were also discarded. Table 1 shows the data structure and descriptive statistics of the traits.

The (co)variance components were estimated by the restricted maximum likelihood method under a two-trait animal model using the AIREMLF90 program (Misztal *et al.*, 2002). The model included random direct additive genetic effects, the fixed effects of contemporary group and age of animal nested in the respective contemporary group as a covariate (linear effect). Direct heritability estimates for each trait were obtained by averaging across all two-trait analyses.

Results and discussion

The direct heritability estimates for the studied traits ranged from 0.12 to 0.47 (Table 2). These results, which are pioneers for Senepol cattle, indicate that selection can be used for increasing feed and water efficiency. However, genetics gains for ADWI and RWI are expected to be quite superior to those for ADFI and RFI due to the significant differences in heritabilities. Berry and Crowley (2013) performed a meta-analysis of genetic parameters for feed efficiency in beef cattle and reported higher values than the ones found in the present study (pooled heritabilities of 0.40 ± 0.01 for ADFI and 0.33 ± 0.01 for RFI). No study was found in the literature with genetic parameters for water intake and efficiency in cattle.

RFI was genetically and phenotypically correlated with RWI (Table 2). These results indicate that selection for animals with better feed efficiency could also lead to genetic progress for water efficiency. Despite water is often thought of as an irrelevant factor in beef cattle production, increasing water efficiency could be strategic, especially, in a long-term context. According to Nardone *et al.* (2010), all effects of global warming on water availability could force the livestock sector to establish a new priority in producing animal products that need less water. Both RFI and RWI presented phenotypic correlations (r_p) near to zero with ADG (Table 2). Genetically (r_g), RFI was

Table 1. Description of the final data set of studied traits.

Trait[1]	Mean ± SD	Number of animals with records	Number of contemporary groups
ADG (kg d^{-1})	0.87 ± 0.21	587	51
ADFI (kg d^{-1})	7.49 ± 1.16	587	51
ADWI (L d^{-1})	24.68 ± 3.99	587	51
RFI (kg d^{-1})	0.00 ± 0.79	587	51
RWI (L d^{-1})	0.00 ± 2.96	587	51

[1]ADG, average daily gain; ADFI, average daily feed intake in dry matter basis; ADWI, average daily water intake; RFI, residual feed intake in dry matter basis; RWI, residual water intake.

Table 2. Heritability (diagonal), phenotypic correlation (below the diagonal) and genetic correlation (above the diagonal) estimates for the studied traits.

Traits[1]	ADG	ADFI	ADWI	RFI	RWI
ADG	**0.15 ± 0.09[2]**	0.61 ± 0.77	0.70 ± 0.69	0.06 ± 1.12	0.45 ± 0.79
ADFI	0.28 ± 0.04	**0.23 ± 0.11**	0.75 ± 0.41	0.68 ± 0.91	0.57 ± 0.40
ADWI	0.29 ± 0.04	0.57 ± 0.03	**0.47 ± 0.12**	0.39 ± 0.90	0.90 ± 0.11
RFI	-0.11 ± 0.05	0.78 ± 0.02	0.30 ± 0.04	**0.12 ± 0.10**	0.50 ± 0.65
RWI	-0.09 ± 0.05	0.29 ± 0.02	0.84 ± 0.01	0.37 ± 0.04	**0.39 ± 0.12**

[1] ADG, average daily gain; ADFI, average daily feed intake; ADWI, average daily water intake; RFI, residual feed intake; RWI, residual water intake.
[2] standard error.

not correlated with ADG, whereas RWI was (Table 2). Berry and Crowley (2013) reported similar estimates (pooled) for r_p and r_g between RFI and ADG. For r_p, these authors found a range of -0.06 to +0.04; while for r_g, a range of -0.15 to +0.53. Guimarães *et al.* (2017) also estimated r_p close to zero between RFI and ADG for Senepol cattle. Kennedy *et al.* (1993) pointed out that although RFI is phenotypically independent of the component traits, except ADFI, it is not genetically independent. As RWI is calculated similarly to RFI, the same could be said for this trait.

The r_p and r_g between the pairs RFI-ADFI and RWI-ADWI were all positive (Table 2). Berry and Crowley (2013) and Guimarães *et al.* (2017) found results of similar magnitude and sign of the ones estimated in the present study. ADFI was highly positive correlated with ADWI (Table 2), both genetically and phenotypically. It suggests that ADWI could be used to estimate ADFI in cattle what would be useful since measuring the former is easier and cheaper than the latter. This would be especially advantageable in grazing systems where evaluating ADFI in large scale is not yet a feasible alternative. ADG presented similar correlations with both intake traits (Table 2), corroborating the findings of Berry and Crowley (2013).

Conclusions

Genetic improvement for feed and water efficiency in Senepol cattle can be achieved through selection. Genetic progress for water efficiency is expected to be superior to the one for feed efficiency. Feed intake and efficiency can be genetically improved by selecting animals for water intake and efficiency.

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First national recording of health traits in dairy cows in the Czech Republic

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Summary

This study presents a basic description and analysis of 20 common health disorders/diseases monitored in 289 802 dairy cows in the Czech Republic. The data were provided by farmers on a one-time basis from 1 183 herds and were collected between July 2015 and June 2016. In 55 % of cows, no disorders/diseases were treated, while in 45 % of cows, at least 1 of 20 monitored disorders/diseases was recorded. The most frequent disease was mastitis (19.8 % treated lactations, i.e., lactational incidence risk, *LIR*), followed by metritis (*LIR* 11.3 %) and foot and claw diseases (*LIR* 11.0 %). Treatment of metabolic disorders was rather seldom (*LIR* 1-3.2 %). Not all farms recorded all diagnoses; while almost 90 % of farmers reported the incidence of mastitis, less than 50 % of them recorded the incidence of metabolic diseases. Additionally, the comparison of the incidence of foot and claw diseases with studies based on hoof trimmer records showed possible under-reporting. Despite those limitations, our basic analysis is important for intended genetic evaluation of health traits and gave us an idea about the health conditions and disease recording in Czech dairy cattle.

Keywords: cattle, lactational incidence risk, mastitis, reproduction disorders, metabolic disorders, foot and claw diseases.

Introduction

Health traits in dairy cattle attract attention not only for their influence on farm profitability and production efficiency but also for the impact of the diseases and their veterinary treatment on animal welfare, food safety and quality (Egger-Danner *et al.*, 2013). Growing attention is also paid to the impact of drugs used in veterinary medicine, such as the spread of antibiotic-resistant strains of bacteria that can negatively impact human health (Egger-Danner *et al.,* 2014).

Health traits have generally low heritability; however, there is a possibility that health traits can be selected, as they show sufficient genetic variability and that genetic improvement can occur; however, for this purpose, we need a large amount of reliable data (Heringstad and Osteras, 2013).

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The incidence of diseases in Czech dairy cattle is high, where more than 80% of culling is due to health reasons. The most frequent reasons for culling in 2016 were reproduction disorders (21.5%), dystocia (10.1%) and udder diseases (8.5%); the remaining 43.9 % were other unspecified health reasons (Kvapilík *et al.,* 2017). According to Bauer *et al.* (2016), Czech farmers use approximately 20 different farm management software packages for recording different farm data, including data on health situations (61% of farmers), veterinary treatments and drug applications (59% of farmers). Based on the Law of Veterinary Care, the farmers are obliged to keep records about the use of medication bound by a prescription and to keep records of the reason for those diagnoses. These records have not been standardized, nor have they been gathered or stored in a joint database yet, which would enable their processing and utilization for the purposes of genetic evaluation and selection. Our aim was to describe the health conditions and to gather and evaluate data on the incidence of 20 common diseases/disorders in Czech dairy cows over one year.

Materials and methods

The data on disease incidences were provided retrospectively by farmers via electronic survey. The observation period covered the period from the 1st of July 2015 until the 30th of June 2016. The survey contained information on the identification numbers of farms and cows, health status of each cow (treated/not treated), diagnosis (chosen from 20 common diseases listed in Table 1), use of antibiotics and their dose and way of application. Other data (breed of cow, date of calving, parity, milk yield) were filled in from the database of lifelong performance.

The first occurrence/incidence of each disease (or group of diseases in case of foot and claw) during lactation, was coded as 0- not treated, 1- treated (lactational incidence risk – *LIR*). Repetitions of the same diagnosis during the same lactation period were not considered.

Data were edited, so that only lactations which started with calvings from July 2015 until 7 days (for dystocia, parturient paresis, retained placenta), 20 days (for metritis) or 60 days (for other diseases) before the end of observation period were included in the evaluation. To differentiate the farms with incomplete data, each herd with a minimum of 20 lactations was required to have at least 1 record of disease/group of diseases. For smaller farms (<20 lactations) no minimum *LIR* was required. For editing of the database and basic calculations we used *SAS 9.4*.

Results and discussion

The data set contained information from 1 183 herds and 289 802 cows, which account 78 % of the total number of dairy cows in the Czech Republic. The distribution of breeds was as follows: 138 643 Holstein (H100; 48%), 64 304 of Czech Pied cattle (C100; 22%), and the rest were crossbreds (28%) or other dairy breeds (Ayrshire, Braunvieh, Montbéliarde, Normande, Red Holstein; 2%). A total of 130 244 cows (45%) had at least 1 diagnosis, while 159 558 (55 %) cows were stated as “not treated”. Description of edited data structure and *LIR* of diseases are presented in Table 1. The most frequently treated disease was mastitis (*LIR* = 19.8 %), followed by metritis (*LIR* = 11.3%) and groups of foot and claw diseases (*LIR* = 11.0%).

Lactation incidents of mastitis and reproductive disorders were comparable to frequencies stated in other national studies (Govignon-Gion, *et al.,* 2012, Egger-Danner *et al.,* 2012, Vukasinovic *et al.,* 2017, Zwald *et al.,* 2004). On the other hand, *LIR* of foot and claw diseases was rather low. As the study of Krpálková *et al.* (2016) showed,

Table 1. The structure of edited data and lactational incidence risk LIR of monitored diseases.

Disease / disorder	No. of herds	No. of lactations	No. of treated lactations	% of treated lactations
Mastitis	1 026	209 147	41 505	19.8
Dystocia and/or retained placenta	815	192 741	9993	5.2
Metritis	802	191 438	21 549	11.3
Endometritis and/or cystic ovaries	810	165 198	17 627	10.7
Milk fever	581	136 877	2 290	1.7
Other recumbency	357	54 405	521	1.0
Primary ketosis	348	63 373	1704	2.7
Subclinical primary ketosis	337	58 180	1830	3.2
Secondary ketosis	263	37 468	564	1.5
Foot and claw[1]	907	187 450	20 673	11.0

[1]group of 9 diseases/disorders including lameness, interdigital hyperplasia, claw ulcer, toe ulcer, typical sole ulcer (Rusterholz), sole ulcer in atypical location, white line disease, interdigital phlegmon, digital dermatitis.

the frequency of claw diseases often exceeds 50%, and better care combined with more control of legs leads to higher recording of diseases. Additionally, van der Spek *et al.* (2013) examined hoof trimmer records and found that more than half of the scored cows had at least one claw disorder. The same authors pointed to the importance of trimming status, which is a heritable trait correlated with claw disorders and therefore an interesting trait to include in the genetic evaluation. Additionally, *LIR* of metabolic diseases was rather low, compared to the meta-analysis of Pryce *et al.* (2016), where the median incidence of ketosis was 3.3 %, the incidence of subclinical ketosis was up to 34 %, and the median incidence of milk fever was 2.8 %.

The quality of data is determined by their objectivity, reliability and validity. In retrospective studies, the quality of on-farm documentation plays a key role. According to Pryce *et al.* (2016), many farm computer systems still do not ensure that data captured for health traits are consistent and accurate, and thus there is a potential for underestimated/over-reported incidences. Reporting of disease is more likely when it is treated by medication, where evidence is mandatory. As mentioned by Egger-Danner *et al.* (2012), the main reasons for incomplete data were missing documentation, a fact that farmers emphasize different health aspects at different times, or situations, when not all farms record all diagnoses. The last reason was also present in our study, where almost 90 % of farmers reported the incidence of mastitis, but less than 25 % of them recorded the incidence of metabolic diseases except for milk fever, which was reported by almost 50 % of farmers.

A clear and unambiguous definition of diagnosis is very important. It is not unusual that the farmer describes the symptoms, applies the medication, but hesitates to name the disease. Additionally, Pryce *et al.* (2016) mentioned under-reporting of metabolic diseases due to differences in producer interpretation of symptoms. Likewise, Krpálková *et al.* (2016) noted the possibility of bad recognition and consequent under-reporting of the incidence of foot and claw diseases by farmers, especially in large herds.

Generally, differentiating between farms with incomplete recording and farms with very low incidence rates is a challenge (Egger-Danner *et al.,* 2012), especially in small farms. Basic measure for data validation is therefore their careful editing, which consisted mainly in determination of their minimum incidence per herd, year and/or

season (Egger-Danner *et al.*, 2012, Vukasinovic *et al.*, 2017). Only data from farms with regular and complete registration of diagnoses should be included in the genetic analysis.

Conclusions

This study presents the first step on the way to the national recording of health traits in dairy cattle and for subsequent use of such data for genetic parameter estimation and genetic evaluation. Despite the limitations, our analysis provides valuable information for future processing and validation of data and identifies the weak points that could negatively affect the recording and reporting of incidence of diseases/disorders in cattle populations.

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Use of plausibility checks in milk recording organisations

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Summary

This survey looks at the plausibility checks used at data capture of a few key data sets in milk recording organisations around the world. The aim of these checks is to keep the data integral, with no internal discrepancies. We found that the most important plausibility checks are almost universal, but in the more novel methods to capture data the possibilities to check the data for integrity are not yet fully utilised.

Key-words: milk recording, data capture, plausibility check.

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Introduction

The ICAR dairy cattle milk recording working group has the responsibility to keep the relevant ICAR Guidelines up to date. In order to know how the milk recording organisations around the world are in fact working, the group has conducted studies on recording practices and on the management of milk recording during the last few years (Bucek *et al*. 2015, Bucek *et al*. 2016, Kyntäjä *et al*. 2015, Zottl *et al*. 2015, Zottl *et el*. 2016). After these studies, we still perceived a need for more data on how the integrity of data is managed.

The survey

The survey was done on a survey tool website, and the participants were invited by email to fill in the form. The addresses were obtained from the ICAR membership list website. We received 25 acceptable answers from 22 very different countries, which gives a good overview on the overall situation. Some characteristics of the respondent organisations are given in tables 1 to 4.

Table 1. Size of the respondent organisations.

Number of recorded cows	Share of respondents, %
Less than 100,000	32
100,000 – 249,000	16
250,000 – 499,000	36
More than 500,000	16

Table 2. Organisation of data processing.

Organisation of data processing	Share of respondents, %
We process our data ourselves, and for no other organisation	40
We process our data ourselves, and also for other organisations	16
We outsource data processing as the sole client	8
We outsource data processing to a company that has several clients	36

Table 3. The most important data capture method in the organisation.

Data capture method	Share of respondents, %
Paper sheets	24
Specialised device	12
PC programme	32
Webpage or mobile app	24
Direct data transfer	8

Table 4. Person responsible for data capture.

Number of recorded cows	Share of respondents, %
Farmer or his representative	20
Technician	40
Shared responsibility	40

Plausibility checks for the most important milk recording events

The events chosen for this survey were calving, milk recording and milk analysis, i.e. those events that are the basis for yield calculation and allow to make the necessary connections for pedigree purposes. In many countries calving data is not strictly speaking gathered by the milk recording organisation, but nevertheless it is a crucial element in yield calculations and was thus included.

Calving

The respondents were asked who registers calvings in their respective country and how data are synchronised between milk recording and an eventual government register. The existence of a government register was reported by 88% of all respondents. Farmers record calvings into the government register in 80% of the respondent organisations, and in 72% also for milk recording. In 52% of the organisations, one record from the farmer is enough for both milk recording and government purposes. Technicians record calvings into the government register in 8% of the respondent organisations, and in 28% of the organisations for milk recording purposes (Table 5).

Plausibility checks for calving are quite similar across the respondent organisations, with more than 90% of respondents using the first seven checks asked. Most often these checks are performed when the data enters the milk recording database, but especially some of the simpler checks are done already at the capture device.

Herd recording

With herd recording, the checks are used for making sure all data from the herd is integral and captured. None of these checks were used in more than 76% of all respondent organisations (Table 6).

Individual cow recording

Individual cow milk yields are usually checked with a comparatively identical set of plausibility checks in the respondent organisations. Depending on the check, these are done at the capture device or at the database entrance. With direct data transfer, there are some additional possibilities for data checks that are not yet fully in use. Direct data transfer is in use in 88% of the respondent organisations (Table 7 and 8).

Milk analysis

Milk analysis is largely done in separate laboratories who deliver the results through direct data transfer. For milk recording purposes, the results often have to be corrected in order to calculate a 24-hour average content and daily yields of solids. Most respondents correct fat contents either based on milking times only or on a more complex formula. A surprisingly high number of respondents also reports adjusting the analysed protein contents (Table 9).

The most popular plausibility checks done for milk analysis results are there to make sure we know which cow the analysis belongs to, and that the milk analysed is normal. Most organisation check the latter by fat and protein only, while some use or more sophisticated model (Table 10).

Table 5. Plausibility checks for calving.

Plausibility check	Total (%)	Where is the check done?		
		At capture device (%)	At transfer from farm (%)	At entrance to database (%)
Cow belongs to herd	96	48	8	40
Cow is female	96	48	8	40
Capture delay	92	44	0	48
Age of cow	92	28	8	56
Calving interval	92	36	0	56
Sire from AI	92	40	0	52
Calf breed	92	36	0	56
Days from service	88	32	0	56
Days dry	72	36	0	36
Synch with government	64	12	4	48

Table 6. Plausibility checks for herd recording.

Plausibility check	Share of respondents (%)
All registered cows are listed for recording	76
All milked cows have milk or an excuse	76
All samples have a milk weight	76
All milked cows have a sample	64
All milk weights have a sample	64
Total milk corresponds to bulk tank	24

Table 7. Plausibility checks for individual milk yields in a 2x setting without data transfer.

Plausibility check	Total (%)	Where is the check done?	
		At capture device (%)	At entrance to database (%)
Cow belongs to herd	96	64	32
Calving to recording	96	40	56
Recording interval	88	40	48
Daily yield within limits	88	44	44
Cow not already recorded	84	48	36
Cow not recorded as dry	84	48	36
Comparison to previous yield	64	20	44
Evening vs. morning milk	52	28	24

Table 8. Plausibility checks for individual milk yields in direct data transfer.

Plausibility check	Total (%)	Where is the check done?	
		At transfer programme (%)	At entrance to database (%)
Cow belongs to herd	76	40	36
Calving to recording	76	28	48
Recording interval	76	28	48
Daily yield within limits	76	28	48
Cow not already recorded	72	28	44
Cow not recorded as dry	72	28	44
Sufficient number of milkings	64	28	36
Comparison to previous yield	52	20	32
Capture starts with complete milking	48	28	20
Milk flow	28	12	16
Milk secretion rate	28	8	20

Table 9. Adjustment of analysed parameters for milk recording purposes.

Correction in place	Share of respondents (%)
Fat according to milking times	44
Protein	44
Fat according to a more complex correction	40
Cells	20
PAG	4

Table 10. Plausibility checks for milk analysis results.

Plausibility check	Share of respondents (%)
Vial ID is connected to a cow	88
Cow has a milk yield	88
Fat content within limits	88
Protein content within limits	84
Cow belongs to herd	80
Calving to sampling, days	52
Lactose content within limits	44
Cell count within limits	44
Urea within limits	28
pH within limits	12
Freezing point within limits	12

Conclusions

The survey shows that even though the milk recording organisations are very diverse as to size and technological advancement, the most important plausibility checks usually seem to be quite similar. Some checks are more dependent on the local situation. There are some possibilities with the more novel data capture methods that could be used wider than they are now.

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See the change, be the change: Overcoming roadblocks to innovation in the New Zealand beef cattle breeding industry

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Summary

This paper will analyse the challenges facing beef breeds and breed societies in New Zealand today, and identify ways the industry can more effectively embrace innovation moving forward. The paper will also address New Zealand's unique position as a microcosm of industry issues worldwide and suggest how New Zealand Breed Associations can help overcome roadblocks to industry innovation.

Key-words: Genomics, performance, beef, breed, society, collaboration, technology, infrastructure, commercialisation, phenotype, genotype, pedigree, purity.

Introduction

While most genetic innovations are specific to the breeds and industries of individual countries, there has been widespread, global move towards innovation in this field fuelled by remarkable advancements in technology over the last decades. Genomic advancements have made a marked impact on the beef industry worldwide, but while the technology has been commercially around for over ten years, the New Zealand beef market has only recently begun to apply genomic prediction.

Agriculture is a lifeblood industry for our economy, and yet, the beef industry in our country has been one of the slowest to adopt genetic innovations vital to their growth and prosperity. There are a variety of reasons for this, and most of these roadblocks still exist today. Differing perspectives among commercial farmers and breeders cause tension, misunderstanding, and lack of collaboration. The industry is facing new challenges, but our deficits are all too familiar and difficult to overcome. The industry's lack of unified vision and infrastructure magnifies these problems and obscures the way forward.

The industry needs a shared vision and strong leadership to unite parties with differing interests and to communicate the value and benefits of genetic improvements. We need a collaboration of minds that will pursue a greater good with passion and integrity. To accomplish this, we also need to integrate industry infrastructures and leverage technology to increase the effectiveness of our efforts. And we need to retain our industry talent by making the New Zealand market the most exciting place to be. To

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accomplish all of these things, it's essential to start with an honest assessment of the thoughts and practices that are holding us back from innovation, as well as realistic ways we can evolve to meet the change and thrive.

Rich industry history: asset turned liability

New Zealand is an agriculture nation. These industries are a huge part of our society, as evidenced by the impact the falling milk price had on our economy in 2014. The fact that the industry is such a crucial part of our national economy means that there is high pressure to perform predictably and profitably. The government encourages businesses to evolve and increase their capabilities, but in reality, leadership is so busy working in the businesses that they have little time to work on the businesses.

One of the contributing factors to this industry issue is the aging farmer demographic. The average age of beef breeders in New Zealand is 58—for other sectors of the larger industry that age may be even higher. While there is much to learn from experienced leaders, the temptation is to keep doing things the way they have been done for the past 40 years. In today's technological and economic climate, that is simply not an option.

