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Prediction of individual milk proteins including free amino acids in bovine milk using mid-infrared spectroscopy and their correlations with milk processing characteristics

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ABSTRACT

The aim of this study was to evaluate the effectiveness of mid-infrared spectroscopy in predicting milk protein and free amino acid (FAA) composition in bovine milk. Milk samples were collected from 7 Irish research herds and represented cows from a range of breeds, parities, and stages of lactation. Mid-infrared spectral data in the range of 900 to 5,000 cm^{-1} were available for 730 milk samples; gold standard methods were used to quantify individual protein fractions and FAA of these samples with a view to predicting these gold standard protein fractions and FAA levels with available mid-infrared spectroscopy data. Separate prediction equations were developed for each trait using partial least squares regression; accuracy of prediction was assessed using both cross validation on a calibration data set ($n = 400$ to 591 samples) and external validation on an independent data set ($n = 143$ to 294 samples). The accuracy of prediction in external validation was the same irrespective of whether undertaken on the entire external validation data set or just within the Holstein-Friesian breed. The strongest coefficient of correlation obtained for protein fractions in external validation was 0.74, 0.69, and 0.67 for total casein, total β -lactoglobulin, and β -casein, respectively. Total proteins (i.e., total casein, total whey, and total lactoglobulin) were predicted with greater accuracy than their respective component traits; prediction accuracy using the infrared spectrum was superior to prediction using just milk protein concentration. Weak to moderate prediction accuracies were observed for FAA. The greatest coefficient of correlation in both cross validation and external validation was for Gly (0.75), indi-

cating a moderate accuracy of prediction. Overall, the FAA prediction models overpredicted the gold standard values. Near-unity correlations existed between total casein and β -casein irrespective of whether the traits were based on the gold standard (0.92) or mid-infrared spectroscopy predictions (0.95). Weaker correlations among FAA were observed than the correlations among the protein fractions. Pearson correlations between gold standard protein fractions and the milk processing characteristics of rennet coagulation time, curd firming time, curd firmness, heat coagulating time, pH, and casein micelle size were weak to moderate and ranged from -0.48 (protein and pH) to 0.50 (total casein and a_{30}). Pearson correlations between gold standard FAA and these milk processing characteristics were also weak to moderate and ranged from -0.60 (Val and pH) to 0.49 (Val and K_{20}). Results from this study indicate that mid-infrared spectroscopy has the potential to predict protein fractions and some FAA in milk at a population level.

Key words: mid-infrared spectroscopy, protein fractions, free amino acids, milk quality

INTRODUCTION

Detailed milk product quality is not considered in the Irish national dairy cow breeding objective, at present, despite its fundamental importance for adding value to the Irish agri-food industry. This is simply due to lack of routine access to data on detailed milk quality parameters, possibly owing to the expense of generating such data using gold standard methods. Consideration of milk quality parameters in national breeding goals is particularly important for exporting countries such as Ireland to consistently achieve a high-quality product suitable for value-added international markets.

The concentration of protein and the composition of protein fractions in milk influence the production

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efficiency of cheese, infant milk formula, and both CN and whey protein supplements. Wedholm et al. (2006) stated that the concentration of CN in milk protein has a favorable effect on the quantity of protein transferred from milk into cheese curd and high concentrations of α_{S1} -CN, β -CN, and κ -CN and of β -LG B were found to significantly increase cheese yield. Elofsson et al. (1996) demonstrated a low β -LG concentration reduces the fouling rate of heating equipment. β -Lactoglobulin, which is not present in human milk, is a major milk allergen and therefore efforts have been made to reduce the level of this protein in cow milk (Jabed et al., 2012). Therefore, milk protein composition is of increasing importance to the dairy industry due to the expected global demand for cheese (FAOSTAT, 2014). Protein plays an important role in immunity, growth, and development of infants (Lönnerdal, 2003). Therefore, milk protein composition is particularly important for infant formula production (de Wit, 1998) as the composition of bovine milk is different from human milk (Jensen, 1995). Infant formula production is the fastest growing sector in the world dairy market (FAOSTAT, 2014), and the international market for infant milk formula is worth approximately US\$5 to \$6 billion annually. Protein composition also affects milk processing characteristics such as the heat coagulating time of bovine milk (Singh, 2004).

