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Diagnosing pregnancy status using infrared spectra and milk composition in dairy cows

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ABSTRACT

Data on Holstein (16,890), Brown Swiss (31,441), Simmental (25,845), and Alpine Grey (12,535) cows reared in northeastern Italy were used to assess the ability of milk components (fat, protein, casein, and lactose) and Fourier transform infrared (FTIR) spectral data to diagnose pregnancy. Pregnancy status was defined as whether a pregnancy was confirmed by a subsequent calving and no other subsequent inseminations within 90 d of the breeding of specific interest. Milk samples were analyzed for components and FTIR full-spectrum data using a MilkoScan FT+ 6000 (Foss Electric, Hillerød, Denmark). The spectrum covered 1,060 wavenumbers (wn) from 5,010 to 925 cm^{-1}. Pregnancy status was predicted using generalized linear models with fat, protein, lactose, casein, and individual FTIR spectral bands or wavelengths as predictors. We also fitted a generalized linear model as a simultaneous function of all wavelengths (1,060 wn) with a Bayesian variable selection model using the BGLR R-package (https://r-forge.r-project.org/projects/bglr/). Prediction accuracy was determined using the area under a receiver operating characteristic curve based on a 10-fold cross-validation (CV-AUC) assessment based on sensitivities and specificities of phenotypic predictions. Overall, the best prediction accuracies were obtained for the model that included the complete FTIR spectral data. We observed similar patterns across breeds with small differences in prediction accuracy. The highest CV-AUC value was obtained for Alpine Grey cows (CV-AUC = 0.645), whereas Brown Swiss and Simmental cows had similar performance (CV-AUC = 0.630 and 0.628, respectively), followed by Holsteins (CV-AUC = 0.607). For single-wavelength analyses, important peaks were detected at wn 2,973 to 2,872 cm^{-1} where Fat-B (C-H stretch) is usually filtered, wn 1,773 cm^{-1} where Fat-A (C=O stretch) is filtered, wn 1,546 cm^{-1} where protein is filtered, wn 1,468 cm^{-1} associated with urea and fat, wn 1,399 and 1,245 cm^{-1} associated with acetone, and wn 1,025 to 1,013 cm^{-1} where lactose is filtered. In conclusion, this research provides new insight into alternative strategies for pregnancy screening of dairy cows.

Key words: Fourier transform infrared spectroscopy, milk, milk component, pregnancy

INTRODUCTION

Changing metabolic and energy requirements due to pregnancy in cows are likely to also change milk yield and milk composition. For example, a decline in milk yield during gestation in pregnant cows has been reported in several studies, becoming more evident after the third month of pregnancy (Olori et al., 1997; Loker et al., 2009) when the requirements of the fetus demand a significant amount of nutrients (Moe and Tyrrell, 1972). Pregnancy also affects milk composition with an increase of fat, protein, lactose, and casein as pregnancy advances (Olori et al., 1997). Consequently, the effect of pregnancy has been suggested as an adjustment factor to increase the accuracy of genetic evaluations on production traits (Bohmanova et al., 2009). In fact, several countries have included pregnancy stage in their genetic evaluations for milk yield, fat, and protein (Interbull, 2016).

Confirmation of pregnancy in dairy cows is fundamental to successful breeding programs such that early diagnosis tools that would help farmers to determine pregnancy are needed. Current pregnancy diagnosis tests include direct methods (transrectal palpation and ultrasonography) and indirect methods (milk progesterone and pregnancy-associated glycoproteins in blood or milk; Fricke et al., 2016). All these methods are costly, require animal handling, and have limited efficacy. Nonreturn to estrus after insemination is commonly used as

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a convenient measure of pregnancy success; however, estrus detection depends on several factors, thereby decreasing the reliability of this measure (Senger, 1994). The ideal pregnancy test should be highly sensitive, highly specific, inexpensive, and simple to conduct on farm (Fricke et al., 2016).