The aging farmer demographic plays a big role in the industry's tendency to perpetuate the *status quo*, but it is not the only factor. Risk is a major concern for commercial and breeder farmers. Ideas, systems, and technology that have not been proven through other farmers' implementation are a risk, and they are often avoided in favour of a "sit and hold" mind-set. This cautious tendency, though understandable, is another major factor that holds back innovation. The lack of education for breeders on the topics of genotyping and genomics also contributes to the problem. It's hard to be motivated to implement change when the risks and the benefits are unclear. Without supportive industry and government bodies, clear and accessible information resources, and incentives for innovation, farmers will continue to choose the safer bet: reduced risk now with the unfortunate side effect of short-changed futures.

Tension between short-term and long-term outcomes

There is a tension between short-term and long-term priorities in our industry that must be addressed if we want to achieve survival in the present as well as prosperity in the future. Many of our major players continue to doubt the value of genetic innovation to the industry's present and future bottom line. Genetic innovations require precise data recording, and this long-term data investment is getting left by the wayside in the rush for lucrative commercialisation. The very nature of the beef market (as it is now) demonstrates this slant toward commercial rather than performance breeding in New Zealand. This movement toward commercialisation in the industry is dangerous when considered in context with the growing global market for niche beef products and the growing international competition in commercial markets.

Internationally, the average consumer income is rising—particularly in the middle class—and the middle-class demographic is growing. Mario Pezzini, Director of the OECD Development Centre, states that the size of the global middle class will increase from 1.8 billion in 2009 to 3.2 billion by 2020 and 4.9 billion by 2030. He surmises that the bulk of this growth will come from Asia—a country that will represent 66% of this global middle-class population by 2030. With this growth in the middle class, there is and will continue to be a corresponding growth in the market for organic food and quality branded meat. The New Zealand beef industry is ideally positioned to fill this niche, and it is less than ideally positioned to fill broader commercial demand.

In the commercial market, giants like the United States fill and exceed demand. Alternative forms of protein have the potential to become both formative competitors in the larger global meat market and, possibly, an answer to world hunger. Each of these tasks is beyond the reach of the New Zealand industry alone. In looking to the future, it is vital that we clarify our role in the larger global markets and pursue that role without reserve. We have the potential to improve the eating quality (EQ) of our product with the assistance of genomics and, in doing so, to build a compelling sense of authenticity and story that will appeal to the current market and outdo synthetic meats. By building a library of data, we can support the integrity and quality of New Zealand beef, connecting us with the larger story and increasing our relevance in the global market. But this potential will only be realised if we recognise and pursue it.

Most of the major players in the New Zealand beef industry still struggle to see the value of investing in genetic improvement and performance recording. The market for selling beef bulls for breeding is not very large, and a significant proportion of bulls sold are bred in herds that do not record performance. In addition, beef bull purchasers do not always recognize the value of genetically improved bulls, making it more difficult to incentivize farmers' investment in performance breeding and data recording. Breeds that do record performance and have embraced genomics are stagnating in animal numbers, and many minor breeds are getting smaller and abandoning recording altogether. Data recording has few immediate returns, but it is vitally important long-term.

The move toward commercialisation may be driven by a short-term view toward survival, but it is also affected by the complexity that exists for breeders who are pursuing genetic advancement. The implications of genomics on pedigrees and record-keeping may form an even great obstacle to their application than money matters. Early adopters of genetic innovations will face the challenge of maintaining herd book integrity in a new and largely uncharted context where errors may be linked with serious consequences.

There is also a rising conversation surrounding the legal ramifications of inaccurate or misleading herd book entries. Breed societies have an obligation in regard to the management of herd books. Any person relying on the integrity of the herd book that suffers loss due to the breakdown of its integrity has the right to sue the breed society that guaranteed the herd book and to claim for damages. Even considered in isolation, this trend towards greater accountability demonstrates the long-term value and security of investment in accurate, conscientious data recording.

The Linchpin: Collaboration

Finally, the lack of collaboration within the New Zealand livestock industry is perhaps the most significant roadblock to its implementation of genetic improvements. The industry is fragmented by factions that use dissimilar technology and processes to achieve their unique benchmarks. The lack of integration and functionality in the information infrastructure serving the beef industry makes it difficult to apply not only genetic innovations, but also technologies that would help bridge the gap.

In Ireland, the Irish Cattle Breeding Federation unified infrastructure and brought dairy and beef together. Within this federation, information is shared freely and data has been centralised to empower collaboration and keep track of animal movements from breeder all the way to consumer. All the industry's technologies talk with each another, and up-to-date data analysis guides decision-making. This collaboration, in turn, increases scientific funding in the industry, and the cycle continues. Whether or not

unification of beef and dairy under one roof is feasible or desirable in the New Zealand industry, we can learn from Ireland's admirable accomplishments: collaboration drives success, and it attracts funding.

The relationship between commercial farmers and breeders is an example of a lack of collaboration that holds the New Zealand industry back from innovation. The tension between commercial farmers and the beef seedstock farmer can not be easily defined as one farmer versus another (many breeders are both pedigree and commercial farmers in their own right), but the progressive farmers who are pursuing genetic advancement through genotyping and performance recording often become the recipients of ill feeling from those who aren't. To the exclusively commercial farmers, the performance breeder is a fat cat, or worse, a tall poppy. If the industry as a whole is to pursue innovation, or even better, the niche market, then it is important that it put the farmers who are investing in innovation and performance on a pedestal and to showcase the successes they achieve. If genetic innovation via performance recording is shown to be a step toward the future and successful, then the tension between commercial and seedstock will decrease because every facet of the industry craves longevity and success.

There is also a lack of unity among performance breeders and breed societies. PBBNZ brings 97% of the beef breed societies in New Zealand under one roof, and it is officially owned by seven of these societies. Despite this fact, there are tensions within breed societies when the interests of the members conflict with the society, or with each other. Many breeds still work in silos rather than together. Scientists, research bodies, government, service providers, breeders, commercial farmers, processors, markets, and consumers all have something to gain and something to give. In order to reap the benefits that each of these industry segments has to offer the others, we must start by sowing, by investing what we can in the effort. We need to work together and invest in technologies that support our collaboration rather than sabotaging it. This will take determination and persistence, but it can be accomplished.

Conclusion

The New Zealand beef breeder industry is a natural microcosm for the larger international industry, and it is ideally positioned to pioneer industry-wide application of genetic improvements. Kiwis are naturally very accepting of change (our country has an extensive track record for turning challenges into opportunities) and our kiwi breeders have the ability to lead the way in pedigree and performance advancements. With our unique client base, PBBNZ can play a major role in spreading innovation. We're already helping breeds integrate the technology and management techniques necessary to apply these innovations, and we understand the legal and logistical concerns surrounding purity and pedigree. In addition, the infrastructure and economies of scale the industry needs, is at the very core of our business. With our connections to breed societies, the science industry, and technology, PBBNZ could be an ideal conduit for collaboration and advancement. PBBNZ is looking to the future and seeking to champion innovation.

The New Zealand beef industry is not suited to end world hunger or to saturate the global commercial meat market, but it can play a significant role on the world stage. With the proper channelling of resources, New Zealand could feed 3-5% of the niche beef market of the world, and in doing so, gain a significant voice in the growing conversation around the integrity and quality of beef products. Pedigree and performance recording have the potential to get us there, but we can't do it alone. We need the support of the international community in our pursuit to strengthen the New Zealand beef industry. Funding, leadership, and talent are essential to these pursuits,

but too many times in the past these precious resources have passed us by. There are many ways that we, as an industry, need to evolve in order to meet the rising challenges of the day. There are many significant roadblocks. But with a vision, determination, and the support of the international community, we can innovate and, just maybe, transform our corner of the global industry.

Documenting changes in dairy breeds in the United States including genomic examination using breed base representation

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Summary

Breed composition of the US dairy population has changed over the last two decades. The changes occurred for various reasons including:

- Shifting demand for milk products.
- Increased interest in different traits.
- Promotion of breeds with little previous presence in the US.
- Desire to capitalize on heterosis.

A few producers are experimenting with rotational crossing schemes, but more are transitioning to another breed by using semen from the targeted breed for multiple generations. Domestic semen sales from Holsteins declined from 93.4% in 1996 to 85.9% in 2016. Jersey sales rose from 4.6 to 13.1% during the same time. For several decades U.S. dairy owners have had a high percentage of their herds made up of a single breed. Herds having ≥75% of the milking animals of a single breed were designated as herds of that breed. The remaining herds, coded as multiple-breed herds, were only 4% 20 years ago, but have climbed to 11.5% today, most coming from the Holstein-herd category. Allelic examination is revealing the extent to which breeds are represented in the crossbreds. The procedure is based on assembling *Purebred Reference Groups (PRG)* representing 5 individual breeds (Ayrshire, Brown Swiss, Guernsey, Holsteins, and Jerseys). The procedure, referred to as Breed Base Representation (BBR), estimates for each animal genotyped the percentages of alleles in common with those in the 5 PRGs. The BBR of the primary breed for Ayrshires and Jerseys has declined below 100% over the last decade, but the changes have been smaller for the other 3 breeds.

Key words: breed composition, breed base representation, genomics

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Introduction

Breed composition of the US dairy population changed across the last two decades. This happened for various reasons including

- Shifting demand for milk products.
- Increased attention to animal longevity and fertility.
- Promotion of a few European breeds.
- Desire to capitalize on heterosis.

A few producers have experimented with 2- or 3-breed rotational crossing schemes. However, more are transitioning to another breed by using semen from the targeted breed for multiple generations. Dairy semen sales provides an indication of these changes. Holstein semen declined from 93.4% of domestic sales in 1996 to 85.9% in 2016. Jersey semen sales rose from 4.6 to 13.1% at the same time (Table 1). Several breed associations now enroll crossbreds, not eligible previously for their traditional herd books.

It has been shown that when the identification of the sire, maternal grandsire, or the maternal great-grandsire are not reported, genomic testing with a 50K chip can reveal these in over 99%, near 97%, and 92% of the cases, respectively (VanRaden and Cooper, 2015). This confirms breeds can be identified among the ancestors, even when on-farm recordkeeping is weak or non-existent. DNA testing, although not entirely random, could reveal changes in the frequency of crossbreeding.

Genomic examination can be used to show the extent to which alleles of various breeds appear to be represented in an animal's genetic makeup (VanRaden *et al.*, 2013). The procedure is based on assembling 5 *Purebred Reference Groups (PRG)*, registered artificial insemination (AI) bulls representing the individual breeds (Ayrshire (AY), Brown Swiss (BS), Guernsey (GU), Holsteins (HO), and Jerseys (JE)). Most are recent bulls, all with milking daughters and having no other breed recorded in their 5-generation pedigree. The procedure, referred to as Breed Base Representation (BBR), estimates the percentages of alleles in common with those in the 5 PRGs for each animal genotyped.

A BBR percentage for the primary breed well below 100% reveals either crossbreeding or an outcross bloodline, but differentiating which has not been the focus of additional investigation. Animals vary and breeds have many common alleles, so assigning contributions from breed sources is not precise, i.e., actual breed contributions which sum to 100% can differ somewhat from the BBR percentages derived. The actual percentage for the primary breed could be off by 5%, or even more, due to limitations of the method.

Material and methods

Summaries were derived that illustrate the changing breed composition of U.S. dairy cattle population. These give the percentage of milking herds and cows by breed 10 and 20 years ago and today. Herds are designated as a single-breed herd if ≥75% of the milking cows are of a one breed. Otherwise, they are coded as a multi-breed herd. Summaries were derived also to reveal the percentage of cows that are designated by breed or as crossbreds separately in single- and multiple-breed herds compared to 10 and 20 years ago.

The demand to have genetic estimates for crossbreds has led to requesting more accurate information on breed composition and so BBR has become an integral part of Jersey breed programs. Therefore a detailed summary of the breed composition of

Table 1. Percentage of U.S. domestic semen sales in 1996, 2006, and 2016 by breed[1]

Year	Ayrshire	Brown Swiss	Guernsey	Holstein	Jersey	Milking Shorthorn	Total units of semen
1996	0.3	0.9	0.6	93.4	4.6	0.1	12,677,139
2006	0.2	0.8	0.2	90.9	7.6	0.1	18,709,887
2016	0.2	0.5	0.1	85.9	13.1	0.1	18,930,168

[1]*https://www.naab-css.org/file.aspx?id=3a8dab80-6ab1-49eb-8577-dcdae5469717*

Table 2. Percentage of milk recorded herds designated by breed on January 1, 1998, 2008 and 2018.

Year	AY	BS	GU	HO	JE	MS	Multiple breeds	Number of herds
1997	0.6	1.0	0.9	89.4	4.0	0.2	4.0	38,920
2007	0.5	1.1	0.7	86.5	4.8	0.2	6.2	23,005
2017	0.4	0.9	0.5	81.3	5.3	0.1	11.5	15,530

Table 3. Percentage of milk recorded cows in herds designated by the herd's breed on January 1, 1998, 2008 and 2018

Year	AY	BS	GU	HO	JE	MS	Multiple breeds	Number of cows
1997	0.2	0.4	0.3	93.4	3.3	0.1	2.4	4,446,460
2007	0.1	0.3	0.2	90.5	4.3	0.1	4.5	4,414,821
2017	0.1	0.2	0.1	80.9	7.7	<0.1	10.9	4,380,995

genotyped cows and AI bulls over recent years was derived by the Council on Dairy Cattle Breeding. One objective of this study is to show some genomic characteristics of the U.S. population over the last 2 decades.

Results and discussion

For several decades U.S. dairy owners have had a high percentage of their herds comprised of a single breed. Herds with 75% or more of their milking animals of one breed were designated as herds of that breed. Herds with no individual breed achieving 75% were coded as multiple-breed herds. Multiple-breed herds have shown considerable growth in the U.S. over the last 2 decades, primarily at the expense of Holstein herds. In 1998, herds designated as multiple-breed herds was 4.0% (Table 2). Today it has increased to 11.5%, most of which came from the Holstein designation. Holstein herds declined by 8.1 percentage points while multiple-breed herds increased by 7.5 percentage points.

Table 3 shows the percentage of cows in the single- and multiple-breed herd designations. Because AY, BS, GU, and MS have smaller herd size, the percentage of cows in these single-breed herds is lower than the percentage of herds shown in Table 2. Single-breed Jerseys represent 5.3% of herds, but have 7.7% of the cows. Four traditional breeds had about half as many cows on test as they had 20 years ago.

Table 4. The percentage of cows[1] by assigned breed in single-breed herds along with the recorded breed of their sires and dams.

Animal	AY	BS	GU	HO	JE	MS	Other breeds	Crossbreds	Number of animals
Cows	0.1	0.4	0.1	87.4	10.1	<0.1	0.1	1.7	3,629,788
Sires	0.1	0.5	0.1	87.4	11.6	0.1	0.2	0.0	3,064,037
Dams	0.1	0.4	0.1	87.1	10.5	<0.1	0.1	1.6	3,119,106

[1]For cows calving from January 1, 2017 to December 31, 2017

Table 5. The percentage of cows[1] by assigned breed in multiple-breed herds along with the recorded breed of their sires and dams.

Animal	AY	BS	GU	HO	JE	MS	Other breeds	Crossbreds	Number of animals
Cows	0.9	2.2	0.7	42.3	20.8	0.6	1.1	31.5	382,042
Sires	1.2	3.3	0.9	52.7	34.8	0.8	6.3	0.1	297,973
Dams	0.9	2.4	0.8	51.3	19.9	0.6	1.2	22.7	314,694

[1]For cows calving from January 1, 2017 to December 31, 2017

Table 6. Breed Base Representation showing the average percentage for the primary breed of genotyped cows with birth dates in 1997, 2007 and 2017 and number genotyped.

Year	AY	BS	GU	HO	JE	Crossbreeds
1997	-	97.9	100.0	98.4	99.1	-
2007	97.6	98.8	97.0	99.0	98.1	66.6 HO
2017	95.9	98.2	97.2	99.0	95.0	78.9 HO

Table 7. Breed Base Representation showing the average percentage for the primary breed of genotyped bulls with birth dates in 1997, 2007 and 2017.

Year	AY	BS	GU	HO	JE	Crossbreeds
1997	99.9	99.8	99.8	99.6	99.5	-
2007	98.0	99.7	99.7	99.6	99.3	-
2017	97.8	99.0	98.3	99.2	97.4	50.0 HO JE

Table 4 provides an examination of the breed designation of animals in the single-breed herds. It shows the breed code assigned to cows calving from July 1, 2016 to June 30, 2017, and the codes assigned to their sires and dams.

Table 5 provides a similar examination of the breed composition in the multiple-breed herds.

Tables 6 and 7 provide evidence of changes in breed composition in cows and bulls, respectively. These results confirm what seems expected from other developments reported in the industry (e.g. semen sales in Table 1). The mean BBR for genotyped Jersey cows declined from 99.1 in 1997 to 95.0 in 2017, apparently as a result of using Jersey (JE) semen on other breed animals. The BBR for Ayrshires was 95.9 in 2017, as a result of more alleles coming from Scandinavian red breeds, although based on limited number of genotyped animals.

There is evidence that BBR is impacted by the chip used for genotyping. Animals with alleles coming from other breeds (e.g., BBR of the primary breed around 87%) often receive a BBR about 4% higher if re-genotyped using a higher density chip. If the BBR is ≥97%, common for most animals, there is no consistent change when re-genotyped with a higher density chip.

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Association of milk MIR-derived body energy traits with fertility parameters in cows

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Introduction

The milk produced by dairy cows is a complex emulsion of proteins, carbohydrates, lipids, vitamins, enzymes, inorganic elements and, amongst others, water. Further to its composition, it is increasingly apparent that certain physiological processes may leave molecular signatures in the milk, some of which could be identified and used to inform cow management. The use of mid-infrared (MIR) spectroscopy to detect such molecular signatures has escalated over the last decade due to advantages of time, cost and labour over reference methods (de Marchi *et al.* 2014).

Building on the predictions of McParland *et al.* (2011), SRUC have developed and deployed prediction tools for energy balance and energy intake used, to date, to generate predictions for over 13.6 million animal test-date records from over 4,500 UK dairy herds as part of collaboration with National Milk Records (NMR). The literature shows us that the severity of negative energy balance in early lactation can strongly influence fertility in dairy cattle; Butler 1981 describes the delay in the commencement of normal luteal activity and ovulation when negative energy balance is severe; Berry *et al.* (2003) and Pryce *et al.* (2001) exemplify negative genetic correlations between body condition score (BCS, a proxy for energy balance) and interval to first service and positive relationships between BSC and pregnancy rate to first service. As such the objective of this study was to explore how MIR-derived energy predictions were associated with various fertility parameters and assess their use as early warning indicators of potential fertility issues.

Materials and methods

MIR-derived energy trait predictions for energy balance and energy intake were estimated as part of a routine pipeline using national data using prediction equations developed previously (Smith *et al.*, in draft) and now in use by NMR. For this analysis, we focussed on Holstein only dairy cows. Fertility records (obtained from those used in national evaluations, Wall et al, 2003) were then aligned to MIR predictions based on the same day in milk and for animals with complete milk records for lactations 1, 2 and 3. Fertility traits available were calving interval (CI), days in milk at first insemination (DFI), number of inseminations that resulted in a subsequent calving (NI) and correlated trait used in the evaluation of fertility - test milk near 110 days (TM110). This gave a dataset of predicted energy, milk and fertility traits on over 63,000 test-dates on 20,735 animals.

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Bivariate models were performed between each of energy balance and energy intake and three fertility evaluation traits (CI, DFI, NI) and TM110, then trivariate models between energy traits and a subset of fertility traits. Models were fitted with a pedigree with 4 generations which totalled 86,803 animals and consisted of:

$$y_{ijklm} = \mu + L_i + M_j + C_k + C_l^2 + a_m + p_m + e_{ijklm}$$

Where y_{ijklm} is the observations for trait 1 and 2 (and 3 in the trivariate); μ is the trait mean, L_i is the fixed effect of the i^{th} lactation number, M_j is the fixed effect of the j^{th} month of calving, C_l, the fixed effect of the l^{th} age at current lactation, C_l^2 is the fixed effect of the l^{th} age at current lactation squared, a_o is the random additive genetic effect and p_o the random permanent environmental effect of the m^{th} individual cow and e_{ijklm} accounts for the error term. ASREML V3 (Gilmour *et al.* 2009) was used to undertake these analyses.

Results and discussion

The heritabilities for the two MIR-derived energy traits and the four fertility traits are given in Table 1, alongside their summary statistics from the dataset analysed. All heritabilities were significant and largely in line with other studies; Bastin *et al.* (2016) reported heritability for effective energy intake of 0.10, Wall *et al.* (2003) that for calving interval, days at first insemination and number of inseminations at 0.033, 0.035 and 0.02 respectively. Estimated heritability for MIR-derived energy balance range from 0.22 (Bastin *et al.* 2016), 0.10 (McParland *et al.* 2015); of which our estimated heritability is slightly below this at 0.06.

Despite being largely non-significant, the bivariate analyses revealed some correlations which are biologically meaningful and in line with previous estimates (Table 2). The negative genetic correlation (-0.19) between energy balance and calving interval is similar to that by Bastin *et al.* (2016) of -0.20 between energy balance and days open, Wall *et al.* (2013) of -0.14 between body condition score (BCS) and calving interval, but slightly lower than estimates of Pryce *et al.* (2001) of -0.36 between BCS and calving interval. In the trivariate analyses (with TM110) the negative phenotypic correlation between energy balance and calving interval was significant.

In *early* lactation the genetic correlation between energy balance and milk yield tends to be negative due to the energetic demands of milk production (Butler *et al.* 1989; Veerkamp *et al.* 1997). In this analyses, however, the genetic and phenotypic correlation between energy balance and milk yield taken at 110 days in milk (TM110) was positive in the bivariate model (0.11, 0.03) and significantly positive (0.35, 0.08) in the trivariate model with calving interval. This suggests that at this stage, following recovery from NEB, resumption of ovulation and increased feed intake may cover the energetic demands of milk production which otherwise were obtained from body reserves. Berry *et al.* (2003) suggests that selection for milk yield in later lactation is likely less detrimental to fertility due to reduce energy demands on the animals and hence greater energy balance.

Despite being small, the negative genetic correlation between energy balance and days to first insemination in the bivariate model and a significantly negative genetic correlation between them when in a trivariate analysis with TM110 is consistent with a negative relationship between BCS and interval to first service by Berry *et al.* (2003) (-0.47 to -0.30), BCS an days to first service by Pryce *et al.* (2001) (-0.54) and Wall *et al.* (2003) (-0.08). The negative genetic correlation between energy balance and number

Table 1. Summary statistics and estimated heritabilities.

