

## Minimization of fertility economical loss by MIR based energy balance prediction

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

### Abstract

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

Mid Infrared (MIR) spectroscopy is using the infrared light from the electromagnetic spectrum which shows specific absorption patterns when sent through a milk sample caused by frequency dependent interactions with the chemical bonds of the chemical milk components. With the help of milk MIR spectra, a wealth of information can be obtained by establishing relationships with reference methods. MIR is a non-expensive and routinely-used method for major milk components and also for fine milk composition analysis. During the last 15 years different researchers were developing new calibration models with the help of milk MIR spectral data linked to milk main components such as fatty acids (Grelet C. *et al.* 2014) or minerals (Soyeurt H. *et al.* 2009), milk biomarkers

### Introduction

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

## Material and methods

From the LKV BW cattle database about 288 dairy farms were selected. The selected farms are taking part in the health monitoring program "GMON Rind BW". The data covered the complete years of calving period and the variability of days in milk for the population. The selection milk samples covered a complete period of 6 years and considered the relevant breeds Holstein, Simmental and Brown Swiss as well as the season variability. For this study indicator data and insemination data was combined together, followed by adding the veterinary diagnosis information to the selected data. Once the data was prepared the spectral data for the selected milk

recording samples was added. All milk recording data from LKV B.W. is analyzed on Bentley Spectrometers. The spectral data set was first standardized by applying the OptiMIR/EMR method (Grelet *et al.*, 2015) and pre-processed by Savitzky-Golay first derivative to remove the offset differences between samples for baseline correction, before performing Legendre polynomial modeling. To identify the main variables that were positively or negatively associated with pregnancy information a Pearson correlation analysis with all MIR predictions available at EMR level but also at LKV B.W. was performed using the “corrplot” R package (Figure 1.). In order to identify the pregnant and open cows it was applied first the definition code for the pregnancy status such as: code 0 = non-pregnant, code 1 = pregnant because of calving date registration and official insemination date within the 9 months period and code 2 = open because of multiple inseminations. The 212 OptiMIR wave numbers of spectral data were used for the comparison between the pregnancy statuses (Figure 2.). Then pregnancy status data were combined with EB-NEL data.

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

## Results and discussions

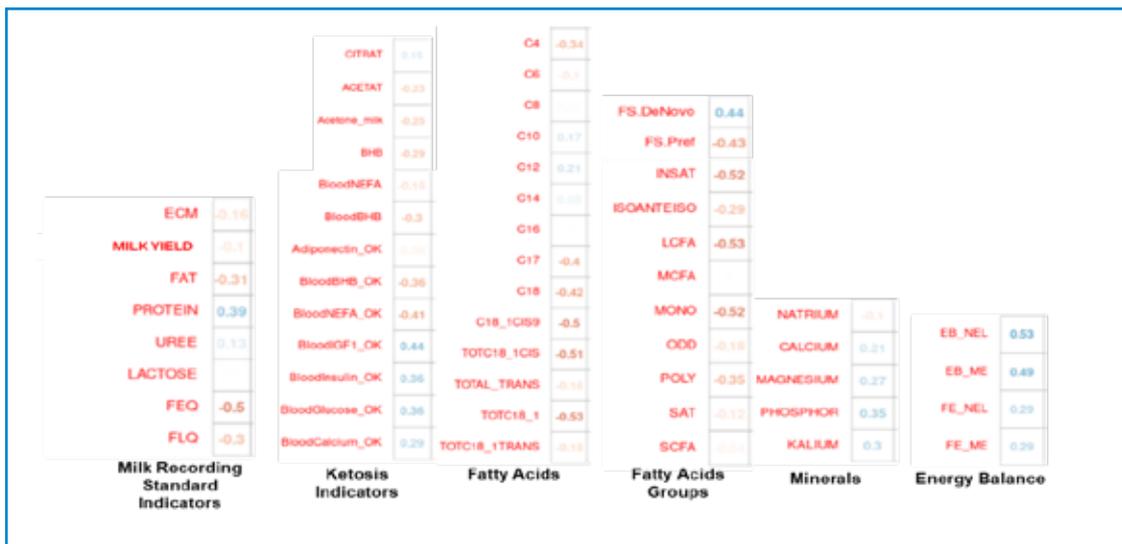


Figure 1. Pearson Correlations comparing open versus pregnant cows.