Fourier transform infrared (**FTIR**) spectroscopy is already globally used to routinely assess milk composition in milk recording programs (ICAR, 2016); for instance, FTIR data are routinely used to determine milk components (fat, protein, casein, lactose, TS, urea, citric acid, free fatty acids, and some individual fatty acids), freezing point, pH, and ketosis screening. In addition, FTIR spectroscopy has been used to predict many other detailed phenotypes such as fatty acid profiles, protein fraction compositions, free AA, and milk coagulation properties (De Marchi et al., 2014). Other phenotypes having direct relationships with milk composition have also been studied, such as body energy status and feed and energy intake (McParland and Berry, 2016). In relation to health and fertility of cattle, Bastin et al. (2016) studied the use of FTIR spectroscopy in milk as an effective indicator of health and fertility, associating acetone and BHB with ketosis and associating various fatty acids (e.g., C18:1 *cis*-9 and C10:0) with fertility. Lainé et al. (2017) reported a direct effect of pregnancy on milk composition of Holsteins and on their milk FTIR spectrum: the absorbance of 212 waves were affected by pregnancy, especially in the infrared spectral region from wavenumber 1,577 to 968 cm^{-1} (transition from mid- to long-infrared sections of the spectrum). Finally, Lainé et al. (2014) used residuals from spectral data (mid-infrared region) preadjusted for several fixed effects (e.g., parity, breed, month of test day, DIM) to discriminate the residual spectra of open cows from the residual spectra of pregnant cows with high specificity and sensitivity.

What has not yet been studied is the possibility of discriminating between pregnant and open cows simply using whole-spectrum FTIR profiles. Therefore, the objectives of this study were to assess and to compare the prediction accuracies of a reproductive outcome (pregnancy status of dairy cows) using data on milk components (fat, protein, casein, and lactose) indirectly derived from spectral wavelength absorbances as well as direct single-band and whole-spectrum FTIR data.

MATERIALS AND METHODS

Field Data

Production and female fertility data were collected from farms in the northeastern Bolzano/Bozen province in Italy by the Breeders Federation of Alto Adige/Südtirol (Associazione Provinciale delle Organizzazioni Zootecniche Altotesine/Vereinigung der Südtiroler Tierzuchtverbände, Bolzano/Bozen, Italy). Management systems were rather heterogeneous, ranging from the traditional small farms of the mountainous areas to more modern and larger operations elsewhere. A good description of the dairy farms in the region is provided by Sturaro et al. (2013) and by Stocco et al. (2017a). Milk yield and composition records were obtained from the official milk recording system and consisted of daily milk yield (kg/d) and of fat, protein, casein, and lactose percentages analyzed from FTIR spectra according to internationally approved methods (ICAR, 2016). Data included records generated from 2010 to 2016 on Holstein, Brown Swiss, Simmental, and Alpine Grey cows.

Data Editing

The interval between consecutive inseminations was required to be greater than 3 d in accordance with ICAR (2016) guidelines. Only records made ≤91 d after each insemination were kept because the percentage of open cows was very low by wk 13 after insemination (7, 6, 3, and 3% for Holstein, Brown Swiss, Simmental, and Alpine Grey, respectively). The proportions of pregnant and open cows by weeks after insemination for each breed are available in Appendix Figure A1. Gestation length was restricted to be within 30 d from the average for each breed (Holstein = 281 d, Brown Swiss = 290 d, Simmental = 285 d, and Alpine Grey = 287 d). The calving interval was restricted to be less than 700 d for all breeds. Only records with DIM ≤305 d were considered, and parity was classified as 1, 2, 3, or ≥4. A detailed description of fertility traits as well as data editing is reported by Toledo-Alvarado et al. (2017).

Pregnancy Definition. A cow's pregnancy status (**PS**) was coded as a binary variable based on whether a subsequent insemination was not recorded within 90 d after breeding and confirmed by subsequent calving (PS = 1) versus an insemination being registered within the subsequent 90-d period (PS = 0). Otherwise, PS was set to be unknown.

FTIR Spectra. All milk samples were analyzed using a MilkoScan (Foss Electric, Hillerød, Denmark) in the laboratory of the Federazione Latterie Alto Adige/Sennereiverband Südtirol (Bolzano/Bozen Italy). The spectrum covers from the short-wavelength infrared (**SWIR**) to the long-wavelength infrared (**LWIR**) regions with 1,060 spectral points from wavenumber 5,010 to 925 cm^{-1}, which correspond to wavelengths ranging from 1.99 to 10.81 μm and frequencies ranging from 150.19 to 27.73 THz. The spectrum transmittanc-