Trait	Mean	Max	Min	h^2 *	S.E. *
Energy balance (EB)	5.95	86.04	-120.03	0.057	0.008
Energy intake (EI)	153.82	255.79	32.02	0.078	0.009
Calving interval (CI)	374.32	599.00	301.00	0.029	0.006
Test milk near 110 (TM110)	34.51	84.40	3.00	0.144	0.015
Days at first insemination (DFI)	65.96	466.00	20.00	0.029	0.006
Number Inseminations till conception (NI)	2.02	13.00	1.00	0.024	0.006

*Average from all bivariate analyses. All significant at $P < 0.05$

Table 2. Results of bivariate analyses between energy balance and fertility traits (upper table) and energy intake and fertility traits (lower table). 'r_p' is the phenotypic correlation and 'r_g' the genetic correlation. Significant results highlighted in bold.

Bivariate	r_p	S.E.	r_g	S.E.
EB and CI	0.006	0.005	-0.197	0.125
EB and TM110	**0.033**	0.005	0.114	0.087
EB and DFI	0.004	0.005	-0.01	0.121
EB and NI	0.007	0.005	-0.156	0.134
Bivariate	**r_p**	**S.E.**	**r_g**	**S.E.**
EI and CI	**0.038**	0.005	0.072	0.118
EI and TM110	**0.085**	0.005	0.137	0.080
EI and DFI	**0.022**	0.005	0.151	0.109
EI and NI	**0.022**	0.005	0.070	0.124

Table 3. Results of trivariate analyses between energy balance and a selection of fertility traits. Traits listed as tr1, tr2 and tr3 in order they appear in the first column. rp is the phenotypic correlation and ra the genetic correlation. Significant results highlighted in bold.

Trivariate	r_p_tr1tr2	r_p_tr1tr3	r_p_tr2tr3	r_a_tr1tr2	r_a_tr1tr3	r_a_tr2tr3
EB, CI, TM110	**-0.018**	**0.082**	**0.062**	-0.135	**0.351**	**0.164**
EB, CI, DFI	0.005	0.003	**0.417**	-0.184	-0.009	**0.709**
EB,TM110, DFI	**0.033**	**0.004**	**0.046**	**0.121**	**-0.020**	**0.403**
EI, CI, TM110	**0.0376**	**0.0852**	**0.0587**	0.0714	0.141	**0.2995**
EI, CI, DFI	**0.0363**	**0.0216**	**0.4171**	0.0791	0.1597	**0.719**

of inseminations till subsequent conception (-0.16 in bivariate) was in line with estimated correlations of 0.34 to -0.17 between BCS and number of services by Berry *et al.* (2003).

The phenotypic correlation between energy intake and each of the four fertility parameters was significant and positive (as in trivariate for EI with CI, TM110 and DFS) the largest of which was between energy intake and weight of milk, consistent with Veerkamp *et al.* (1997 and 1999). The positive phenotypic correlations between energy intake and CI, DFI and NI respectively were all extremely small (< 0.04). Bastin *et al.* (2016) estimated a genetic correlation between energy intake (from 5 days in milk) and days open at -0.28 whereas results here were obtained from around 110 days in milk and are therefore more consistent with the results of Bastin *et al.*, (2013) whereby the genetic correlation between effective energy intake and days open in mid-lactation was 0. It may be that as the demand for feed intake declines throughout lactation and that excessive feeding and over-conditioning is also detrimental to calving interval length.

The ability of the MIR spectra to relay information on energy balance is likely due to the changing molecular composition of key fats in milk as a result of mobilisation of body tissue in times of energy deficit (Bastin *et al.* 2016); a state which is well reported to affect fertility. Exploration of such correlations has been hindered in the past due to the cost and labour required to generation energy balance estimates; however the large resource of MIR-derived energy traits routinely obtained has made such exploration very feasible and initial studies such as these help indicate where to run larger models with more power. Such initial multivariate analyses have also highlighted the conclusion of others (Berry *et al.* 2003, Veerkamp *et al.* 1999) that the stage of lactation is also very important when understanding the direction of correlations between traits and as such should be considered when determining the appropriate combination of traits to select on. In this study using estimates of energy and fertility traits at approximately 110 days in milk, multivariate analyses showed positive correlations of energy traits with TM110 but antagonistic genetic correlations between energy balance and CI, DFS and NI, highlighting the potential use of MIR-derived traits as indicators of traits collected as part of fertility records. We aim to further explore correlations between the energy traits across lactation and fertility and wider functional traits as data size and structure allows. As the MIR-derived energy traits tend to have higher heritabilities than the fertility traits selection on these as indicator may be more effective as long as it is performed at a time which is least detrimental to fertility.

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Prediction of blood beta-hydroxybutyrate content in early-lactation New Zealand dairy cows using milk infrared spectra

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Summary

The objective of this study was to evaluate the ability of mid-infrared predictions of blood BHB concentration to serve as a tool for large-scale phenotyping and management tool in New Zealand dairy farms. The data were on 553 cows (Holsteins and Holstein x Jersey crossbreds), from 2 farms located in the Waikato and Taranaki regions of New Zealand, operated under a seasonal-calving, pasture-based dairy system. Milk infrared spectra were collected once a week on all cows. A blood "prick" sample was taken from the ventral labial vein of each cow 3 times a week for the first 5 wk of lactation. The content of â-hydroxybutyrate (**BHB**) in blood was measured immediately using a hand-held device. All blood samples were collected at approximately the same time of the day (7 am, before a fresh allocation of pasture and supplementary feed were offered), between June and October 2016. Concentrations of blood BHB measured on the day before and after the milking when the spectra data were acquired were averaged and used for developing prediction models. After outlier elimination, 1,910 spectra records and relative BHB measures were available for calibration.

Calibration models were developed by PLS regression using two-thirds of the cows (corresponding to 1,297 spectra records) and validated on the remaining one-third. Cows in the calibration and validation set were randomly selected. A moderate accuracy was obtained for prediction of blood BHB. The R^2 of calibration was 0.58, with a ratio of performance to deviation (**RPD**), calculated as the ratio of the SD of the PLS model calibration set to the SE of prediction, of 1.54. In validation, the R^2 was 0.49 with RPD = 1.39. The relatively low number of samples with high values of BHB is a limiting factor in the development of infrared prediction models. Hence, as an alternative approach, part of the samples in the calibration set were excluded from the analysis, in order to obtain a more balanced distribution of the BHB values. The subset of samples excluded from the calibration set ranged from 25 to 50%. The R^2 in calibration increased (up to 0.63) as the proportion of samples excluded

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increased, but this led to a reduced R^2 in validation (0.42), indicating that this approach is not expected to improve the predictive ability of models when they are applied at the population level.

This study has shown that the prediction of blood BHB content from milk is possible and moderately accurate and can be potentially used as management tool at farm level. To evaluate the role of infrared predictions as indicator traits of blood BHB content in future selective breeding programs, genetic parameters of the infrared predicted blood BHB need to be estimated.

Keywords: Infrared spectroscopy, blood â-hydroxybutyrate, ketosis, prediction model.

Introduction

In New Zealand dairy cows, hyperketonaemia (defined as blood β-hydroxybutyrate (**BHB)** concentration ≥ 1.2 mmol/L) is common during the postpartum period with an estimated herd-level incidence of 68% during the first 5 wk of lactation (Compton *et al.*, 2015). As hyperketonaemia is associated with increased occurrence of clinical ketosis, other health disorders, and reduced fertility (Compton *et al.*, 2015), reliable diagnostic methods and strategies to reduce hyperketonaemia are needed.

Currently available commercial calibration equations for the infrared (**IR**) prediction of milk ketone bodies have never been tested in New Zealand. Hence, IR prediction of milk BHB are not yet available. Moreover, due to its pasture-based dairy farming system, where cows are grazed outdoors all year round, New Zealand is likely to benefit from the development of dedicated calibration equations. Considering that the average content of milk BHB is below the limit of detection of IR spectrometers (Broutin, 2015) and prediction of milk BHB, therefore, relies on correlated traits (e.g., concentration of fat and protein, lactose, fatty acid profile etc.), an alternative approach is to use milk IR spectra to predict blood BHB concentration instead of milk BHB content.

The objective of this study was to evaluate the ability of milk IR spectra to predict the concentration of BHB in blood in pasture-grazed, early-lactation New Zealand dairy cows to serve as a future tool for large-scale phenotyping for selective breeding purposes and for on-farm management purposes.

Material and methods

The study involved 553 cows (Holsteins and Holstein x Jersey crossbreds), from 2 seasonal-calving, pasture-based dairy farms located in the Waikato and Taranaki regions of New Zealand. Milk IR spectra were collected once a week (on PM/AM composite samples) for all cows using a Milko-Scan FT1 (Foss Electric A/S, Hilleroed, Denmark).

A blood “prick” sample was taken from the ventral labial vein of each cow 3 times a week for the first 5 wk of lactation. The concentration of BHB in blood was measured immediately using a hand-held device (FreeStyle Optimum™ Blood Glucose Monitoring System with Blood β-Ketone Test Strips, Abbott Diabetes Care Ltd., UK). All blood samples were collected at approximately the same time of the day (7 am, before a fresh allocation of pasture and supplementary feed were offered), between June and October 2016. Concentrations of blood BHB measured at the milking prior to and the day after milk spectral data collection were averaged and used for developing prediction models. The number of spectra records per cow ranged from 1 to 5.

The noise or non-informative regions of the spectra between 1,628 and 1,658 cm^{-1}, 3,105 cm^{-1} and 3,444 cm^{-1}, and 2,966 to 5,010 cm^{-1} were removed before the chemometric analysis. Spectra with a global Mahalanobis distance greater than 3 (N = 45) were considered outliers and eliminated. After outlier elimination, 1,910 spectra records and corresponding BHB measures, from 542 cows, were available for calibration. Spectra were transformed using extended multiplicative scatter correction and a 1st derivative calculated over a window of 5 points. Calibration models were developed by PLS regression with a 10-fold cross-validation, implemented in the R package PLS (Mevik and Wehrens, 2007) using two-thirds of the cows (corresponding to 1,297 spectra records) and validated on the remaining one-third. Cows in the calibration and validation set were randomly selected. The procedure used to create the subsets guaranteed that all the records from a cow were in either the calibration or the validation subset.

Preliminary statistics indicated that blood BHB concentrations were not normally distributed, with a higher proportion of low concentrations. Visual inspections of the data indicated that most low BHB concentrations were between 0.3 and 0.6 mmol/L. Prediction models were developed using the full calibration sets, or after randomly removing 25% and 50% of the data with low concentrations, following the approach used by Grelet *et al.* (2016). The values were then log-transformed to approach a normal distribution. To evaluate the predictive ability of models when applied to a random population, the distribution of the samples in the validation set was not modified.

The root mean squared error of prediction (**RMSEP**) in calibration and validation, the coefficient of determination between the predicted and measured concentrations in calibration (**R^2c**) and validation (**R^2v**), and the ratio of performance to deviation (**RPD**, i.e. the ratio of the SD of measured BHB concentrations to RMSEP) were calculated.

Results and discussion

The average blood BHB concentration was 0.8 ± 0.4 mmol/L, and ranged from 0.15 to 4.0 mmol/L. With the defined threshold of blood BHB ≥ 1.2 mmol/L, the prevalence of hyperketonemia during the first 5 wk of lactation was, on average, 10.4%.

Fitting statistics of the prediction model for log-transformed blood BHB concentrations are reported in Table 1. In calibration, the R^2c was 0.58 with a RMSEP = 0.14, whereas, in validation, the R^2v was 0.49 with a RMSEP = 0.16, which translated into an RPD

Table 1. Average fitting statistics (SD in parentheses) of quantitative predictions models for log-transformed blood BHB concentration obtained in calibration and validation[1].

Dataset[2]	N	#terms	RMSEP	R^2	RPD
Full					
Calibration	1,297	24	0.14	0.58	1.54
Validation	613	24	0.16	0.49	1.39
25% of low conc. removed					
Calibration	1,100	24	0.15	0.61	1.60
Validation	613	24	0.15	0.43	1.27
50% of low conc. removed					
Calibration	902	24	0.16	0.63	1.65
Validation	613	24	0.16	0.42	1.23

[1]N: number of records in the datasets; #terms: number of optimal partial least square components; RMSEP: root mean squared error of prediction; RPD = RMSEP/SD of measurements.
[2]Prediction models were developed using the full calibration set, or after randomly removing 25% and 50% of the data with BHB concentrations were between 0.3 and 0.6 mmol/L.

of 1.39. These results indicate that the models are not expected to provide accurate quantitative values at the individual cow level, but the moderate R^2v indicates that they could potentially be used to distinguish low and high BHB concentrations, or used to predict aggregate values (i.e., herd average) with reasonable accuracy. The prediction accuracy in our study is either similar to, or higher, than that reported in previous studies (Broutin, 2015; Belay *et al.*, 2017), with a comparable or lower prediction error. When part of the samples (25 and 50%) in the calibration set were excluded from the analysis to obtain a more balanced distribution of the BHB concentrations, the R^2c increased (by 3 to 5%) as the proportion of samples excluded increased, but this reduced the R^2v (by 6 to 7%). Hence, this approach is not expected to improve the predictive ability of models when they are applied at the population level.

The values of R^2v in this study are relatively low if compared with those achieved for prediction of milk ketone bodies (Grelet *et al.*, 2016), but we consider the results to be satisfactory as blood components were predicted from milk spectra. In addition, the dataset used in our study included samples from 2 farms and one season only, hence accuracy and robustness of prediction models can likely improve with additional calibration samples.

Conclusions

This study indicates that the prediction of blood BHB concentrations from milk spectra is possible and moderately accurate. Further data will likely improve the robustness and accuracy of prediction models. To evaluate the role of IR predictions as indicator traits of blood BHB concentrations in future selective breeding programs, genetic parameters of the IR predicted blood BHB need to be estimated.

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Prediction of serum metabolic profile biomarkers in early lactation dairy cows using mid-infrared spectroscopy of milk

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Summary

Metabolic diseases in early lactation have significant negative effects on dairy cow health and welfare, and farm profitability. The most commonly described metabolic diseases are ketosis, hypocalcaemia, and hypomagnesaemia. Subclinical metabolic diseases, which are not associated with obvious clinical signs, are of particular interest due to their relatively high prevalence and significant effects on animal welfare and performance. Currently one of the most common methods for monitoring the metabolic health of cows is serum metabolic profiling, which utilises well-established associations between the concentrations of several metabolites in serum, and the presence of both subclinical and clinical metabolic disease.

An emerging technology to evaluate subclinical metabolic disease is mid-infrared spectroscopic analysis (MIR) of milk samples. In this cross-sectional study we investigated the use of MIR spectroscopy of milk for estimating the concentrations a number of serum metabolites commonly employed in metabolic profiling. A single plain/clotted blood sample was taken from 1027 cows from 5 farms in the Gippsland region of south-eastern Victoria, Australia, on the same day as milk recording. All cows had calved within 8 weeks of sampling. Serum samples were analysed for beta hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), calcium, magnesium, blood urea nitrogen, total protein, albumin and globulins.

Milk samples were analysed by MIR spectroscopy using a Bentley Instruments FTS Combi. Calibration models were constructed using partial least square (PLS) regression, and external validation was performed using both a farm exclusion (a calibration equation derived using data from 4 farms was used to predict the outcome on the 5th farm) and a random sampling method (a random sample of 20% of cows was excluded from the calibration dataset and used for validation). The R^2 and root mean square error (RMSE) values for MIR predictions using random external validation were 0.49 (0.19) for BHB, 0.51 (0.24) for NEFA, 0.88 (0.79) for Urea, 0.18 (0.14) for calcium, 0.23 (0.10) for magnesium, 0.28 (2.01) for albumin, and 0.31 (4.76) for globulins.

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Our results demonstrate that MIR spectroscopic analysis of milk shows promise for evaluating short-term protein status of animals through accurate estimation of BUN concentration, and reasonable prediction of energy balance by estimation of serum BHB and NEFA concentrations.

Keywords: Mid-infra-red spectral prediction, metabolic profiles, ketosis.

Introduction

Metabolic diseases in early lactation have significant negative effects on dairy cow health and welfare, and farm profitability (McArt *et al.*, 2015, Suthar *et al.*, 2013). The most commonly described metabolic diseases are ketosis, hypocalcaemia and hypomagnesaemia. Subclinical metabolic diseases, which are not associated with obvious clinical signs, are of particular interest due to their relatively high prevalence and significant effects on animal welfare and performance (Compton *et al.*, 2013, Macrae *et al.*, 2006, McArt *et al.*, 2012, Suthar *et al.*, 2013).

Serum metabolic profile testing (MPT) utilises well-established epidemiological associations between the concentrations of several metabolites in serum and the presence of both subclinical and clinical metabolic disease, and provides objective information on the metabolic health and nutritional status of cows (Payne *et al.*, 1970). The metabolites evaluated in MPT vary, but often include beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFAs) as indicators of energy balance, albumin, globulins and blood urea nitrogen (BUN) as indicators of protein status, and magnesium (Mg) and calcium (Ca) and as indicators of mineral status (Anderson, 2009, Whitaker, 2004). NEFA and BHB are particularly important, as elevated concentrations of one or both of these metabolites are indicative of mal-adaptation to the period of post-partum negative energy balance and are associated with an increased risk of subsequent negative health and production outcomes (Ospina *et al.*, 2010, Sordillo and Raphael, 2013, Chapinal *et al.*, 2012)

Despite the advantages of MPT, blood testing animals on a regular basis is invasive, logistically challenging and potentially costly. Given the ready availability of milk, its use as a biofluid for diagnostic purposes is of increasing interest. A milk fat to protein ratio (FTP) of greater than 1.4 has been described as an indicator of negative energy balance and subclinical ketosis in early lactation (Schcolnik, 2016). More recently, mid-infrared spectroscopy (MIRS) of milk has shown promise for predicting the risk of subclinical ketosis and evaluating energy balance in early lactation (McParland *et al.*, 2011, Grelet *et al.*, 2016, van Knegsel *et al.*, 2010, Belay *et al.*, 2017, de Roos *et al.*, 2007). The aim of our study was to determine if MIRS of milk is a better predictor of subclinical ketosis risk and negative energy balance in early lactation dairy cows than milk fat to protein ratio, and whether MIRS can be used to estimate the concentrations of other serum metabolites.

Materials and methods

All procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2004). Approval to proceed was obtained from the DEDJTR Agricultural Research and Extension Animal Ethics Committee (Department of Economic Development, Jobs, Transport and Resources, 475 Mickleham Road, Attwood, Victoria 3049, Australia).

Sample collection

Blood samples were taken from 1028 cows on the same day as milk-recording between July and October 2017. The cows were located on five farms in the Gippsland region of south-eastern Australia. All five farms implement a feeding system reliant on grazed pasture plus other forages and more than one tonne grain/concentrates fed in the bail per cow per year. Three of the farms (farms 3, 4 and 5) had rotary milking platforms which allowed blood samples to be collected during milking. Samples were taken immediately after milking on the other two farms. Samples were collected at the morning milking on farms 1 and 3, and during the afternoon milking on farms 2, 4 and 5. Blood was collected from the coccygeal vein into 10ml serum clot activator vacutainer tubes (Becton Dickinson). Samples were allowed to clot for a minimum of one hour at room temperature before centrifugation at 1200 g for ten minutes at 18°C. All samples were processed within six hours of collection. Serum samples were refrigerated at 4°C then transported on ice to Regional Laboratory Services (Benalla, Victoria, Australia). Samples were analysed for BHB, NEFA, total Ca, Mg, total protein and albumin using colourimetric analysis, with reagents supplied by Randox Laboratories. Globulin concentrations were calculated as total protein concentration minus albumin concentration. Milk samples were collected as part of normal milk-recording by the Herd Improvement Co-Operative Australia (Maffra, Victoria, Australia). Samples were preserved with bronopol and analysed fresh using mid-infrared (MIR) spectroscopy (Bentley Instruments NexGen FTS Combi) by TasHerd Pty Ltd (Hadspen, Tasmania, Australia). MIR spectra were expressed in absorbance, with 899 spectral points between 649cm^{-1} and 3998cm^{-1}. Spectral regions associated with the O-H bending and stretching regions of water were excluded prior to analysis (Afseth *et al.*, 2010, Belay *et al.*, 2017). A total of 563 spectral wavelengths between 928cm^{-1} and 3025cm^{-1} were included in the analysis.

Statistical analysis

Descriptive statistics

Descriptive statistics of the animals included in the analysis are summarised in Table 1.

The distribution of serum metabolite concentrations were visually assessed for normality using frequency histograms. NEFA and BHB concentration distributions were both skewed, with lower values over represented. This type of distribution leads to decreased accuracy in predicting high values (Grelet *et al.*, 2016), so a logarithmic (10) transformation was applied.

MIR predictions

All MIR spectral data analysis was performed with MATLAB R2017a (MathWorks, Natick, MA) utilising PLS Toolbox (Eigenvector Research, Manson, WA).

Table 1. Number of cows that had metabolic profiles and milk MIR spectral data by farm, including stage of lactation (days in milk means and ranges) and percentage of animals in their first lactation.

Data	No. of cows	Percentage primiparous	Days in milk		
			Mean	Min	Max
Farm 1	480	17.7	23.15	4	53
Farm 2	137	27.0	19.94	3	39
Farm 3	81	29.6	27.48	5	56
Farm 4	149	12.1	21.01	4	39
Farm 5	180	17.8	29.51	6	52
Total	1027	19.1	23.86	3	56

Pre-processing of spectra

Preliminary analysis of the spectral data was conducting using principal component analysis (PCA). A single outlier spectrum with a zero absorbance was identified from the PCA plot and removed from the data set, leaving 1027 samples. MIR spectra were pre-processed with Savitzy-Golay 2nd derivative transformation and smoothing, removal of linear trend and auto scaling.

Calibration and validation

The relationship between blood metabolite concentrations and milk MIR spectra was investigated using partial least square (PLS) regression analysis.

Cross-validation (CV) was performed using a venetian blinds method, which split the data into 20 subsets and performed CV on two samples per subset. Each model was assessed for over-fitting using a permutation test with 50 iterations, the statistical significance ($P<0.05$) of which was tested using a Wilcoxon sign test.

The number of latent variables (LV) included in each model was based on maximising the percentage of variance captured while minimising the root mean square error of cross-validation ($RMSE_{CV}$). The optimum number of LVs was determined by examining a plot $RMSE_{CV}$ as a function of number of LVs

External validation (EV) was performed using two methods. The first method involved randomly sorting the data then splitting it into calibration (n=822) and validation (n=205) sets. Each dataset was designed to have a representative number of samples from each farm and parity category (primiparous or multiparous), and was balanced for days in milk. The second method involved using data from one farm to validate calibration equations constructed using data from the remaining four farms.