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

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

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

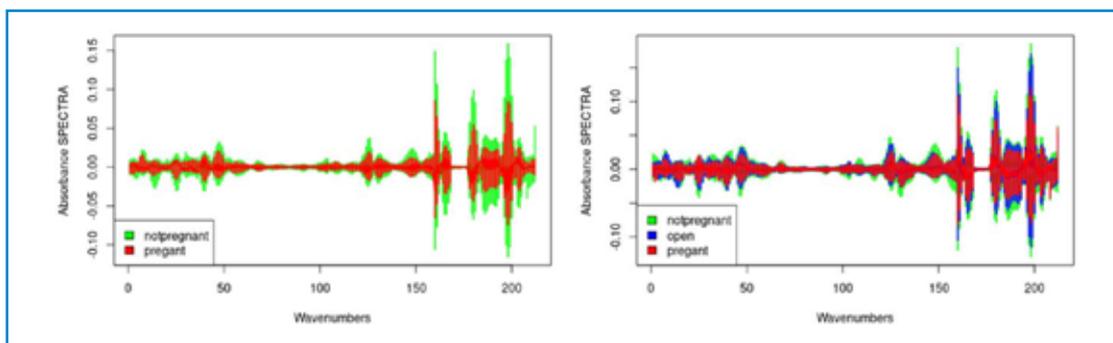


Figure 2. Spectral differences of cows after 2<sup>nd</sup> insemination between not-pregnant, open (not successful insemination) and pregnant data at 22-56 days in milk (DIM)

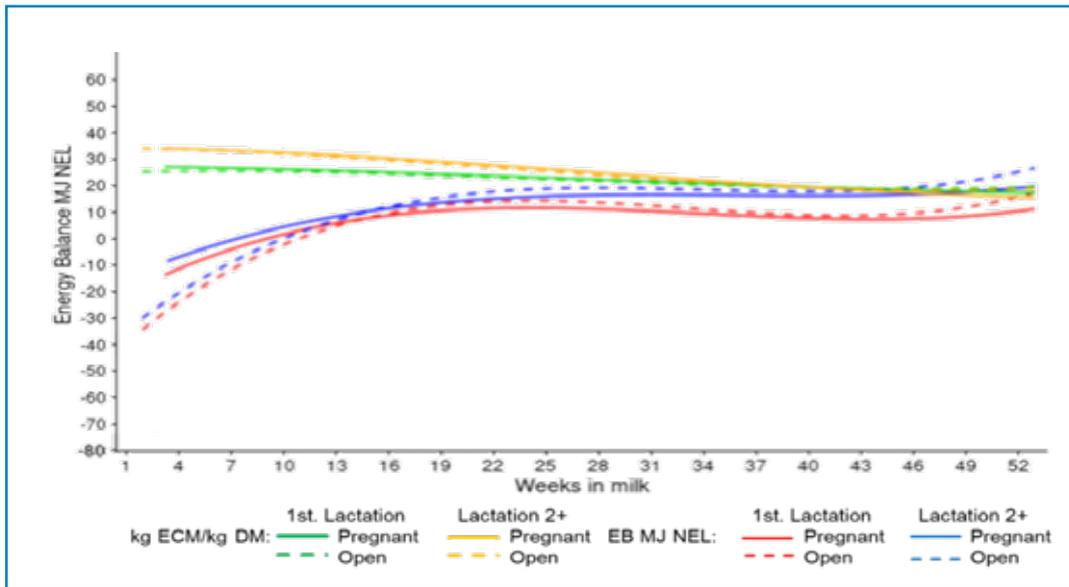


Figure 3. Mean ECM (kg/day) and EB-NEL MJ/day by primiparous and multiparous and open and pregnant cows in different lactations

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

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

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

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

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

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

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

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

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

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

## Conclusions

## Acknowledgments

## List of references

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

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

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

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

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

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

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

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

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

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

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



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

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