Comparison with milk fat to protein ratio

The usefulness of milk MIR predictions of serum NEFA and BHB for estimating the degree of fat mobilisation and the risk of ketosis respectively, was determined by comparing the accuracy of MIR prediction outputs with that of milk fat to protein ratio.

Multiple linear regression models were constructed to predict serum BHB and NEFA concentrations using combinations of MIR prediction of BHB (MIR_{BHB}), MIR prediction of NEFA (MIR_{NEFA}) and milk fat to protein ratio. Farm identification, parity category and weeks in milk (WIM) were included as fixed effects in each model. The significance ($P<0.001$) of each predictor variable within each model was determined using ANOVA F-tests. The overall accuracy of the models was assessed by comparing the adjusted R^2 values.

Results and discussion

Descriptive statistics

Of the 1027 animals included in the analysis, 480 (46.74%) were from one farm. The remaining 547 animals were evenly distributed between the remaining four farms. Of the animals sampled, 757 (73.7%) had been calved 30 days or less, which is the period of highest risk for development of metabolic disease (LeBlanc *et al.*, 2006). The overall percentage of primiparous animals in the dataset was 19.1%, with a range of 12.1% to 29.6% between farms.

MIR calibration and validation

The results of PLS regression models investigating the relationships between blood metabolite concentrations and MIR spectra from milk samples are shown in Table 2. The coefficient of determination of CV (R^2_{CV}) and EV (R^2_{EV}) for the model predicting serum BHB in the random calibration and validation analysis were 0.49 and 0.49 respectively. The results for the NEFA model were similar with an R^2_{CV} of 0.46 and an R^2_{EV} of 0.51. The most promising results were for the model predicting serum urea concentration, which had an R^2_{CV} of 0.89 and an R^2_{EV} of 0.88. The prediction models for both Ca and Mg had comparatively low accuracies with R^2_{CV} and R^2_{EV} of 0.18 and 0.18, and 0.17 and 0.23 respectively. Serum albumin and globulin concentration prediction models performed slightly better with R^2_{CV} and R^2_{EV} values of 0.26 and 0.28, and 0.20 and 0.30 respectively.

The results of the second external validation method, which involved using prediction equations derived from the data from four farms to predict the outcome on the excluded farm, were consistently lower than the results of the random external validation. This is important from a practical point of view, as it suggests that if MIRS is to be employed commercially to predict the metabolic health of cows from multiple herds, the results may be more accurate if data from a subset of animals in each herd being tested are included in the calibration/reference dataset. The relative accuracies of prediction models using farm exclusion external validation were similar to the results of random external validation, with urea, BHB and NEFA models having the highest R^2_{EV} values.

Table 2. Results of partial least square regression models for the prediction of serum metabolite concentrations from milk MIR spectra, using a random external validation method.

		Cross-validation (n=822)			External Validation (n=205)	
Metabolite	No. LVs[1]	R^2_{cv}[2]	$RMSE_{cv}$[3]	p-value	R^2_{ev}[4]	$RMSE_{ev}$[5]
BHB	12	0.49	0.16	< 0.05	0.49	0.19
NEFA	12	0.46	0.25	< 0.05	0.51	0.24
Urea	20	0.89	0.77	< 0.05	0.88	0.79
Calcium	16	0.18	0.14	< 0.05	0.18	0.13
Magnesium	16	0.17	0.11	< 0.05	0.23	0.10
Albumin	16	0.26	2.07	< 0.05	0.28	2.01
Globulin	16	0.20	5.56	< 0.05	0.31	4.76
Alb:Glob	14	0.23	0.15	< 0.05	0.27	0.13

[1] Number of latent variables included in the model
[2] Coefficient of determination for cross-validation
[3] Root mean square error of cross-validation
[4] Coefficient of determination for external validation
[5] Root mean square error of external validation

Comparison of MIR with milk fat:protein

The results of fixed effect models constructed to compare the value of milk MIR predictions of serum BHB and NEFA concentrations with milk fat to protein ratio for predicting the risk of subclinical ketosis and negative energy balance respectively, are shown in Table 3.

MIR_{BHB} was considerably better at estimating serum BHB, and therefore the risk of ketosis, than milk fat to protein ratio. Similarly, MIR_{NEFA} was significantly better at estimating serum NEFA concentrations, and therefore the degree of negative energy balance, than milk fat to protein ratio. As mentioned previously, the accuracies of models using farm exclusion external validation were much lower than the accuracies of models using random external validation. However, even when the accuracies of

Table 3. The accuracy (R^2) of predicting SCK risk and fat mobilisation using milk fat:protein (FTP), and MIRS predictions of serum BHB (MIR_{BHB}) and NEFA (MIR_{NEFA}) concentrations.

	Risk of subclinical ketosis (BHB)		Negative energy balance (NEFA)	
Data	FTP	MIR_{BHB}	FTP	MIR_{NEFA}
Random	0.21	0.47	0.44	0.59
Farm 1	0.19	0.32	0.34	0.36
Farm 2	0.21	0.40	0.43	0.61
Farm 3	0.00	0.35	0.00	0.03
Farm 4	0.17	0.27	0.31	0.41
Farm 5	0.06	0.29	0.20	0.29

prediction models were low, MIRS out-performed milk fat to protein ratio. For example, the R^2_{EV} of the MIR_{NEFA} model for farm 3 was only 0.08, yet it was still a better indicator of serum NEFA concentration than milk fat to protein ratio.

ANOVA testing of the fixed effect models demonstrated that MIR_{BHB} were a poor predictor of serum NEFA concentrations, and therefore negative energy balance. Similarly, the usefulness of MIR_{NEFA} for the prediction of serum BHB concentration, and therefore SCK risk, was also poor. This suggests that the PLS prediction models for NEFA and BHB are significantly different, and are likely utilising different parts of the MIR spectra. This highlights the need for further studies to better understand the association between biomarkers in serum and milk, and the potential use of milk MIR spectral data for further investigation of the genetic parameters of metabolic diseases.

Conclusion

In this study we assessed the accuracy of MIRS performed as part of routine milk-recording for predicting the metabolic health and nutritional status of early lactation dairy cows. We found that MIRS of milk provided accurate estimation of BUN concentration, and good prediction of energy balance by reasonable estimation of serum BHB and NEFA concentrations. The accuracy of MIRS of milk for predicting serum albumin and globulin concentrations, and mineral concentrations (as estimated by serum Ca and Mg concentrations), was poor. We also noted that the accuracy of external prediction was much higher when animals from all farms were represented in both the calibration and validation data sets. This has important implications for the commercial application of MIRS, and implies that it is important to include data from as many herds as possible in the calibration data set.

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Accuracy of genomic predictions for sheep milk fatty acid composition

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Summary

The increasing consumer's demand for high quality and healthier foods is drawing a great attention on milk fatty acid (FA) composition. However, the inclusion of these traits as breeding goals in traditional selection plans is hampered by cost and logistic problems. Medium infrared spectroscopy (MIR) is a valid and cheap alternative to the traditional laboratory gas chromatography (GC) methodology for predicting milk fatty acids composition. Moreover, genomic selection (GS) could represent a valid option for breeding for these traits. Objective of this research was to estimate breeding values for milk FA composition in dairy sheep using two different phenotypes (GC vs MIR) and two breeding strategies (traditional vs GS). Milk FA composition, pedigree relationships, and SNP genotypes were available for 769 Sarda breed ewes, divided in two groups: 669 in training and 100 validation cohorts, respectively. Traditional EBV were estimated using a BLUP animal model whereas GEBV were estimated using a single step approach. Prediction accuracies for validation animals were rather low (<0.30), but always higher for GEBV in comparison with EBV. Moreover, no differences were observed between GC and MIR phenotypes.

Keywords: Fatty acids, genomic selection, estimated breeding value.

Introduction

Milk fatty acid (FA) content and composition represent interesting potential breeding objectives for dairy animals because of healthy properties of these components for humans (i.e. C18:2cis9,trans11 was associated to antiatherogenic effect) (Banni *et al.*, 2003). Moreover, previous studies in dairy sheep confirmed the existence of genetic variability for sheep milk FA profile (Boichard *et al.*, 2014). However, FA recording on large scale using the standard gas chromatography (GC) methodology is problematic due to the high costs, logistic problems and a huge variability due to the several effects that can affect their content in milk. In addition to the diet, breed, stage of lactation, flock and farming area affect milk FA composition in sheep (De La Fuente *et al.*, 2009). A valid and cheaper alternative to the GC method for milk FA measurement is the medium infrared spectroscopy (MIR). Moreover, this simplified measurement method could be combined with genomic selection (GS) for enlarging the number of animals involved in the breeding plan. Objective of this research was to estimate breeding values for milk FA composition in dairy sheep using two different phenotypes (GC vs MIR) and two breeding strategies (traditional selection vs GS).

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Material and methods

Data

Milk FA composition (see table 1) for 769 Sarda breed ewes was measured by GC using a 7890A GC System (Agilent Technologies, Santa Clara, CA, USA) or predicted by MIR spectra. One record per ewe was available. Animals were genotyped using the Illumina Infinium Ovine SNP50 v1 BeadChip: after data editing, 44,619 SNPs across 27 chromosomes were retained for the analysis. Animals were divided in two groups: 669 ewes were considered as training cohort (TC) and the 100 youngest animals as validation cohort (VC). The phenotypic values of VC animals were masked in order to mimic the candidate animals without own records.

Breeding values

EBV and genomic breeding values (GEBV) were estimated with the following animal model:

$$y_{ijklnop} = \mu + PAR_j + DIM_k + LM_l + ALT_n + anim_o + FTD_p + e_{ijklnop} \quad (1)$$

where $y_{ijklmnop}$ is the FA trait; μ is the overall mean; *PAR* is the fixed effect of the *j*-th parity class (eight classes = 1, …, 7, >7); DIM is the fixed effect of the *k*-th days in milking interval (five intervals: < 110, 110 to 140, 141 to 170, 171 to 200, >200); LM is the fixed effect of the *l*-th class of lambing month (1: January; 2: February to March; 3: October to November; 4: December), ALT is the fixed effect of the n-th altitude of location of flocks (mountain>= 500 mt above the sea level; hill= < 500 and >= 200 m a.s.l; *plain* < 200 m a.s.l.); *anim*, is the random additive genetic effect of the o-th animal; (*o* = 1, …, 6,252), *FTD* is the random effect of the p-th flock-test date combination (*p* = 1, …, 66); and $e_{ijklmnop}$, is the residual term.

EBV were estimated with a BLUP methodology by structuring the animal genetic covariance with the pedigree relationship matrix (**A**). GEBV were estimated with a single step approach (ssGBLUP) combining genomic and pedigree relationship matrix (**H**). Variance components were estimated using airemlf90, whereas for GEBV prediction blupf90 program was used. Moreover, prediction accuracies of GEBV were expressed as square root of reliability, calculated from prediction error variance (PEV) and then averaged in TC and VC for each fatty acid.

Results and discussion

Prediction accuracies are reported in Table 1. As expected, larger values were observed for TC in comparison with VC animals, for both phenotypes (GC and MIR) and breeding strategies (BLUP vs ssGBLUP). In the BLUP approach, EBV accuracies were in most of cases generally larger for GC compared to MIR phenotypes, whereas GEBV accuracies showed an opposite trend. Accuracies for VC were rather low, slightly larger for ssGBLUP in comparison with BLUP (0.23±0.05 and 0.17±0.06, respectively). Values obtained in the present work are in agreement with previous reports for genomic prediction of meat FA composition in beef cattle (Chiaia *et al.*, 2017; Zhu *et al.*, 2017). Results of the present study, although low in absolute terms probably because of the reduce size of sample of animals considered, showed that MIR predicted FA MIR are valid substitutes of GC measures for breeding purposes. Moreover, the inclusion of genotype information to the breeding value prediction can improve its accuracy, also in young animals without phenotypic information.

Table 1. EBV accuracy of sheep milk fatty acids obtained with gas chromatography (GC) or predicted by medium infrared spectra (MIR). Accuracy for training cohort (TC) and validation cohort (VC) predicted using pedigree (BLUP) or pedigree and SNP genotypes combined with single-step genomic approach (ssGBLUP).

	TC (n=669)[1]				VC (n=100)[2]			
	BLUP		ssGBLUP		BLUP		ssGBLUP	
Trait	GC	MIR	GC	MIR	GC	MIR	GC	MIR
C4:0	0.59	0.66	0.66	0.78	0.21	0.23	0.31	0.36
C6:0	0.33	0.43	0.60	0.68	0.12	0.15	0.27	0.31
C8:0	0.52	0.42	0.61	0.66	0.18	0.15	0.27	0.30
C10:0	0.56	0.49	0.60	0.62	0.19	0.17	0.27	0.28
C12:0	0.57	0.55	0.56	0.55	0.20	0.19	0.25	0.25
C18:0	0.65	0.57	0.53	0.54	0.22	0.20	0.24	0.24
C18:1t11	0.70	0.60	0.42	0.41	0.23	0.20	0.19	0.19
C18:1c9	0.58	0.26	0.62	0.50	0.20	0.09	0.27	0.22
C18:2ω6	0.11	n.a.	0.26	0.11	0.04	n.a.	0.14	0.14
C18:3ω3	0.11	0.24	0.22	0.39	0.04	0.09	0.12	0.18
CLAc9t11	0.60	0.58	0.40	0.41	0.20	0.20	0.19	0.19
MUFA[3]	0.35	n.a.	0.57	0.48	0.12	n.a.	0.26	0.22
PUFA[4]	0.36	0.56	0.37	0.50	0.13	0.19	0.18	0.22
ω6:ω3[5]	0.73	0.46	0.54	0.50	0.23	0.16	0.24	0.22
TFAnoVA[6]	0.49	0.48	0.47	0.58	0.17	0.17	0.21	0.26
Denovo[7]	0.46	0.43	0.55	0.56	0.16	0.15	0.25	0.25

[1] Cohort of sheep with own records born before 2012.
[2] Cohort of sheep born after 2012 with own records masked to mimic a validation set of younger sheep.
[3] Sum of the individual monounsaturated fatty acids.
[4] Sum of the individual polyunsaturated fatty acids.
[5] Ratio between the sum of individual PUFA ω6 fatty acids and the sum of individual PUFA ω3 fatty acids.
[6] Trans Fatty Acid (TFA) without Vaccenic acid (VA).
[7] Fatty acids synthetized de novo in mammary gland.

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Milk mid-infrared spectra based biomarkers contributing to genetic improvement for udder health, fertility and longevity

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Summary

Recent research showed the usefulness of using estimated breeding values (EBV) for mid-infrared (MIR) based biomarkers in genetic improvement. Similarly, research has also shown that genetic variation is contained in the absorbance traits along the MIR band of wavelengths. Targeted extraction of the useful genetic variance can be achieved by the combination of EBV. Direct estimation of EBV for absorbance traits was demonstrated. Our first objective was to show that the reduction of the rank of the (co)variance structure among spectral traits is possible by imposing linear functions, even if these functions represent lower accuracy MIR biomarkers. MIR based biomarkers traits were derived from ongoing research in the FP7 GplusE project. In this study, the pathway from MIR spectra to the use in genetic improvement will be described. First, blood reference phenotypic data was collected on Holstein cows, at early lactation for IGF-1, glucose, urea, cholesterol, fructosamine, beta-hydroxybutyric (BHB) acid and non-esterified fatty acids (NEFA). These traits were calibrated against corresponding MIR spectral data. Calibration R_{cv}^2 ranged from 0.21 to 0.51, very low from a chemometrical point of view, but potentially sufficient to extract useful spectral variation. This was validated, using EBV that were based on these MIR predictions for 144,623 records (closest to days in milk 25), from 73,378 cows, in the Walloon region of Belgium. Single-trait, but multi-lactation (1, 2, 3+) models yielded h2 estimates ranging from 0.07 to 0.27. At least 20 daughters with novel traits and official EBV for udder health, fertility and longevity, with minimum reliabilities of 70% were required; a total of 124 bulls met this criteria. Standard selection index theory would usually rely on prediction error variance minimisation and estimated population (co)variances. Alternatively in this study, Partial Least Squares were applied to EBV for the milk MIR based biomarkers to develop novel genetic predictors for udder health, fertility and longevity, by extracting genetic variation along the wave band after rank reduction. Using all bulls, correlations between best predictors and EBV for udder health, fertility and longevity were at least 0.63, 0.67 and 0.62. Using selection index theory and based on significant increases of prediction abilities of longevity (0.76 compared to 0.68 from udder health or fertility alone) using milk MIR based blood biomarkers, their potential contribution to genetic improvement of udder health, fertility and longevity will be demonstrated.

Keywords: milk MIR spectra, blood biomarkers, genetic improvement, udder health, fertility, longevity.

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Introduction

Genetic improvement for functional and health traits depends on the availability of relevant data (Egger-Danner *et al.*, 2014). Recent efforts allow the development of appropriate genetic evaluation systems for udder health, fertility and longevity in many breeds and population. However given the specific context of these traits, achieving earlier high reliabilities can be problematic. For example, fertility can only be assessed later during lactation, udder health often relies on somatic cells, or on unreliable mastitis records and longevity of a cow is truly known at her death. For this reason, alternative, early indicator traits could be considered useful under the hypothesis that they are available very early during the productive life of a given cow.

Milk mid-infrared (MIR) spectral data has been identified as a major potential source of relevant data through its easy, cheap and routine use in milk analysis. Calibration of milk based biomarkers has been reported by several authors. In a more limited fashion, MIR predicted milk based biomarkers also start to be used. However, reference traits are blood biomarkers. Therefore, the European FP7 Project GplusE (*http://www.gpluse.eu*) has collected blood biomarkers for insulin growth factor 1 (IGF-1), glucose, urea, cholesterol, fructosamine, beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Efforts to calibrate those biomarkers against milk MIR spectral data are ongoing (C. Grelet, personal communication) but MIR predicted blood biomarkers are not expected to be reliably predicted such. In the context of this study used predictors were based on of 0.21 to 0.51; very low from a chemometrical point of view, but potentially sufficient for the extraction of useful spectral variation.

The objective of this study was to test if the targeted combination of estimated breeding values (EBV) for biomarkers predicted with low accuracies from MIR spectra, increased their usefulness in the genetic evaluation of dairy cows for udder health (UDH), fertility (FERT) and longevity (LONG).

Material and methods

All research was based on data collected in the Walloon region of Belgium. Spectral MIR records were collected since 2012 and standardised. Using the available equation IGF-1, glucose, urea, cholesterol, fructosamine, BHB and NEFA were predicted from MIR data. for 144,623 records (test-day closest to days in milk 25), from 73,378 cows. Single-trait, but multi-lactation (1, 2, 3+) models were fitted and yielded h^2 estimates ranging from 0.07 to 0.27. Official EBV for UDH, FERT and LONG were obtained from the official genetic evaluation in the Walloon Region of Belgium (www.elinfo.be). Some of the EBV for these bulls were from MACE genetic evaluations by INTERBULL. At least 20 daughters for each bull with novel trait data were required. Moreover, novel traits and UDH, FERT and LONG had to have a minimum reliability (REL) of 70%. A total of 124 bulls met these criteria. By imposing rather strict REL, observed correlations between EBV for different traits were expected to be closer to genetic correlation avoiding the need to use the correction proposed by Calo *et al.* (1973). Standard selection index theory would usually rely on prediction error variance minimisation and estimated population (co)variances. However in this specific data high to extremely high correlations between the 21 biomarker EBV leading to major multicollinearity issues. Therefore, alternatively in this study, Partial Least Squares (PLS) were applied to EBV for the MIR based biomarkers to develop novel genetic predictors, for udder health, fertility and longevity, by extracting genetic variation along the wave band after rank reduction. Cross-validation was done by choosing 10% randomly and predicting from the 90% other records, a process that was repeated 1000x. The Proc PLS procedure from SAS was used to do the computations.

Results and discussion

Reliabilities

As given in Table 1, based on the restrictive selection of bulls, average REL were for all traits over 80%, the lowest values were reported for MIR predicted Blood Urea, a trait with low heritability (0.07 – 0.08). For the highest heritable traits, the average REL were close to those found for LONG, FERT and UDH (93.2%, 93.8% and 97.8%) with h^2 of 0.11, 0.04 and 0.14.

Correlations

Correlations between EBV for individual biomarkers in a given lactation ranged in absolute values between 0.01 (BHB and UDH in first lactation) and 0.39 between (Fructosamine and FERT in 3+ lactation (Table 2). Generally the values were rather low compared to the theoretical links between blood biomarkers and UDH; FERT and in consequence also LONG. However, one should not forget that we based these EBV on predictions with rather low .

Table 1. Heritabilities and average reliabilities (REL) in percent for the MIR predicted blood biomarkers reported for the 124 selected sires.

	Lactation 1		Lactation 2		Lactation 3+	
Blood biomarker	Average REL	h^2	Average REL	h^2	Average REL	h^2
IGF-1	92.0	0.21	93.0	0.26	92.7	0.24
Glucose	92.8	0.27	92.9	0.27	92.7	0.25
Urea	80.6	0.07	80.7	0.08	81.0	0.07
Cholesterol	85.1	0.09	82.3	0.08	88.9	0.17
Fructosamine	92.6	0.25	93.2	0.27	93.3	0.27
BHB	90.9	0.20	89.3	0.16	89.8	0.16
NEFA	88.0	0.14	88.6	0.13	88.9	0.12

Table 2. Correlations for the MIR predicted blood biomarkers with udder health (UDH), fertility (FERT) and longevity (LONG) reported for the 124 selected sires.

	Lactation 1			Lactation 2			Lactation 3+		
Blood biomarker	UDH	FERT	LONG	UDH	FERT	LONG	UDH	FERT	LONG
IGF-1	0.05	0.18	0.14	0.07	0.18	0.16	0.18	0.25	0.22
Glucose	0.06	0.23	0.15	0.09	0.24	0.17	0.20	0.31	0.23
Urea	0.13	0.21	0.14	0.11	0.07	0.14	0.17	0.16	0.15
Cholesterol	-0.08	-0.06	0.09	0.02	0.03	0.20	0.05	0.03	0.20
Fructosamine	0.18	0.36	0.24	0.19	0.35	0.24	0.26	0.39	0.25
BHB	-0.01	-0.15	-0.09	-0.02	-0.12	-0.06	-0.08	-0.12	-0.07
NEFA	-0.12	-0.31	-0.23	-0.17	-0.30	-0.23	-0.24	-0.32	-0.25

Predicting udder health, fertility and longevity from milk MIR blood biomarkers

One feature of the use of PLS is its ability to deal with multicollinearity. When comparing regressions coefficients obtained by ordinary least square regression (results not shown) and those reported here (Table 3). Despite the very high genetic correlations between lactations (results not shown) for the same biomarkers, we see that only very few coefficients showed the expected strong opposition that was found in ordinary least square regression.

Predictions of UDH, FERT and LONG obtained using the coefficients in Table 3 explained 0.41, 0.45 and 0.39 of the total variance (R_{cv}^2). The associated cross-validation regression coefficients were 0.64, 0.67 and 0.62. In following paragraphs these predictions using milk MIR based blood biomarkers will be called pUDH, pFERT and pLONG (Figures 1, 2 and 3).

Table 3. Centred and scaled PLS (number of latent variables = 13) coefficients for the MIR predicted blood biomarkers with udder health (UDH), fertility (FERT) and longevity (LONG) reported for the 124 selected sires.

	Lactation 1			Lactation 2			Lactation 3+		
Blood biomarker	**UDH**	**FERT**	**LONG**	**UDH**	**FERT**	**LONG**	**UDH**	**FERT**	**LONG**
IGF-1	0.30	0.12	-0.12	-0.93	-1.35	-1.30	0.37	0.40	0.78
Glucose	-0.51	-0.26	-0.54	-0.02	0.15	0.17	1.42	1.68	1.74
Urea	0.15	0.39	0.00	0.01	-0.18	0.21	-0.03	0.01	-0.01
Cholesterol	-1.53	-1.14	-1.19	0.60	0.98	0.88	0.74	-0.04	0.33
Fructosamine	-0.01	0.18	0.24	-0.63	-0.44	0.85	0.11	-0.39	-1.68
BHB	0.66	0.27	0.28	-0.18	-0.53	-0.12	0.25	1.14	0.52
NEFA	-0.85	-0.56	-0.83	0.12	0.08	0.25	0.12	-0.34	-0.02

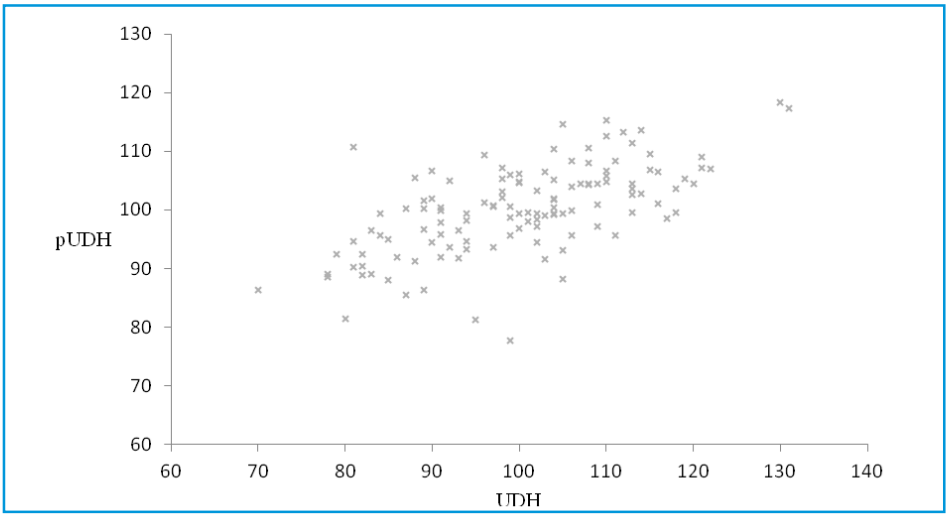

Figure 1. EBV for udder health (UDH) and predicted EBV udder health (pUDH) using milk MIR blood biomarkers (correlation between UDH and pUDH 0.63)

Different predictions of longevity

An important breeding goal trait is longevity, also called herd life, or with a slightly different definition, survival. Unfortunately, it is also a very difficult trait as relevant data is late available. Predictor traits are very commonly used and the result of this prediction called indirect longevity or herd-life (Jairath *et al.*, 1998). Different types of predictors are commonly used, ranging from type traits to functional traits. In this study we have put into perspective the use of usual traits as functional traits UDH and FERT, but also the novel traits. Therefore, pLONG was added as an alternative predictor.

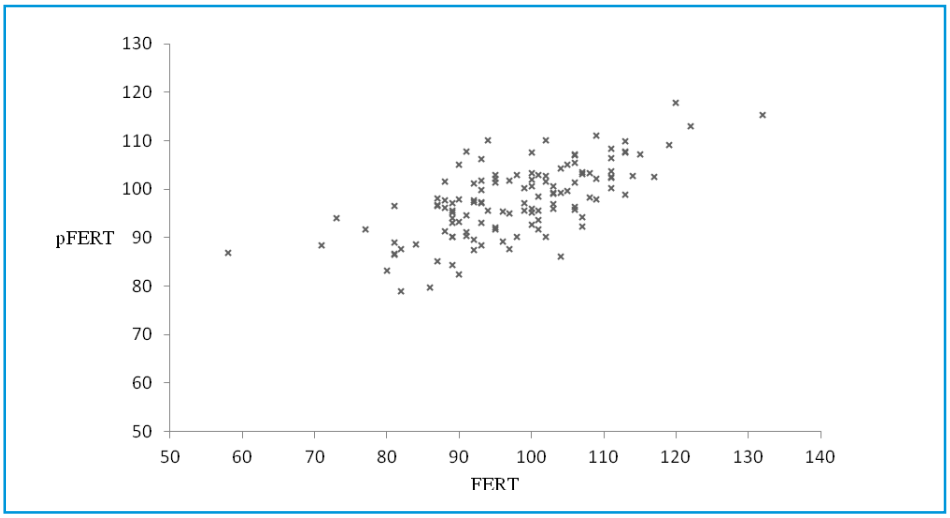

Figure 2. EBV for fertility (FERT) and predicted EBV fertility (pFERT) using milk MIR blood biomarkers (correlation between FERT and pFERT 0.67)

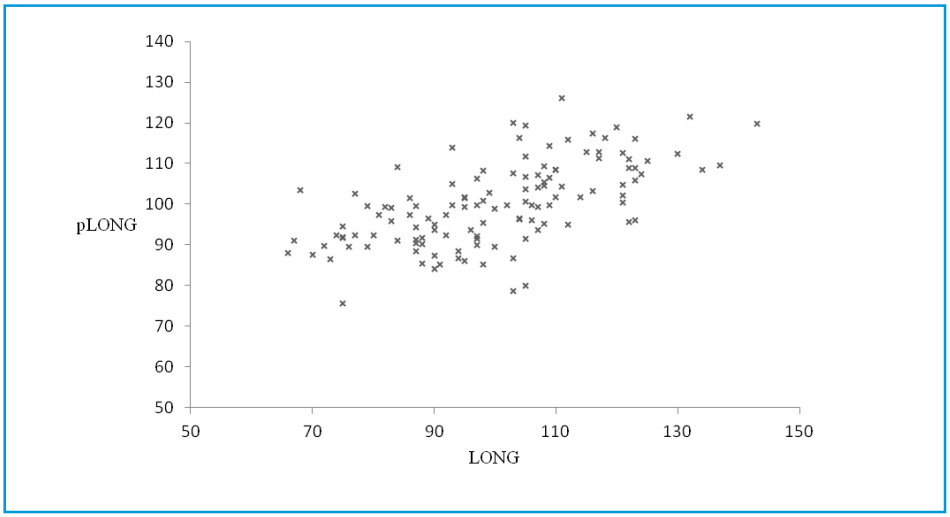

Figure 3. EBV for longevity (LONG) and predicted EBV longevity (pLONG) using milk MIR blood biomarkers (correlation between LONG and pLONG 0.62)

Results based on the 124 sires, PLS and a cross-validation strategy to retain results with the optimum number of latent variables, are in Table 4. Results based on R_{cv}^2 showed that the combination of two traits in the predictor was always a better choice than using UDH, FERT or pLONG alone. Moreover, combining the three was based on this criteria the best choice with an approximate genetic correlation between direct and indirect longevity of 0.76. Most studies reported lower values.

However, high correlations are not directly sufficient to compare predictors. Another point is the information content (linked to data and heritability) that indirect longevity contains. In this study, average REL were used to represent this (Table 4.). Results showed once more that the addition of pLONG has a substantial effect.

Table 4. Results under a cross-validation scenario obtained for different predictors of longevity (indirect longevity) based on udder health (UDH), fertility (FERT) and predicted longevity (pLONG) obtained from milk MIR predicted blood biomarkers (results reported for the 124 selected sires).

Traits used in the predictor	R_{cv}^2	r_{cv}	LV[1]	REL_PRED[2]	REL_ORG[3]
UDH	0.60	0.36	1	35.4	97.8
FERT	0.68	0.46	1	43.2	93.8
pLONG	0.62	0.38	1	32.8	86.5
UDH, FERT	0.72	0.52	1	49.7	95.7
UDH, pLONG	0.70	0.49	1	44.9	92.0
FERT, pLONG	0.74	0.55	2	49.8	90.9
UDH, FERT, pLONG	0.76	0.57	1	53.2	92.7

[1] LV = latent variable
[2] REL_PRED = theoretical average reliability in % associated to this (combination of) trait(s) when used as predictor of longevity
[3] REL_ORG = theoretical average original reliability in % associated to this (combination of) trait(s)

Conclusions and perspectives

The innovative use of PLS instead of classical selection index type regressions proved to be beneficial, limiting the effect of multicollinearity. There are indeed still opportunities to improve the selection index theory, especially when predictor traits in the information vector are extremely highly correlated.

This research showed that even lower accuracy milk MIR based blood biomarkers can make a useful contribution in the estimation of EBV for important breeding goal traits. The targeted combination of EBV for these milk MIR predicted blood biomarkers increased their potential contribution to breeding goal traits, and therefore, their usefulness for genetic evaluation. This research demonstrated this in the context of longevity. Even if the gains observed through approximate reliabilities of indirect longevity remain rather modest, one should not forget that MIR data is available very early; as soon as the cow is milked. This study used MIR spectral data from the first test-day on, so potentially for a two year old. This should have a relevant impact on genetic improvement.

The definition of milk MIR predicted blood biomarkers may still be preliminary. Currently, improvements of their calibrations are foreseen (C. Grelet, personal communication). Furthermore, the genetic model used to analyse the 7 blood biomarkers could be changed to a full 21 traits model. Alternative phenotype definitions could also help to better extract information from spectra. Moreover, it might be suboptimal to first do a phenotypic calibration from MIR spectra, then genetic evaluations on these calibrated traits. A direct massive multi-trait genetic model taking absorbances directly for given MIR wavenumbers as traits (Soyeurt *et al.*, 2010) could be a better approach. However, rank reduction techniques will be necessary to handle the very large number of traits.

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Defining and using novel milk composition based heat stress resilience traits in the context of genomic selection for more robust dairy cows in Wallonia

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Summary

Recent research showed the usefulness of using estimated breeding values (EBV) for mid-infrared (MIR) based biomarkers in genetic improvement. A novel class of biomarkers was defined based on modelling responses of milk composition (e.g., mid-infrared (MIR) based) to stress expressed on continuous scales using reaction norm models. Heat stress is an important aspect of dairy production even in temperate climates as shown in recent studies. Implementation of genomic selection for tolerance to heat stress is therefore not only an issue for Australian dairy cattle, a country that recently introduced such an evaluation. The question remains open if using milk composition based heat stress resilience genomically enhanced EBV (GEBV) is not a viable option. Genetic parameters were estimated for production, udder health, and milk composition traits. Data included 155,977 test-day records for milk, fat, and protein yields, fat and protein percentages, 9 individual milk fatty acids (FA), 7 FA groups, 5 minerals, and 3 ketone bodies predicted by mid-infrared spectrometry, and 7 FA groups. Data were from 21,375 first-lactation Holstein cows in 473 herds in the Walloon region of Belgium and were collected between 2008 and 2014. Test-day records were merged with daily temperature-humidity index (THI) values based on meteorological records from public weather stations. The maximum distance between each farm and its corresponding weather station was 13 km. Linear reaction norm models were used to estimate the intercept and slope responses of 23 traits to increasing THI values. Most yield and FA traits had phenotypic and genetic declines as THI increased, whereas C18:0, C18:1 cis-9, and 4 FA groups (unsaturated FA, monounsaturated FA, polyunsaturated FA, and long-chain FA) increased with THI. Moreover, the latter traits had the largest slope-to-intercept genetic variance ratios, which indicate that they are more affected by heat stress at high THI levels, and therefore good candidate traits. Among all traits, C18:1 cis-9 was the most sensitive to heat stress. As this trait is known to reflect body reserve mobilization, using its response to THI could be a very affordable milk biomarker of heat stress for dairy cattle, expressing the equilibrium between intake and mobilization, and therefore adaptation, under warm conditions. By including novel milk based composition traits to traditional production traits, correlations between EBVs and GEBVs of those milk based traits and udder health, fertility and longevity increased considerably. This study demonstrated that milk composition resilience heat stress traits could be used

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as early indicators of robustness traits. Our results also suggest that marker information tend to lead to higher accuracies of prediction. Therefore, new options to improve robustness through genomic selection in Walloon Holsteins are now available.

Keywords: Milk MIR spectra, resilience, genetic improvement, genomic selection.

Introduction

Robustness is a key element towards increased farm profitability through improved cattle health and welfare and is closely linked to increased adaptive capacity to climate disturbances. Animal breeding is used to improve animal features as needed for tomorrow. Genomic prediction is the method of choice to provide genetic merit estimates to breeders, especially for novel traits. It allows the identification of young animals quickly, especially for traits difficult to record such as resilience (e.g. resilience to thermal stress). Although few recent genomics studies have identified regions of the genome that appear to be involved with resilience, they were focused on limited populations and mostly used temperature humidity index (THI) with milk yield traits decays as proxy traits of resilience (Hayes *et al.,* 2009). Thermoregulatory and production responses to heat shock are only marginally related, and the biological basis of physiological changes is still elusive (Baumgard *et al.*, 2016). Phenotypes based on physiology, metabolism and health, when available, may be more accurate to identify valid genomic regions and genes associated with resilience to heat stress (Nguyen *et al.*, 2016).

Current advances in the use of mid-infrared (MIR) spectroscopy had allowed the large-scale accurate prediction of milk based biomarkers. Many of those milk MIR predicted biomarkers were found indicators of fertility and health traits reflecting the physiological and health status of the cow such as for the negative energy balance (e.g., fatty acids profile), for body fat mobilisation (e.g., C18:1 cis-9) and for (sub) clinical ketosis (e.g., acetone contents in milk) (Bastin *et al.*, 2016). The main objective of this study was to assess the potential of the integration of milk predicted phenotypes, meteorological, and genomic data for the selection of robust dairy cows in the Walloon Region of Belgium. For that we firstly evaluated the reaction of traditional milk production and novel milk phenotypes predicted from mid-infrared spectra to THI towards the identification of the potential novel milk composition based heat stress resilience traits. Conventional (EBV) and genomic (GEBV) slope breeding values for the high influenced predictors were estimated and investigated for their potential contribution to genetic improvement of robustness traits.

Materials and methods

Data

A total of 155,977 test-day records from 21,375 primiparous Holsteins routinely registered between 2005 and 2012 by the Walloon Breeding Association (AWE, Ciney, Belgium) were available. Each record included traditional milk records (milk yield, fat, and protein ratios) and Milk MIR spectra expressed in absorbance with 1,060 spectra points. Prediction equations were used for the prediction at each test-day of i) 9 individual fatty acids (FA): C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C18:1 cis9 and 7 groups of FA: saturated (SFA), unsaturated (UFA), monounsaturated (MUFA), polyunsaturated (PUFA), short chain (SCFA), medium chain (MCFA), and long chain (LCFA), ii) 5 minerals: calcium, phosphor, potassium, sodium, magnesium, and iii) 3 metabolites: β-hydroxybutyric acid, acetone, citrates.

The predictive ability of used calibration equations was described in Soyeurt *et al.* (2011) for FA, Soyeurt *et al.* (2009) for minerals, and Grelet *et al.* (2016) for metabolites.

Daily meteorological records were supplied by the Belgian Crop Growth Monitoring System consortium (Brussels, Belgium). Data was collected from meteorological stations located at 5.1 and 13.3 Km in average, and in maximum distances from the herds used in this study. Average 3 day lag of temperature humidity index (THI) value was assigned to each test day record from the nearest meteorological station to the herd. THI was calculated following NRC (1971):

$$\mathbf{THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]} \quad (1)$$

where **T** and **RH** are daily average temperature (°C) and relative humidity (%), respectively.

Aiming to obtain greater data consistency, retained datasets contained only data from cows with known parents, having at least 5 test days per lactation with known traditional production, MIR spectra and THI values for each test-day record. A minimum of 3 test days recorded under heat stress conditions (THI > 62), a threshold point for Holstein population in temperate region (Hammami *et al.*, 2013) was required to retain the lactation record.

Genotypes based on 54,609 SNP markers (50K chip) were acquired on 2,380 animals including 1,883 bulls and 497 cows. Genotype quality control edits were implemented and 28,796 (52.7%) SNP markers remained to estimate genomic relationship coefficients between animals. Pedigree information was recovered from historical breeding records from AWE, and was comprised of 67,907 individuals. A total of 63,037 animals remained after pruning.

Statistical analysis

To evaluate the impact of thermal conditions on traditional milk, traditional and predicted milk MIR-based traits at genetic level, individual reaction norm coefficients were estimated using random regression of the phenotypic values of the cows on THI. The magnitude of this impact was quantified by the genetic variation for the slopes of the linear reaction norms. Univariate linear reaction norm models were applied separately to each studied trait. The model can be written in matrix notation as follows:

$$\mathbf{y = Tt + Ll + Cc + Mm + Q_{hs}Ww + Q_{hs}(Z_1a + Z_2p) + e} \quad (2)$$

where **y** was the vector of observations; **t, l, c, m** were vectors for fixed effects: herd x test-date, lactation stage (classes of 15 DIM), classes of age at calving, classes of year x season of calving; **T, L, C, M, W** were respective incidence matrices linking fixed effects to **y; w** was the vector of the fixed second-order Legendre polynomial coefficients effects to model the population mean for standardized THI (range 28-78); $\mathbf{Q_{hs}}$ was the covariate matrix for first-order Legendre polynomials for standardized THI; **a** and **p** were vectors of random regression coefficients for additive genetic and environment permanent respectively; $\mathbf{Z_1}$ and $\mathbf{Z_2}$ being their respective incidence matrices linking them to random regressions for each records; and e was the vector of residuals.

We applied these analyses into two different scenarios; by including (**SC1**) or not (**SC2**) genotype information. EBVs in SC2 and GEBVs in SC1 were computed for each animal. A study was performed by using 177 genotyped bulls with 20 or more

daughters with records as reference population, whereas 1,601 young bulls with no progeny as validation population. Partial least square (**PLS**) analyses were performed for both scenarios in order to specify the existence of relationship between novel milk composition heat stress resilience traits (predictor variables) and udder health, longevity and fertility traits (response variables). To compare SC1 and SC2, we investigated GEBVs prediction efficiency across THI levels.

Results and discussion

Genetic and phenotypic variation of traditional milk production and novel MIR milk predictor traits under heat stress

Many studies focused mainly on the genetics analysis of production traits (milk, fat and protein) as associated to the meteorological information in the test date from the milk recording data (Ravagnolo & Miztal, 2000; Hayes *et al.*, 2009). All authors confirmed that there is:

1. Genetic and phenotypic variability in the response to THI;
2. An antagonistic relationship between the level (intercept) and the tolerance to stress (slope).

Table 1. Slope-intercept ratios for additive genetic (G) and permanent environmental (P) effects.

Traits	G variances ratios ($\sigma_a^2{}_{hs}$ / $\sigma_a^2{}_0$) (%)	P variances ratios ($\sigma_p^2{}_{hs}$ / $\sigma_p^2{}_0$) (%)
Production traits		
Milk (Kg)	0.073	0.237
Fat %	0.077	0.294
Protein %	0.065	0.285
Protein yield (Kg)	0.037	0.553
Fat yield (Kg)	0.028	0.704
Individual & groups of FA		
C18:1 cis9	0.105	1.341
C4:0	0.055	0.622
C18:0	0.051	0.684
C8:0	0.043	0.562
C6:0	0.041	0.588
C10:0	0.037	0.528
C12:0	0.035	0.533
C17:0	0.032	0.697
C14:0	0.027	0.558
C16:0	0.027	0.593
MUFA	0.079	1.212
LCFA	0.073	1.076
UFA	0.072	1.180
PUFA	0.038	0.669
SCFA	0.036	0.583
SFA	0.025	0.591
MCFA	0.024	0.592
Ketone bodies		
Acetone	0.108	1.132
BHB	0.071	0.630
Citrates	0.027	0.407
Minerals		
Ca	0.042	0.627
Mg	0.031	0.493
Na	0.026	0.477
P	0.023	0.462
K	0.021	0.526

The slope solution was identified as heat tolerance pseudo-phenotype. Baumgard *et al*. (2016) reported that reaction of production traits to heat loads do not fully capture the whole effect of heat stress. The ratio of slope to intercept variances of a trait measures the magnitude of the individual response to THI. In our study, ratios of specific milk predictors (Table1) were higher by 1.5 fold (for C18:1 cis-9, acetone), 1.1 for (MUFA, LCFA and UFA) compared with milk ratio. Those traits are indicators of the energy balance and body reserve mobilization. Based on experimental protocol and using metabolomics and lipidomics data, Tin *et al.* (2016) found also that FA (e.g. C18:1 cis-9, UFA) and ketone bodies (acetone, β-hydroxybutyrate) are those of the 10 biomarkers in milk that can reflect heat induced metabolomic alterations.

Table 2. Correlation between milk composition based heat stress resilience and robustness traits (RT).

RT	Scenarios	EBV	GEBV
Fertility	S^1	0.28	0.31
	S^2	0.30	0.33
	S^3	0.44	0.46
Longevity	S^1	0.31	0.31
	S^2	0.32	0.33
	S^3	0.39	0.39
Udder health	S^1	0.13	0.16
	S^2	0.14	0.16
	S^3	0.40	0.40

S^1: Milk, fat, and protein yields (Nguyen et al., 2016); S^2: S^1 + fat and protein percentage; S^3: S^2 + acetone, C4:0, C18:0, C18:1 cis-9, LFCA, MUFA and UFA.

Association between milk composition based on heat stress resilience and robustness traits

Correlations between traditional milk production traits EBVs (milk, fat, protein yields and fat and protein percentage) with fertility, longevity, and udder health ranged from 0.14 to 0.32 (Table 2). On the other hand, correlations between GEBVs were higher (range 0.16-0.33). By adding the fat and protein percentages in this study, our values are slightly higher than correlations reported by Nguyen *et al.* (2016).

Our study also demonstrated that by including novel milk based composition traits to traditional production traits, correlations between EBVs and also GEBVs of those milk based traits and udder health, fertility and longevity increased considerably (Table 2). In general, it was noticeable that adding genomic information enabled similar or even better predictions of the robustness traits (e.g., 0.46 vs. 0.44 for fertility; Table 2).

Conclusion

This study demonstrated that milk composition resilience heat stress traits could be used as early indicators of robustness traits. Our results also suggest that marker information tend to lead to higher accuracies of prediction. Therefore, new options to improve robustness through genomic selection in Walloon Holsteins are now available.

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Comparison of on-line SCC analysers and herd testing for estimating mastitis detection rates

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Summary

On-line somatic cell count (SCC) analysers automatically test for SCC during milking for individual cows, providing farmers with more measurements than conventional herd testing. The purpose of this study was to determine whether more frequent milk sampling increases detection of elevated SCC due to mastitis. We compared the rate of detection of mastitis events using on-line SCC analysers with that using conventional herd testing with a simulated dataset of 100 random herds of 1,000 cows. Five herd test scenarios (two, four, six, eight, or ten herd-test dates per lactation) were compared to 20 on-line SCC analyser scenarios (varying based on whether cows were milked once or twice daily and the proportion of on-line SCC analysers installed on bails, ranging from 2% (i.e. one in 50 bails) up to 100%). Random mastitis events for each cow were modelled based on composite cow SCC and individual quarter bacteriological data derived from a dataset of 2,345 cow-lactations. We then calculated the average probability of on-line SCC analysers and of herd testing detecting these mastitis events, assuming 280 days of lactation. Herd testing four or ten times per lactation was found to detect 47% or 75% of mastitis cases, respectively. On-line SCC analysers installed on 10% of bails with once-a-day milking detected 84% of cases. We observed that on-line SCC analysers, even on a small proportion of bails, were more likely to detect mastitis events than standard herd testing, in a simulated dataset.

Keywords: mastitis, on-line SCC analysers, herd testing, automated milk sensors, simulation, somatic cell count, bivariate distribution, copula.

Introduction

Analysis of milk from individual cows has traditionally been conducted in off-farm laboratories, using milk samples collected during herd testing. In New Zealand this is typically carried out four times per lactation, or every two months; in Australia and other countries, it may occur eight times per lactation, or monthly. In the last few decades, there has been noteworthy development of small automated devices, such as LIC Automation's CellSense®, which allow direct on-line analysis of milk on farm. These devices enable real-time reporting and detection of trends in somatic cell count (SCC) over the entire lactation as well as more collections and analyses of milk samples (Bewley, 2016).

Mastitis is a costly production disease, causing significant economic loss to dairy industries (Halasa *et al.*, 2007) due to decreased milk production, increased costs of veterinary services and treatment, extra labour, and replacement (Seegers *et al.*,

2003). Clinical mastitis is indicated by signs of disease in the animals or visibly abnormal changes in the milk, but subclinical mastitis is only recognisable through additional testing.

SCC is the standard proxy for subclinical infection. High values indicate mastitis (Moyes *et al.*, 2009). Bulk tank values greater than 400,000 cells/mL are often penalised by dairy companies (Valeeva *et al.*, 2007), while an individual cow threshold of 200,000 cells/mL is commonly used to indicate mastitis (Eberhart *et al.*, 1979; Harmon, 1994). Farmers use cow SCC data to monitor levels of infection in the herd, which informs treatment or culling decisions. Since herd testing in New Zealand typically occurs once every two months, mastitis might not be detected until weeks after the onset of infection. On-line SCC analysers take spot samples during milking, producing an SCC estimate for each cow at the time of milking and providing a more detailed picture of a cow's infection history.

In New Zealand, clinical mastitis is typically caused by *Streptococcus uberis* (SU) or *Staphylococcus aureus* (SA), with other species less commonly isolated (McDougall *et al.*, 2009; Bryan *et al.*, 2011). There are differences in duration, recovery time, and cell count response for each type of infection. *Streptococcus uberis* is found in faecal material, and so is associated more with the environment; intramammary infections are more prevalent during wetter periods, typically near the start and end of lactation. *Staphylococcus aureus* is a contagious pathogen characterised by infections of long duration with a high prevalence observed in late lactation (De Haas *et al.*, 2004).

This is the first study to combine real-world fortnightly and weekly herd test data with periodic bacteriology data from a New Zealand herd to evaluate typical mastitis start times and duration for different pathogen types. An empirical dataset, containing bacteriology data gathered over seven seasons from this well-recorded herd, was used to generate a simulation. Simulations compared the proportion of mastitis events detected using an on-line SCC analyser and using conventional herd testing. The proportions were calculated for all pathogen groups combined as well as separately for the SU and SA groups in order to determine whether pathogen type affected detection rates.

Materials and methods

Empirical data

The empirical dataset was obtained from bacteriology and SCC records, generated for 2,345 unique cows milked during the 2004, 2005, 2006, 2007, 2010, 2011, and 2013 seasons at DairyNZ Lye Farm (Waikato, New Zealand). Data from 2008, 2009, and 2012 were not included as large numbers of cows were enrolled into mastitis infection challenge studies and the subsequent infections were not representative of natural infections. Any one quarter of a cow's udder can be infected without spreading it to the rest; therefore, milk samples were collected from each quarter. Bacteriology data were determined using standard microbiology methods (Hogan *et al.*, 1999) on quarter foremilk samples. Milk samples were collected aseptically at four times during lactation: at the first milking after calving, at peak-mid lactation, at mid-end lactation and in the last seven days before dry off. Additional samples were collected from affected quarters if clinical mastitis was detected. Cow-lactations were retained in the dataset if sampled all four times, resulting in a dataset of 1,435 cow-lactations and 26,407 quarter samples over all seven seasons. SCC was measured by the routine herd testing, conducted weekly or fortnightly. A total of 30,442 herd test records were available, averaging 21 herd tests per cow-lactation.

Across all seven seasons, 65.99% of the 1,435 cow-lactations were bacteriologically-positive (testing positive at some point for a recognised mastitis pathogen). Retaining only these bacteriologically-positive cows generated a dataset

containing 947 cow-lactations, with 14.32% of quarters being bacteriologically positive at any point in time. The proportion of quarters infected with SU, SA, coagulase-negative *staphylococci* (CNS), and other types of pathogens (other) were 16.41%, 4.55%, 22.9%, and 56.1%, respectively, with the most frequent other pathogen isolated being *Corynebactetrium bovis* (38.31%). This study focused specifically on SU and SA as these are the most common pathogens associated with clinical mastitis in New Zealand and therefore more likely to cause a mastitis event associated with an elevated SCC or clinical episode (McDougall *et al.*, 2007).

Start dates and duration of mastitis events were based on weekly and fortnightly cell count data from herd tests and corresponding bacteriology data for each animal. An inflammatory event was defined as a period when the SCC was above 200,000 cells/mL, starting from the date on which the SCC first exceeded this threshold and ending when SCC dropped below it. A particular inflammatory event was defined as a bacteriologically-positive mastitis event (from hereon referred to as a mastitis event) if a positive bacteriology test occurred within 21 days either side of it. Mastitis events that were $\leq$ 14 days apart were merged into one event, and those at the end of lactation with a duration of < 14 days were arbitrarily assigned a duration of 14 days. When more than one bacterial species was associated with a single mastitis event, the event was assigned a bacterial species in the following order of dominance: SU, SA, CNS, and any other type of pathogen. A total of 790 subclinical and clinical mastitis events were detected over seven seasons: 228 events attributed to SU, 46 to SA, 171 to CNS, and 345 to other pathogens. Three subsets of the empirical data were used to build three separate simulations: i) All bacteria, ii) SU only, and iii) SA only.

Simulation

All calculations and statistical analyses were conducted using R software version 3.4.0 (R Core Team, 2017). To estimate the detection rate of mastitis by herd testing and by on-line SCC analysers, we simulated 100 herds of 1,000 cows assuming a total of 280 days in milk (DIM) for each cow. Three simulations were conducted, for i) All bacteria, ii) SU only, and iii) SA only. Individual mastitis events from the empirical data were taken as independent data points when fitting the marginal distributions. The distribution of DIM when mastitis was first detected was approximated by a distribution obtained from the sum of a gamma distribution (for the first half of the data) and a normal distribution (for the second half). A gamma distribution was also used to approximate the distribution of the duration of mastitis (except when modelling SA, for which the sum of a gamma distribution and a uniform distribution was used). Parameters for the marginal distributions were estimated based on the data using the fitdistr function in the MASS R package (Venebles and Ripley, 2013).

Plotting the data indicated non-independence of the duration of mastitis and the DIM when mastitis was first detected. Therefore, it was deemed more appropriate to sample from a bivariate joint distribution model than from the two marginal distributions independently. Copulas were used to characterise the dependence structure between the two marginal distributions. The BiCopSelect function from the VineCopula R package (Schepsmeier *et al.*, 2012) was used to determine the best-fitted copulas: Student t for SA and Frank for SU and all bacteria. We sampled from the resulting bivariate joint distributions to produce one single mastitis event during each cow's lactation (restricted to 280 days) in the 100 simulated herds.

Herd test dates for the 2015 season (7,267 farms) were extracted from the LIC herd recording database to simulate four herd test dates for each of the 100 simulated herds. The dataset was restricted to farms with four test dates and a first calving date between 2015-07-01 and 2015-08-31, reducing the number of farms in the dataset

to 2,895. To simulate herd test dates, we sampled from four uniform distributions centred around the four mean dates for each of the first to fourth herd tests. To simulate routines with more herd tests, uniform distributions were built around equally-spaced centres. The length of the range of each uniform distribution was 20% of the interval between adjacent centres.

Farmers may choose to install on-line SCC analysers in only a subset of bails. Therefore, we modelled on-line SCC analyser events for a range of proportions of bails with analysers (p). We assumed that each cow was equally likely to be milked at any bail on any given day. The probability of any given cow being milked at a bail with on-line SCC analysers on a particular day (P) was calculated as $P = p$ for cows milked once a day, or $P = 1 - (1 - p)^2$ for cows milked twice a day. On-line SCC analyser events (the analyser taking a sample at milking) were generated for each cow and each day when $U < P$, where U was independently sampled for each event from a random uniform distribution (range 0 to 1).

Obtaining simulated detection rates

Each cow in each simulated herd was modelled to have a single mastitis event during its 280 days of lactation. We then simulated two, four, six, eight, and ten herd tests for each herd and on-line SCC analyser events for one or two milkings, where p = 0.02, 0.025, 0.05, 0.1, 0.15, 0.2, 0.25, 0.50, 0.75, or 1.00. The average mastitis detection rates for all herds and animals were calculated for all herd test and on-line SCC analyser scenarios. Each of the three simulations used separate distributions of mastitis start dates and duration, based on each of the bacteria classes: i) All bacteria, ii) SU, and iii) SA.

Results

The duration of mastitis events was found to be dependent on the bacterial species and the DIM when they were first detected (Figure 1). Therefore, three bivariate distributions (duration and DIM) were designed for the three subsets of the empirical data: i) All bacteria, ii) SU only and iii) SA only.

The plots for the all bacteria simulation are shown in Figure 2. The parameters for the marginal distributions and the best-fitting copulas were selected based on maximum likelihood. The distribution of DIM (Figure 2A) was modelled as the sum of a gamma (shape =1.042, rate = 0.022) and a normal distribution (mean = 228.7, sd = 41.95). The distribution of duration (Figure 2B) was modelled as a gamma distribution (shape = 0.85, rate = 0.024). The empirical (Figure 2C) and modelled (Figure 2D) bivariate distributions (using a Frank copula (par = -0.35, tau = -0.04)) show that shorter mastitis events were concentrated at the start and end of lactation. The same process was applied to data based on SU infections only and SA infections only to create bivariate distributions based on a Frank copula (par = 1.64, tau = 0.18) for SU and a Student t copula for SA (par = -0.58, par2 = 3.01, tau = -0.4), respectively.

Within each simulation, the average detection rates were compared between herd testing scenarios and different on-line SCC analyser scenarios (Figure 3). As expected, detection rates improved with increasing frequency of herd tests. Similar detection rates were achieved for all bacteria and SU-only simulations and higher rates for SA-only simulations at all frequencies of herd testing. Mean detection rates for herd testing were 58%, 57%, and 73% for all bacteria, SU-only, and SA-only simulations, respectively.

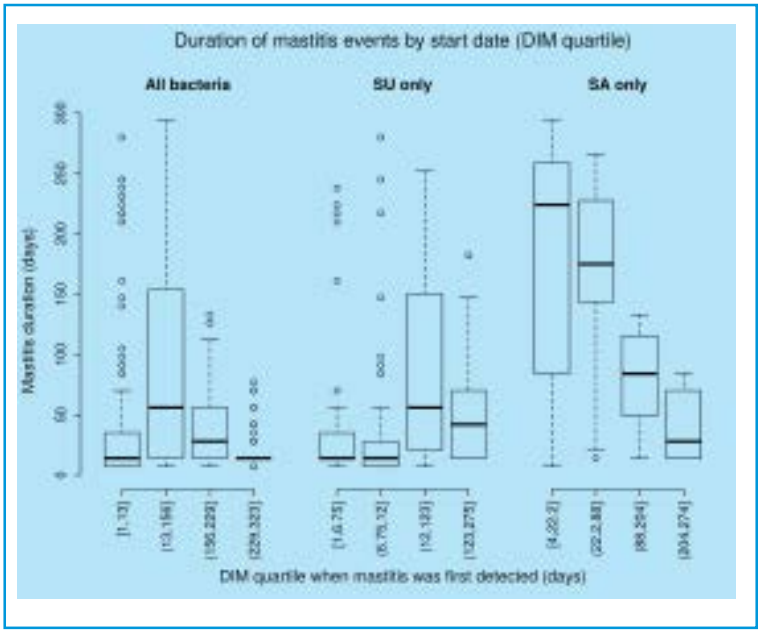

Figure 1. The relationship between mastitis duration and the DIM quartile when mastitis was first detected.

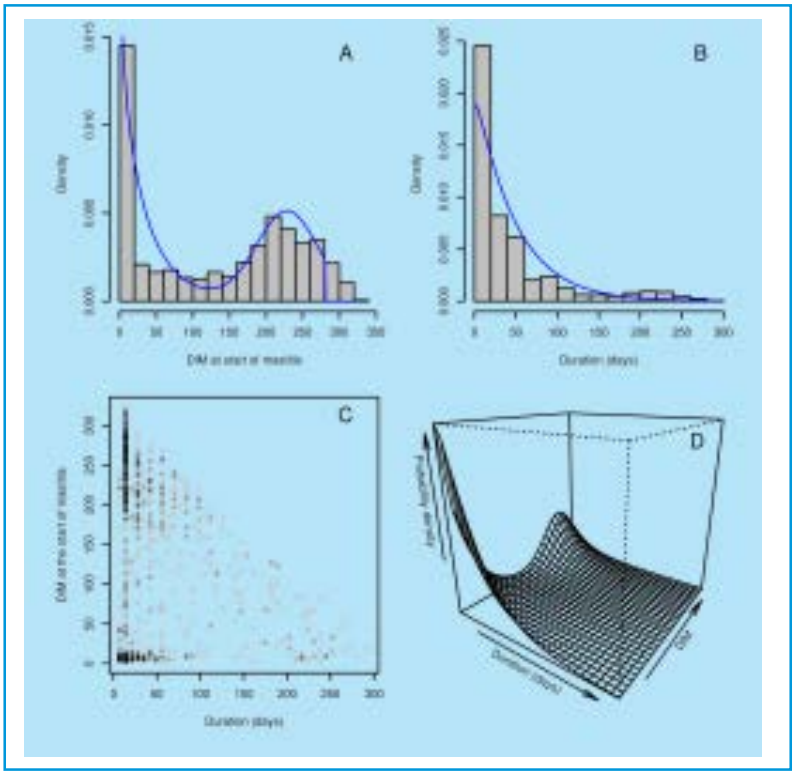

Figure 2. Plots for 'all bacteria' simulation: A) Marginal distribution of DIM when mastitis was first detected (grey bars) and the fitted distribution (blue line: sum of gamma and normal distribution); B) Marginal distribution of duration of mastitis and the fitted distribution (blue line: gamma distribution); C) Scatterplot of the relationship between DIM at the start of mastitis and duration; D) 3D density plot of the probability density function of the bivariate distribution generated using a Frank copula and the marginal distributions.

For on-line SCC analysers, higher detection rates were achieved in simulations of a higher proportion of bails installed, and of cows milked twice instead of once a day (Figure 3). As for herd testing, detection rates were similar for all bacteria and SU-only events, and higher rates observed for SA-only, with mean detection rates of 86%, 86%, and 92% respectively observed for the different simulations.

On-line SCC analysers installed on 2% of bails with once-a-day milking produced a similar detection rate to four herd tests for the all bacteria simulation (46.6% and 47%, respectively). With on-line SCC analysers installed on 10% of bails (still operated with once-a-day milking), a detection rate of 84% of all bacteria events was achieved, significantly higher ($P < 0.001$) than the detection rate of 75%, achieved with ten herd tests.

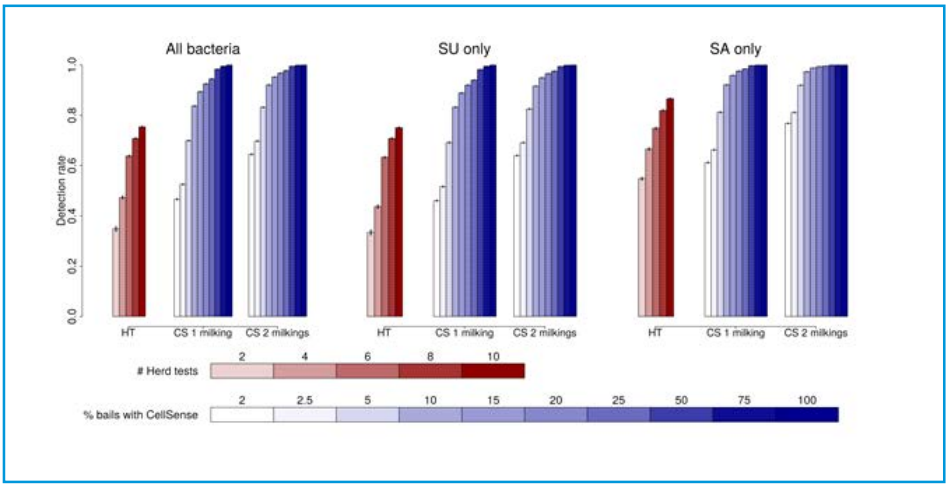

Figure 3. The average mastitis detection rate for different scenarios of herd testing and on-line SCC analysers calculated from 100 simulated herds of 1000 cows. Error bars represent 95% confidence intervals.

Discussion

This is the first New Zealand study to compare on-line SCC analysers and herd testing in detecting SCC events associated with mastitis. Using our modelling approach, we observed that an on-line SCC analyser approach was far more likely than conventional herd testing to detect individual mastitis events, due to the more frequent measurement of SCC. Mean detection rates of 86% and 58% were achieved by on-line SCC analysers and herd testing respectively for all bacteria. On-line SCC analysers installed on at least 10% of bails were more likely to detect mastitis events than any of the simulated scenarios of herd testing. On-line SCC analysers installed on just one out of 50 bails for once-a-day milking had detection rates comparable to four herd tests (both 47%).

This study illustrated a method for simulating mastitis events by using copula functions to link the marginal distributions of DIM when mastitis was first detected and mastitis duration to generate bivariate joint distributions. This bivariate copula model could be extended to become multivariate copulas, incorporating more parameters, which may make simulations more realistic. Copulas are widely used in a variety of disciplines, and their use has developed considerably in recent years (Salvadori and De Michele, 2007; Genest *et al.*, 2009; AghaKouchak *et al.*, 2010). They have been used to model mastitis in dairy cattle; for example, correlated infection times in the four udder quarters of cows have been modelled using four-dimensional copulas (Massonnet *et al.*, 2009;

Prenen *et al.*, 2017). Copulas are suitable for modelling complex biological systems where it is difficult to describe the dependency structure of the joint distribution. Their usefulness in modelling mastitis in dairy cattle should be explored further.

The epidemiology of mastitis varies between pathogen types (Zadoks *et al.*, 2011). It is for this reason that we built separate simulations for three pathogen groups. Indeed, prevalence, timing, and duration of mastitis differed depending on the type of infection. Cows infected with SU were more likely to develop mastitis earlier in lactation for shorter episodes, whereas mastitis in SA cows was more often contracted at the start and persisted to the end of lactation.

This study reported the combined prevalence of subclinical and clinical mastitis. The prevalence of SU in this herd was found to be higher than SA, consistent with previous reports from New Zealand herds (Compton *et al.*, 2007; McDougall *et al.*, 2009; McDougall *et al.*, 2010; Pankey *et al.*, 1996), although exceptions where prevalence of SA exceeds that of SU have also been reported (Bryan *et al.*, 2011). Subclinical and clinical mastitis were not modelled separately in the study due to a lack of available data; however, this would be a potentially interesting approach for future study should such datasets become available.

The prevalence of SA was lower in this herd (4.55% out of bacteriologically-positive quarters) than expected, based on the previously reported prevalence of clinical mastitis during lactation, estimated to be 10% among bacteria-positive glands in one New Zealand study (McDougall *et al.*, 2009). Good management practices on this farm may have resulted in a low prevalence of SA, resulting in a low contagious spread at milking time. The low prevalence of SA led to a scarcity of available data points, resulting in a less precise model for simulation compared to other types of infection.

For simplicity, only one mastitis event was allowed for each simulated cow-lactation; realistically, multiple events can take place, which should be explored in future simulation studies. Additionally, simulations were based on mastitis-positive simulated cows only; future simulations could model herds with both infected and uninfected cows based on the incidence rate in the empirical data. It was also assumed that SCC was elevated every time an animal became infected, which may not be so in all circumstances; for example, cows with weakened immune systems for a period post-calving may not respond with elevated SCC (Zadoks, 2006).

Some mastitis events were not observed for the full event time (right censoring); that is, some animals were lost from the herd, or dried off, or data collection ended before the mastitis event ended naturally. In these cases, total duration for an arbitrary 14 days was assumed. Durations in this study may be underestimated (especially near the end of lactation), and further investigation is required. The term “detection rate” was used to describe the proportion of on-line SCC analyser events or herd test events occurring simultaneously with simulated mastitis events. However, simulation did not take into account accuracy or bias of the two measurement methods; this should be incorporated into future simulations.

Mastitis is a multifactorial disease, and any modelling will inevitably simplify the biological system. In this study, we obtained sampled mastitis events directly from a bivariate distribution approximated using the empirical herd test and bacteriological data from a single farm in New Zealand. This approach captures the complexity of the disease by emulating real-world data. However, the restriction to a single farm as a basis for estimated mastitis parameters and distributions may make the study less applicable to other dairy herds due to variation in mastitis circumstances between farms in terms of climate, herd, and management (Hogeveen *et al.*, 2011). Also, assumptions were made where the literature did not fully describe certain parameters:

the minimum number of days between periods of high SCC for an event to be distinct, the window of time during which bacteriology tests were effective, and the order of dominance of bacterial species.

The time of mastitis detection affects the effectiveness of mastitis control at the herd level, as infections early in lactation can incur higher economic cost than later ones (Rollin *et al.*, 2015). Through increased frequency of testing, on-line SCC analysers are more likely to enable faster and earlier detection of mastitis events and, thus, earlier treatment and recovery or removal from the herd, ultimately reducing financial impact from the disease.

Conclusion

This is the first study to compare the potential detection rate for mastitis using on-line SCC analysers with herd testing. The results from this study demonstrate that installing on-line SCC analysers on even a small proportion of bails is more likely to allow detection of mastitis events than standard herd testing for all pathogen groups studied. Detection rates for both on-line SCC analysers and herd testing were higher in the SA pathogen group than in the all bacteria and SU pathogen groups, due to the persistence of SA infections. Future work could explore the economic and production benefits of on-line SCC analysers in providing SCC trends and earlier detection of mastitis.

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Reticulo-rumen temperature as a predictor of estrus in dairy Gir heifers

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Summary

Reticulo-rumen sensor data were used for estrus prediction in Gir heifers. Temperature-sensing reticular boluses were orally administered to 60 heifers, and the ruminal temperature (RRT) and animal activity were recorded every 10 minutes. The cows were synchronized using a hormonal protocol. Logistic regression with binary distribution was employed in the analysis to determine the response variables: Yi = 1 for presence, or Yi = 0 for the absence of estrus; using the GLM function of the R software. Two models were adjusted for data analysis: Model 1 considered only the effect of RRT as a dependent variable, while Model 2 included the effects of RRT and animal activity. The AIC inferred that Model 2 was the best fit regarding the data. The area under the ROC curve was used as an indicator of the model's ability to discriminate between non-occurrence (0) and occurrence (1) of estrus. Regarding the probability of correct estrus identification, Y2 retained a value of 0.64 and Y1 of 0.62. These values are considered low and evidence that the correct identification capability of estrus is reduced. In other words, the use of other approaches to determine estrus occurrence is required in order to improve model calibration and/or develop more robust methodologies.

Keywords: estrus, livestock precision farming, reproduction, ruminal boluses, zebu cattle.

Introduction

The Gir breed is an important genetic resource for milk production in the tropics. It is widely crossbred with Holstein cattle given its resistance to adverse conditions in the tropical environment. The animals of this breed are sexually late and have short and often nocturnal cycles, hindering the identification of estrus in commercial herds (Pires *et al.*, 2003).

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Precision technologies, such as temperature sensors, can assist in estrus identification. The body temperature increases in 0.4ºC during the estrus of cows (Cooper-Prado *et al.*, 2011; Adams *et al.*, 2013). Temperature-sensing reticulo-rumen boluses can be employed to continuously monitor body temperature variations of animals during time, aiding in the detection of estrus (Sieves *et al.*, 2004), calving (Costa Jr. *et al.*, 2016), and other physiological events. The adoption of precision technologies enables to phenotype traits related to the sexual precocity of zebu females and to take measurements that are associated with postpartum reproductive activities, providing significant information for the selection of these traits in zebu cattle.

Most of the studies that utilized ruminal sensors to predict estrus were conducted using taurine breeds, which exhibit distinct estrus behavior when compared to zebu cattle (Pires *et al.*, 2003). Therefore, the prediction equations developed for taurine breeds may result in different performance values than zebu cattle, leading to low efficiency in the detection of estrus in these animals.

The objective of the present study was to utilize the data obtained from reticulo-rumen sensing for the prediction of estrus in Gir breed heifers.

Material and methods

Temperature-sensing reticular boluses (TX-1442, Smaxtec Animal Care, Austria) were orally administered to cows using a custom bolus gun in the ruminal region of 60 Gir dairy heifers. The sensors retain a temperature measurement range of 0°C to 50°C and an activity index of 0 to 100%. The reticulo-rumen temperature variation (RRT) and animal activity were recorded every 10 minutes.

The RRT data were collected using a telemetry system, through an antenna, with a reading range of 30 meters. The antenna was allocated in the pen where it gathered the RRT data from the animals while they were handled. The readings were sent via radio signal and accessed using the Smaxtec Messenger online software (Smaxtec Animal Care, Austria).

After a 45-day adaptation period, the heifers were synchronized regarding estrus using the following hormonal protocol: D0 - Application of the Intravaginal Progestogen implant (P4) + benzoate (BE); D7 - Application of Prostaglandin (PGF); D9 - Application of Cypionate of Estradiol (CP) + Equine Chorionic Gonadotropin (ECG) + removal of the implant; D11 - Realization of Artificial Insemination + use of the Gonadotropin-releasing Hormone (GnRH).

Behavioural observations were made every 10 minutes during 48 hours, starting two hours after the removal of the Intravaginal Progestogen implant. The sexual behaviour related to estrus consisted of mounting acceptance. Ultrasound imaging was used to identify animals that ovulated after 60 hours following implant removal. A total of 59 heifers ovulated.

During the statistical analyses, all temperature data inferior to 37.72°C were discarded, given the low temperature is associated with water consumption (Bewley *et al.*, 2008). A total of 19,660 records regarding stored information on reticulo-rumen temperature and activity were used.

In order to evaluate the optimal model for prediction capability, the animals were divided into five groups (four groups of 12 cows, and one with 11) at random. The analyses were repeated five times and, in each round, a different group was considered as a validation population. Data analysis relied on the use of a logistic regression model with binary distribution to determine the response variables (Yi = 1 for presence, or Yi

= 0 for the absence of estrus), using the GLM function of the R software. Two models were adjusted for data analysis: Model 1 included only the RRT effect as a dependent variable, while Model 2 involved the effects of RRT and animal activity.

Additionally, the response variable (presence or absence of estrus) was defined by two distinct forms (Y1 and Y2). In Y1, the presence of estrus (1) was considered during the interval between 32 and 45 hours after the removal of the progesterone implant, assuming that zebu females have an average estrus duration of 13 hours (Pires *et al.*, 2003). In turn, in Y2, the presence of estrus was associated with the period from the first to the last accepted mounting, based on behavioural observations. Models 1 and 2 were compared trough Akaike's information criteria (AIC) with Y1 or Y2 as variable responses. The best model was used to determine the occurrence of estrus in the validation population, and the prediction accuracy was calculated as the correct number of predictions considering the total number. The ROCR package of the R software was employed to construct ROC curves, which are used to evaluate the model's ability to predict estrus. When the area under the curve is closer to 1.0, the probability of the occurrence of true-positive results is greater.

Results and discussion

The observations showed that estrus occurred between 30 and 48 hours after implant removal. Diskin *et al.* (2002) stated that the synchronization, combining progesterone and PGF, led to the occurrence of a considerable number of cows in estrus between 36 and 60 hours after the removal of the progesterone implant. The earlier observation of the receptivity period may be attributed to the application of ECG at the time of implant removal, which may have anticipated the occurrence of estrus (Baruselli *et al.*, 2008).

The AIC values indicated that Model 2, which associated the ruminal temperatures with the activity of the animals, provided better fit than Model 1 (only RRT) regarding Y1 and Y2 (Table 1). The inclusion of activity in the model improved the adjustment. Some authors obtained similar results, in which increased activity of the animals was observed during the estrus when compared to other stages of the reproductive cycle (Lovendahl & Chagunda, 2010).

Yoshioka *et al.* (2010) observed a positive correlation between increased activity with estrus and ovulation in beef cattle. When using body temperature sensors, previous studies showed that the ruminal temperature of the animals increased in 0.3°C to 0.7°C during the estrus (Bailey *et al.*, 2009; Cooper-Prado *et al.*, 2011; Adams *et al.*, 2013). These results corroborate with the present study, in which the model that considered the two variables (temperature and activity) exhibited optimal fitness.

Table 1. AIC values employing training populations and estrus period or mounting acceptance as response variables, considering reticulo-rumen temperature (RRT) or reticulo-rumen temperature+activity (RRT+A) as independent variables.

Training population	Estrus Period		Mounting acceptance	
	Model 1 RRT	Model 2 RRT+A	Model 1 RRT	Model 2 RRT+A
1	17251	17014	11686	11469
2	17162	16951	10823	10737
3	17552	17265	11087	10862
4	16849	16564	10821	10655
5	17685	17428	11676	11521
Mean	17300	17044[1]	11219	11049*

[1]Best values according to the AIC.

Considering Model 2 individually, the validation population data was used to test the prediction ability of the model. The area under the ROC curve (AUC) is an indicator of the model's ability to discriminate between the non-occurrence (0) and occurrence (1) of the event (Table 2). An AUC that was higher than 0.50 indicated that the discrimination was not at random. Thus, regarding Y1, the probability of correctly identifying the estrus (true-positives) was 0.62 on average, while the identification probability of false-positives was 0.38. For Y2, the probability of correctly identifying the estrus was slightly improved (0.64 on average), and the probability of false-positives was of 0.36. These values are considered small and demonstrate how the correct identification capability of estrus remains reduced. However, the better definition of the response variable, such as the use of hormonal dosages for the determination of estrus occurrence, is believed to result in an enhanced calibration of the model for the prediction of estrus in zebu cattle.

Table 2 - Parameter estimates and standard errors (between brackets), prediction accuracy, and area under the ROC curve of the estrus period (Y1) or mounting acceptance (Y2) traits regarding Model 2.

Training population	Estimates*			Accuracy	Area under ROC curve
	Intercept	RRT	Activity		
Estrus Period (Y1)					
1	-11.00 (1.66)	0.24 (0.04)	0.045 (0.003)	0.77	0.65
2	-15.26 (1.72)	0.35 (0.04)	0.042 (0.003)	0.78	0.64
3	-17.09 (1.70)	0.39 (0.04)	0.050 (0.003)	0.73	0.60
4	-13.28 (1.71)	0.29 (0.04)	0.049 (0.003)	0.73	0.62
5	-16.10 (1.66)	0.39 (0.04)	0.0450 (0.003)	0.76	0.58
Mounting Acceptance (Y2)					
1	-29.25 (2.17)	0.68 (0.05)	0.053 (0.003)	0.92	0.64
2	-29.68 (2.36)	0.69 (0.06)	0.036 (0.004)	0.87	0.72
3	-43.40 (2.316)	1.03 (0.06)	0.058 (0.004)	0.86	0.52
4	-29.70 (2.29)	0.68 (0.06)	0.049 (0.004)	0.86	0.68
5	-31.65 (2.19)	0.74 (0.05)	0.045 (0.003)	0.89	0.66

*P values < 2e-16.

The accuracies for Y1 and Y2 were 0.75 and 0.88 in average, respectively (Table 2). The lower accuracy of Y1 can be attributed to the subjectivity of the variable since Y1 definition was based on the expected range in time according to the hormonal protocol, and Y2 was based on the observed behaviour. The artificial induction of luteal regression in reproductive protocols, with the use of prostaglandin, can result in lower proportions of synchronization than expected (< 65%), and the estrus can occur in up to 130 hours following treatment (Viana *et al.*, 1997). On the other hand, mounting acceptance is the leading indicator of estrus in zebu cows (Lamothe-Zavaletha *et al.*, 1991).

The model that used the information regarding ruminal temperature and animal activity displayed improved fitness.

The probability of the detection of estrus (true-positives) was similar for both response variables. However, the values indicated the occurrence of errors in estrus detection. Therefore, it is necessary to use other approaches to improve the calibration of the model or to use more robust methodologies.

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Use of body measurements and ultrasound to predict carcass yield in Nelore cattle

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Summary

The objective of this work was to identify which variables are related to the carcass yield, using path analysis., 120 nelore steers were put in a feedlot system until reaching the average weight of 570 kg. At the end of the feeding test the animals were weighed and there were obtained the biometric measurements: height at withers (HW), height at the rump (HR), thoracic perimeter (TP) and body length (BL) and were obtained longissimus muscle area (LMA), subcutaneous fat thickness (FT). After slaughter, information on hot carcass weight and carcass yield (CY) were recorded. The data were analysed by path analysis with the CALIS procedure of SAS (SAS Inst. 9.4). The results showed that LMA is the main variable responsible for the CY variation. The body measurements and FT did not present significant effects on the CY. Negative association were observed between CY and TP/ FT. In view of these results, it is important to collect more data before an adequate model to predict carcass yield can be stablished and used for selection purposes.

Keywords: beef cattle,rib eye area, carcass weight, path analyses.

Introduction

Acording to the literature, (Fernandes *et al.*, 2010; Mourão *et al.*, 2010; Cyrillo *et al.*, 2012) beef cattle linear body measurements appear as a reasonable tool for predicting live weight and the slaughter point of the animals, especially for many producers who do not have access to the carcass ultrasound technique, either for economic reasons and / or property infrastructure. In zebu cattle, including Nelore – the most important beef breed in Brazil, works relating production traits with body dimensions are frequent, but few studies have analyzed the correlations between biometric measurements and carcass yield (CY). The objective of this work was to identify which variables are related to the carcass yield, using path analysis.

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Material and methods

The present study, including 120 nelore steers, was developed at Cachoeirão Farm, located in Bandeirantes, Mato Grosso do Sul State. At the beginning of the experimental phase, the animals, with an average age of 20 months, were grouped in two batches of 60 animals each one, according to live weight. After this, they were put in a feedlot system being submitted to the same sanitary and nutritional management, until reaching the average weight of 570 kg. At the end of the feeding test the animals were weighed and there were obtained the biometric measurements: height at withers (HW), height at the rump (HR), thoracic perimeter (TP) and body length (BL) and were obtained longissimus muscle area (LMA), subcutaneous fat thickness (FT). After slaughter, information on hot carcass weight and carcass yield (CY) were recorded. The data were analysed by path analysis with the CALIS procedure of SAS (SAS Inst. 9.4). Data analysis adopted is a causal diagram illustrating the carcass yield as basic variable and the others as explanatory variables.

Results

The results showed that LMA is the main variable responsible for the CY variation. (Figure 1). The body measurements and FT did not present significant effects on the CY (0.20). Negative association were observed between CY and TP/ FT, -0,10 and -0.12, respectively. Rosa *et al* (2014) analized 35 Nellore bulls, confined for 96 days, with 402 ± 14.90 kg and 18 months old and noted that measures of loin eye area evaluated by ultrasonography, it were found positive correlations with body length (0.32), rump (0.36) and thigh (0.20); withers height (0.20) and pelviano contour (0.38) (P<0.05). Variables of hip height and chest, chest width and pin bones, and heart girth showed positive correlations with two or more productive traits of economic interest, such as slaughter weight, hot carcass weight and dressing percentage (P<0.05).

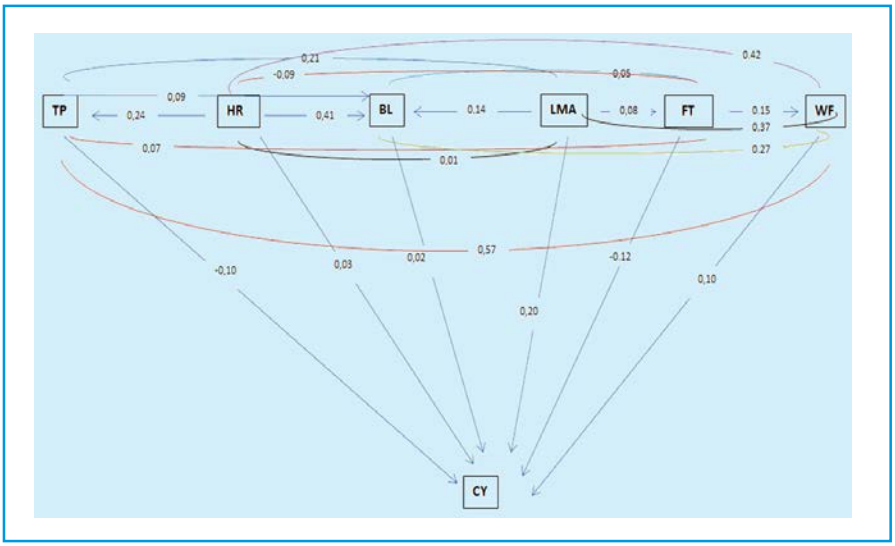

Figure 1. Diagram illustrating the carcass yield as basic variable and the others as explanatory variables.

Conclusion

In view of these results, it is important to collect more data before an adequate model to predict carcass yield can be stablished and used for selection purposes.

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Population dynamics and size stratification in 75-day old *Clarias gariepinus juveniles*

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Summary

The greatest cause of economic losses in aquaculture has been traced to cannibalism and aggressive behaviour which have been more frequently exhibited by Catfish due to its omnivorous nature, as a result of disparity in sizes, sex ratio and/or stocking density. The aim of the aquaculturist is to produce fast growing fry and fingerlings of comparatively uniform sizes in order to avoid cannibalism in the hatchery. This study therefore aims to investigate population dynamics and size stratification within the Clarias population bred in artificial tanks.

The study was carried out at SEJ Farm Ventures in Torikoh, Badagry, Lagos State, Nigeria, located at latitude 6° 28.598' N and longitude 02° 54.440' E of the equator with an average annual rainfall and temperature of 1693mm 27.0°C respectively.

A total of 250, 75-day old juveniles were randomly sampled from the tank using a hand net and isolated for measurements. Data was collected on live body weight and linear body measurements which include Total Length (TL), Standard Length (SL), Head length (HL), Pre Dorsal Length (PDL), Dorsal Fin Length (DFL) Pre Anal Length (PAL) and Anal Fin Length (AFL). Two indices (length-weight relationship and Fulton's condition factors) were also computed based on the measures for predictive assessment of future performances and wellness of the fish.

The minimum and maximum fish weight recorded was 3.30g and 20.30g respectively with a range of 17.0g. The overall (n = 250) mean ± SE for body weight was 10.61±0.28g. The Sturge's formula was used to construct nine class intervals of 2.0g width each, with the grouping resulting in a disproportionate frequency distribution which statistically (P<0.0001) deviated from expected proportional frequencies across the nine sub-groups. Of all the variables measured, weight had the highest variability within the population with a CV of 41.88%, while other measures had CV of between 13.82% and 16.65%.

Although all measures recorded are within the normal distribution without an outlier value, it was observed that based on the mean body weight of the fish studied, only 6.4% are within the 95% Confidence Interval (CI) of the Mean, while 53.2% and 40.4% are respectively below and above the CI.

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The stratification and population structure provide a good discriminatory tool in separating the fish into fairly homogenous sizes for further rearing to minimize cannibalism and optimize profit.

Keywords: catfish, heterogeneity, body measurements, wellness, Nigeria

Introduction

Protein from animal sources are in short supply in Nigeria as a result rapid increase in human population (FAO, 2006), which has led to increase in the demand for fish, the cheapest and most available source of animal protein to supplement the needed animal protein intake. Fish remains the highest contributor of animal protein in Nigeria accounting for over 34% of all the animal protein sources in the country (Edwin *et al.*, 2009).

Due to the reckless fishing methods and destruction of the natural environment, there is need to artificially propagate fish seeds. Thus, the culture of fish has become an innovative technology aimed at producing large quantity of fish as food for the ever-increasing human population in Nigeria. In order to meet the high demand for fish, aquaculture which is the rational rearing of fish in an enclosed and fairly shallow body of water remains the best option to bridge the wide gap between fish demand and domestic production in most countries of the world especially sub Saharan Africa (Dauda *et al.*, 2013). Aquaculture, which could be practiced by artificial methods, most especially to produce fish on large-scale basis in and out of season to ensure regular supply all year round, constitute the major practicable means of providing enough quality seed for rearing in confined fish enclosure such as fish ponds, reservoirs and lake (Charo and Oirere, 2000).

Catfish (*Clarias gariepinus*, Burchell, 1822), one of the most commonly cultivated species of fish provides food for the populace and allows for improved protein nutrition because it has a high biological value in terms of high protein retention in the body, higher protein assimilation as compared to other protein sources, low cholesterol content and one of the safest sources of animal protein (Anoop et al., 2009). However, being omnivores, cannibalism and aggressive behaviour have been more frequently exhibited by this specie as a result of disparity in sizes, sex ratio and/or stocking density.

Since the aim of the fish culturist is to produce fish that grows to table size using most minimal of inputs to maximise profit, it is necessary to know which cluster of fingerlings to pick as brood stock for the production of fast growing fry and fingerlings of comparatively uniform sizes and also avoid cannibalism in the hatchery. This study therefore aims to investigate growth dynamics and size stratification within the Clarias population bred in artificial tanks.

Material and methods

Study site

The study was carried out at SEJ Farm Ventures in Torikoh, Badagry, Lagos State, Nigeria. The farm is located at latitude of 6° 28.598' N and longitude of 02° 54.440' E of the equator. The average annual rainfall and average temperature in Lagos are 1693mm and 27.0°C respectively.

All measurements were taken at the farm and further analyses were conducted at the Department of Zoology and Environmental Biology, Lagos State University, Ojo Lagos, Nigeria.

Experimental units

The African catfish (*Clarias gariepinus*) broodstocks (one male and one female) used in the production of the new seed (hatchling) were from diverse lineage to avoid inbreeding. The hatchlings were raised to 75 days in artificial tanks under intensive management.

All hatchlings were subjected to the same experimental conditions and were fed thrice daily throughout the study period.

Data collection

A total of 250 juveniles were randomly sampled from the tank using a hand net and isolated for measurements. Each fish sampled was measured for the variables studied

Measurements

Data was collected on live body weight and linear body measurements. The weight of the fish was taken using professional digital scale sensitive to 0.01 grams. A flex graduated tape was used to obtain the linear body measurements (Figure 1).

Aside Body Weight (BW), seven morphometric measurements were taken on each fish, which include Total Length (TL), Standard Length (SL), Head length (HL), Pre Dorsal Length (PDL), Dorsal Fin Length (DFL) Pre Anal Length (PAL) and Anal Fin Length (AFL).

Computed indices

Based on the various measurements taken on individual fish, two distinct indices were computed to aid in the appraisal of the wellness of the fish and its potential for later growth and development.

Length weight relationship

Length-weight relationship was expressed as $W = aL^b$, the logarithm transformation of which gives the linear equation $LogW = a + b\ logL$, where W = Weight in gram, L = length in (cm), a = a constant being the initial growth index, and b = growth coefficient. Constant 'a' represents the point at which the regression line intercepts the y-axis and 'b' the slope of the regression line.

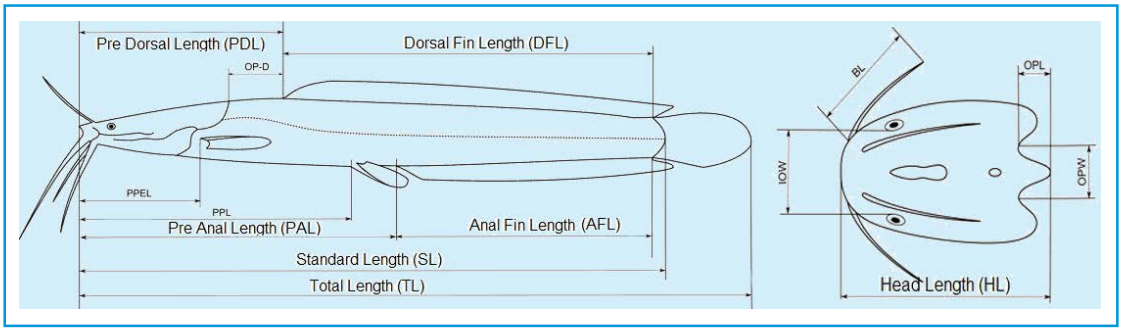

Figure 1. Diagram of Morphometric Measures Studied (Adapted from Agnese et al., 1997).

Condition factor

The condition factor (K) which is defined as the well-being of the fish was calculated. K is a useful index for monitoring of feeding intensity, age, and growth rates. The condition factor is to quantify the health of individuals in a population or to tell whether a population is healthy relative to other populations Stevenson and Woods (2006). The K was determined by:

$$K= W.100L^{3}$$

where W = length of fish in grams and L = Length of fish in centimeters.

Statistical analyses

A preliminary exploratory statistical analysis was conducted on each of the eight variables measured to test for normality and outlier values.

Based on the values obtained on body weight which is the most important attribute of fish in economic terms, the entire population was classified into nine (9) categories of fairly homogenous weights. This classification is based on Sturges (1926) rule of $K = 1+3.322(\log_{10} n)$ where K is the number of class intervals and n is the sample size. The classes are labelled A – I with each class having a width of 2.0g as follows; A (3.1 – 5.0), B (5.1 – 7.0), C (7.1 – 9.0), D (9.1 – 11.0), E (11.1 – 13.0), F (13.1 – 15.0), G (15.1 – 17.0), H (17.1 – 19.0) and I (19.1 – 21.0) respectively.

Descriptive statistical measures of all variables were obtained along with a multivariate correlation matrix for all variables. Due to the very correlation between weight and total length, a regression analysis was conducted to examine the length-weight relationship. A non-parametric was also done to evaluate the deviation of the disproportionate nine categories from an expected uniform sample sizes per category. A one way analysis of variance using group as the predictor variable was done for all variables and a Tukey test was conducted for further mean separation.

All statistical analyses were done using the Minitab 17® (2013) Statistical Software.

Results and discussion

The weight of fish in this study range between 3.30g and 20.30g with a mean $\pm$ SE of 10.61 $\pm$ 0.28g (Tables 1a and 1b). The nine class intervals have a width of 2.0g and the histogram with a fitted normal curve for both weight and total length is presented in Figure 2. Expectedly, all values increase as we go down the various groups with the first group having the least, while the last group had the highest values.

Only 6 percent of the fish studied have weights within 95% Confidence Interval of the entire population, while 53.2% and 40.8% were below and above the Confidence Interval respectively. This implies that variability in fish weight was more pronounced in the lower class intervals than the higher class intervals.

The differences between the groups for all measured variables were highly statistically significant ($P<0.01$) except for some measures in the heavier categories (Tables 1a and 1b) that were not statistically different ($P>0.05$) after a post-hoc test.

Table 1a. Mean p SE of some measured variables[1].

Group	N	Weight (g)	Total Length (cm)	Standard Length (cm)	Head Length (cm)
A	12	4.61±0.15^{i}	8.88±0.11^{h}	7.67±0.13^{g}	2.13±0.05^{g}
B	62	6.06±0.07^{h}	9.54±0.05^{g}	8.41±0.05^{f}	2.35±0.03^{d}
C	44	8.03±0.09^{g}	10.21±0.06^{f}	9.00±0.06^{e}	2.55±0.04^{e}
D	28	9.95±0.11^{f}	11.09±0.08^{e}	9.87±0.07^{d}	2.77±0.04^{d}
E	24	12.09±0.12^{e}	11.71±0.07^{d}	10.44±0.06^{c}	2.99±0.04^{c}
F	22	13.95±0.13^{d}	12.48±0.09^{c}	11.18±0.08^{b}	3.16±0.04^{bc}
G	30	16.16±0.09^{c}	12.97±0.08^{b}	11.61±0.11^{a}	3.32±0.04^{ab}
H	23	17.80±0.10^{b}	13.37±0.08^{a}	11.87±0.09^{a}	3.22±0.08^{b}
I	5	19.66±0.23^{a}	13.54±0.28^{ab}	12.14±0.23^{a}	3.64±0.05^{a}
Overall	250	10.61±0.28	11.11±0.10	9.86±0.09	2.78±0.03

[1]Means with different superscript within the same column differs significantly (P<0.05)

Group alone accounted for more than 70% of variability in measured variables with the highest effect recorded in fish weight (98.50%) and the least recorded in Head Length (74.73%).

The very high positive correlation amongst the variables (Table 2) is indicative of the strength of relationship between the variables and calls for caution in modelling for weight in order to avoid multicollinearity in the model. All the pairwise correlations are high, direct (positive) and are highly significant (*P*<0.01).

The disproportionate subclass sizes recorded across the nine groups was statistically significant (*P*<0.01) with the largest deviation recorded in groups A, B, C and I (Figure 3). While groups A and I fell short of expected frequencies, groups B and C contributed far beyond and above the expected frequencies accounting for almost 64% of the

Table 1b. Mean p SE of some measured variables[1].

Group	N	Pre Dorsal Length (cm)	Dorsal Fin Length (cm)	Pre Anal Length (cm	Anal Fin Length (cm)
A	12	2.55±0.04^{h}	4.85±0.08^{g}	3.99±0.05^{g}	3.39±0.06^{g}
B	62	2.76±0.02^{g}	5.35±0.03^{f}	4.34±0.03^{f}	3.77±0.03^{f}
C	44	3.00±0.02^{f}	5.79±0.04^{e}	4.68±0.04^{e}	4.03±0.04^{e}
D	28	3.24±0.03^{e}	6.28±0.05^{d}	5.10±0.07^{d}	4.31±0.04^{d}
E	24	3.45±0.03^{d}	6.71±0.06^{c}	5.47±0.05^{c}	4.56±0.05^{c}
F	22	3.64±0.04^{c}	7.15±0.05^{b}	5.81±0.05^{b}	4.84±0.05^{b}
G	30	3.83±0.03^{b}	7.43±0.06^{a}	6.14±0.05^{a}	5.06±0.04^{a}
H	23	4.01±0.05^{a}	7.58±0.06^{a}	6.17±0.09^{a}	5.20±0.04^{a}
I	5	4.20±0.08^{a}	7.74±0.12^{a}	6.48±0.10^{a}	5.28±0.11^{a}
Overall	250	3.26±0.03	6.30±0.06	5.13±0.05	4.34±0.04

[1]Means with different superscript within the same column differs significantly (*P*<0.05).

Table 2. Correlation amongst variables studied[1].

	Total length	Standard length	Head length	Pre dorsal length	Dorsal fin length	Anal fin length	Pre anal length
Weight	0.9696	0.9590	0.8503	0.9438	0.9561	0.9246	0.9432
Total Length		0.9847	0.8705	0.9485	0.9636	0.9296	0.9516
Standard Length			0.8812	0.9456	0.9630	0.9220	0.9522
Head Length				0.8902	0.8687	0.8055	0.8787
Pre Dorsal Length					0.9251	0.9069	0.9169
Dorsal Fin Length						0.9210	0.9398
Anal Fin Length							0.8535

[1]All correlations are highly statistically significant (*P*<0.01).

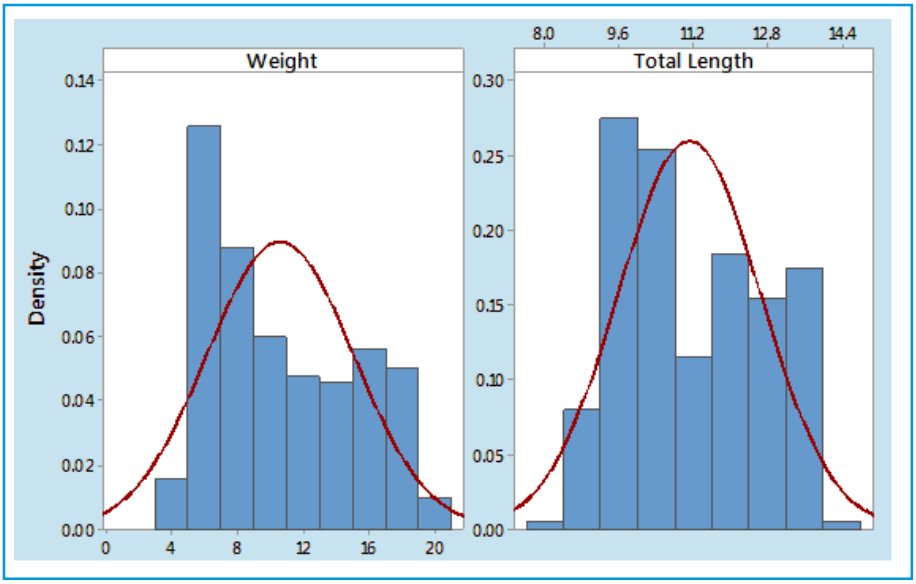

Figure 2. Histogram of weight (g) and total length (cm) across the nine groups.

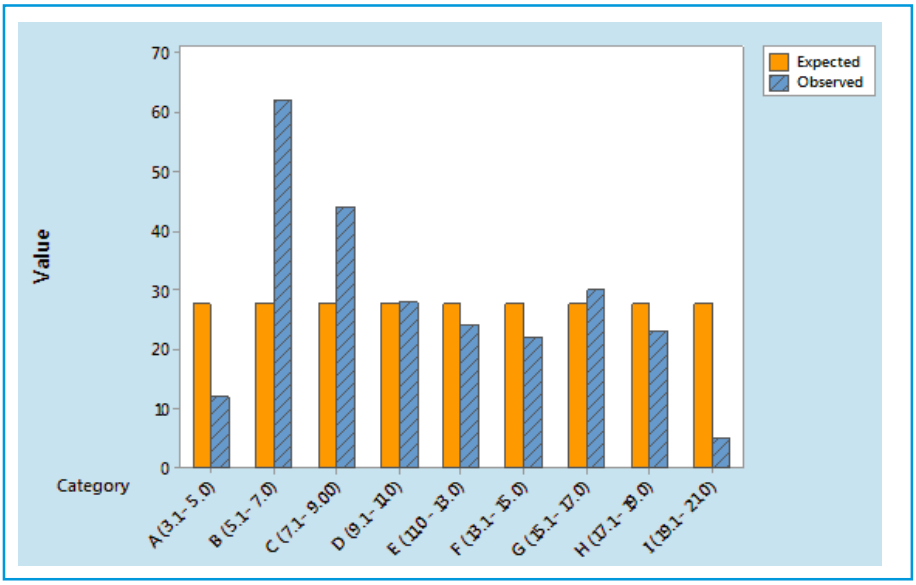

Figure 3. Chart of observed and expected values across the nine weight groups.

deviation from expected values. This is an indication that the growth rate within that population is non-uniform and as such the sizes are highly heterogenous which consequently poses a big threat to the growth and development of the small sized fish and encourage cannibalism within the tank.

The length-weight relationship in the study revealed the a and b values to be -2.18 and 3.04 respectively indicating a positive allometric growth with an r^2 of 0.94 which is close to reports of Torres (1992). The condition factor (K) ranges between 0.66 and 0.80 across the nine groups with an overall mean value of 0.73, implying that the larger fishes had higher values compared to the fishes in the lower groups. This is

indicative of the fact that the larger fishes are performing better metabolically compared to the smaller fishes despite the fact that all were reared under similar conditions and corroborated the work of Keyombe *et al* (2015).

Conclusions and recommendation

The following can be concluded from this study:

- The weight of fish sampled in this study varied widely between 3.30g and 20.30g with coefficient of variation of 41.88 percent and a mean of 10.61g.
- Majority of the fish (53.2 %) has weight below 95 percent confidence interval of the mean weight, implying that less than half of the fish are at or above mean weight.
- There is very high positive and significant correlation between all variables studied, implying that any of the variable can be used to predict or model another variable.
- The length-weigh relationship indicated a positive allometric growth (b = 3.04) with an average condition factor (K) of 0.73.
- The population structure in the study is inimical to profitable fish rearing as it encourages cannibalism and/or under-development of the weaker groups.

It is therefore recommended that the fish be separated into groups of fairly homogenous sizes for further rearing to minimize cannibalism and optimize profit.

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Genetic parameters for foot and claw diseases/disorders in Holstein cows in the Czech Republic: A preliminary study

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Summary

In this study, we analyzed genetic parameter for foot and claw diseases/disorders from 29 377 lactations of 11 776 Holstein cows recorded on 7 farms in the Czech Republic from 2000 to 2016. Three groups of foot and claw diseases/disorders were defined: skin diseases (SD), including digital and interdigital dermatitis and interdigital phlegmon; disorders of the claw horn (CH) including ulcers, white line disease, horn fissures, and double sole; and overall claw disease (OCD) comprising all the recorded disorders.

There were 22 711 records of overall claw disease (OCD), with an average of 3 records per cow and 1.7 records per lactation; 10 196 records of INF, with an average of 2.1 records per cow and 1.4 records per lactation; and 8 489 records of CH, with an average of 1.56 records per cow and 1.56 records per lactation. A total of 61% of cows showed OCD at least once in their lifetime, and OCD was recorded in 43% of all observed lactations. For INF and CH, the respective values were 41.13% and 46.18% of cows and 24.40% and 18.51% of all observed lactations. For the purposes of analyses, foot and claw diseases/disorders were defined as 0/1 occurrence per lactation. For CM, traits were the number of CM cases per lactation (CMn) or defined as 0/1 occurrence per lactation (CM0/1). The longevity trait was defined as pseudo-survival rate (PSR) determined according to whether a cow was alive (1) or absent (0) from her herd on the test day within each lactation. Genetic parameters for analyzed traits were estimated using linear animal models that included the random additive genetic effect of animal (A) and the permanent environmental effect of cow (PE). Fixed effects for claw diseases and clinical mastitis were parity, farm, year and season of calving, and age at calving in classes. For longevity, a model with random regressions within both random effects was employed together with the combined fixed effects of herd, year, season, and parity. The foot and claw disease traits were mostly affected by the herd, the year of calving, age at calving, and parity. The estimated heritability for INF was 8.8%, whereas that for CH and OCD was 7.0% and 7.7%, respectively, and that for CM and longevity was 12.2% and 2.2%, respectively. Genetic correlations between foot and claw diseases/disorders and CM ranged from 3% to 12% according to trait. Permanent environmental correlations between foot and claw diseases/disorders and CM were negative, from -3% to -18%, as were genetic correlations between foot and claw diseases/disorders and longevity (from 0 to -1%). Permanent environmental correlations between foot and claw

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diseases/disorders and longevity were also negative (from 0 to -1%) to approximately 400 days of life, followed by positive estimates (from 0 to 1.5%) particularly between INF and longevity, between 400 and 2000 days of life.

Keywords: Holstein cattle, lactation incidence risk, genetic parameter, foot and claw diseases, longevity, mastitis.

Introduction

Claw and foot diseases/disorders are among the most important health traits in dairy cattle, with noticeable negative impacts on farm profitability and production efficiency, animal welfare, food safety, and food quality (Enting *et al.*, 1997, van der Waaij *et al.*, 2005, Egger-Danner *et al.*, 2013). Claw and foot diseases/disorders impair the milk production, reproduction, and longevity of cows. Reducing the incidence of these conditions can be achieved by improvement in management practices and possibly by genetic selection. Genetic selection depends on sufficient genetic variability that is manifested by a particular claw disease/disorder, as shown in many previous studies (e.g., Buch *et al.* 2011, Chapinal *et al.*, 2013, and Pérez-Cabal and Charfeddine, 2015).

The aim of our study was to estimate the genetic parameters for claw and foot diseases/disorders recorded in the Czech Republic and to analyze the genetic correlations between claw and foot diseases/disorders, clinical mastitis (CM), and longevity.

Material and methods

Cases of foot and claw diseases/disorders from 29 377 lactations of 11 776 Holstein cows were recorded on 7 farms in the Czech Republic from 2000 to 2016, during both regular and emergency visits of trimmers. Three groups of foot and claw diseases were defined: skin diseases (SD), including digital and interdigital dermatitis and interdigital phlegmon; disorders of the claw horn (CH), including ulcers, white line disease, horn fissures, and double sole; and overall claw disease (OCD) comprising all the recorded diseases/disorders. For the purposes of analyses, foot and claw diseases/disorders were defined as 0/1 occurrence per lactation. For CM, traits were the number of CM cases per lactation (CMn) or defined as 0/1 occurrence per lactation (CM0/1). The longevity trait was defined as the pseudo-survival rate (PSR) that was determined according to whether a cow was alive (1) or absent (0) from her herd on the test day within each lactation (Sasaki *et al.*, 2015).

The following linear animal model was used to estimate genetic parameters for foot and claw disease/disorder traits and CM traits:

$$y_{ijklmno} = parity_i + herd_j + year_k + season_l + age_m + pe_n + a_o + e_{ijklmno}$$

where y_{ijklmn} is the analyzed trait: SD, CH, OCD, CMn, or CM0/1; $parity_i$ is the effect of parity class *i* (4 levels: first, second, third, and fourth and higher parity); $herd_j$ is the effect of herd *j* (7 levels); $year_k$ is the effect of calving year *k* (17 levels); $season_l$ is the effect of calving season (4 levels: January–March, April–June, July–September, and October–December); age_m is the effect of age at calving (13 levels); pe_n is the *random* permanent environmental effect on cow traits across *n* parity; a_o is the *random* additive genetic effect of cow *o*; and $e_{ijklmno}$ is the *random* residual effect.

The following random regression linear animal model was used to estimate genetic parameters for longevity:

$$y_{djklt} = \Sigma\, HYSP_{jtlq}\phi_q(d) + \Sigma\, a_{ktq}\phi_q(d) + \Sigma\, pe_{ktq}\phi_q(d) + e_{djklt}$$

where y_{djklt} is the observation of PRS of cow k exhibiting trait t at days in milk (DIM) d; $HYSP_{jtq}$ is the qth random regression coefficient of the herd-year-season-parity group j with trait t (q = 0–2), (herd, 7 levels; year, 17 levels; season, 4 levels: January-March, April-June, July-September, and October-December; parity 6 levels: 1st to 6th and higher parity); a_{ktq} is the q^{th} additive genetic random regression coefficient of cow k exhibiting trait t (q = 0–2); pe_{kltq} is the q^{th} permanent environmental random regression coefficient of cow k with trait t (q = 0–2); e_{djklt} is the residual random effect for each observation; and $\phi_q(d)$ is the q^{th} Legendre polynomial at DIM d.

The pedigree file contained 24 628 records. Data were analyzed using the DMU package (Madsen and Jensen, 2010) or VCE 6.0 program (Groeneveld *et al.*, 2008).

Genetic correlations between traits were estimated using bivariate models.

Results and discussion

There were 22 711 records of OCD, with an average of 3 records per cow and 1.7 records per lactation; 10 196 records of INF, with an average of 2.1 records per cow and 1.4 records per lactation; and 8 489 records of CH, with an average of 1.56 records per cow and 1.56 records per lactation. A total of 61% of cows showed OCD at least once in their lifetime, and OCD was recorded in 43% of all observed lactations. For INF and CH, the respective values were 41.13% and 46.18% of cows and 24.40% and 18.51% of all observed lactations. Recent studies have reported foot and claw diseases/disorders in 40% to 70% of cows (Sogstad *et al*. 2005, Buch *et al.*, 2011; Chapinal *et al.*, 2013, van der Spek *et al.*, 2013). In Czech Holstein, the frequency of claw diseases often exceeds 50% (Krpálková *et al.*, 2016).

The foot and claw disease traits were mostly affected by the herd, year of calving, age of calving, and parity. Differences between herds or years can be explained by differences in management. Krpálková *et al.* (2016) pointed out that better care and management combined with more inspection of legs leads to a higher recording of diseases. Furthermore, the frequency of recorded OCD has increased over the years because of a growing emphasis on claw health. The frequency of OCD increased with parity, with a higher incidence of disease being recorded at the beginning and end lactation and during the dry period. Older calving cows showed a higher frequency of OCD than the younger cows.

The estimated heritability of INF was 8.8%, whereas that for CH and OCD was 7.0% and 7.7%, respectively, and that for CM and longevity was 12.2% and 2.2%, respectively. These estimates are in line with those reported in the literature, which range from 1% to 17% (van der Waaij *et al.*, 2005, Buch *et al.*, 2011, Chapinal *et al.*, 2013, Pérez-Cabal and Charfeddine 2015). Genetic correlations between foot and claw diseases/disorders and CM ranged from 3% to 12% according to trait. Buch *et al.* (2011) reported 0% genetic correlation between CM and dermatitis and 32% genetic correlation between CM and sole ulcer. These values are in agreement with the results of the present study because our estimates of the genetic correlation between CM traits and claw disease traits were higher for disorders of the claw horn (CH), which includes ulcers, than for skin diseases (SD), which includes dermatitis (7% CH × CM0/1; 12% CH × CMn vs. 3% INF × CM0/1; 6% INF × CMn). Permanent environmental correlations between foot and claw diseases/disorders and clinical mastitis were negative from -3% to -18%. Genetic correlations between foot and claw

diseases/disorders and longevity were negative to -1%. Permanent environmental correlations between foot and claw diseases/disorders and longevity were negative (0% to -1%) to approximately 400 days of life, followed thereafter by positive estimates (from 0% to 1.5%), particularly between INF and longevity. Estimates of both genetic and environmental correlations between longevity and foot and claw diseases/disorders were small and non-significant.

Conclusions

There is evidence that susceptibility to foot and claw diseases/disorders is heritable, although our estimates of heritability would benefit from larger datasets. We identified a genetic relationship between foot and claw diseases/disorders and clinical mastitis; however, the genetic correlations between these traits and longevity were very low and non-significant. This study represents the first step in developing a national genetic evaluation of foot and claw diseases/disorders in dairy cattle.

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