Regarding milk processing ability, high free AA (FAA) levels indicate poor quality milk as they arise from protein hydrolysis and are generally in greatest concentration in early and late lactation milk (Davis et al., 1994), when milk quality is poorest (Auldist et al., 1995). Human and bovine milk have different FAA content and composition, with bovine milk generally having a lesser concentration of FAA than human milk (Armstrong and Yates, 1963; Sarwar et al., 1998; Agostoni et al., 2000; Roucher et al., 2013). Therefore, for nutritional reasons, supplementation of infant formula with the required FAA may be of interest in infant formula production. Achieving a milk FAA profile in bovine milk similar to that of human milk through breeding may be an alternative strategy. The FAA profile of milk is therefore of interest to dairy farmers, as milk processors may pay higher prices for milk based on its FAA composition.

Milk compositional traits such as protein fractions have a major influence on milk processing ability-related traits such as rennet coagulating time (RCT; Auldist et al., 2004; Ikonen et al., 2004; Wedholm et al., 2006). It is well documented that milk composition and milk coagulation properties are affected by environmental factors including stage of lactation (Ostensen et al., 1997; Heck et al., 2009). Auldist et al. (1995) documented a stage of lactation effect on both cheese

yield and quality. The majority of milk production in Ireland is seasonal (Berry et al., 2006), as most dairy cows calve in spring (Berry et al., 2013). Therefore, it may be of interest to milk processors on how the correlations between milk compositional traits and processing ability characteristics differ in different stages of lactation.

Despite the importance of quantifying individual proteins and FAA in milk, no inexpensive and efficient method of measuring these components in milk is available. Mid-infrared spectroscopy (MIRS) is a technique that studies the interactions between light and matter at wavelengths in the spectral range of 900 to 5,000 cm^{-1} . It is based on the capability of molecules to reflect, transmit, or absorb part of the electromagnetic radiation when exposed to light. According to the Beer-Lambert law (Swinehart, 1962), the quantity of the electromagnetic radiation absorbed is directly proportional to the amount of the absorbent molecule in the sample. Mid-infrared spectroscopy is an efficient method currently used by milk recording organizations worldwide to predict milk fat, protein, and lactose and has recently been used to predict more detailed milk composition traits such as fatty acids (De Marchi et al., 2011; Soyeurt et al., 2011;), coagulation traits (De Marchi et al., 2013), as well as animal-level characteristics such as energy balance (McParland et al., 2011, 2012) and feed efficiency (McParland et al., 2014). Limited studies exist evaluating the effectiveness of MIRS in predicting milk protein fractions (De Marchi et al., 2010; Bonfatti et al., 2011; Rutten et al., 2011). The gold standard method used in both the studies of De Marchi et al. (2010) and Bonfatti et al. (2011) was HPLC; however, Rutten et al. (2011) used capillary zone electrophoresis. In the studies of Bonfatti et al. (2011) and Rutten et al. (2011), the ratio performance deviation ranged from 1.04 (γ -CN) to 2.12 (protein) and from 0.48 (β -CN) to 1.06 (total whey), respectively. Across studies (Bonfatti et al., 2011; Rutten et al., 2011), the coefficient of determination for cross validation ranged from 0.08 (γ -CN) to 0.80 (protein). However, De Marchi et al. (2009) expressed protein fractions as grams per liter, whereas Rutten et al. (2011) expressed them on a protein percentage basis (g/100 g) and Bonfatti et al. (2011) expressed them in both forms. Higher coefficient of determination values were obtained when protein fractions were expressed in grams per liter rather than on a percentage basis.

The aim of this study was to quantify the effectiveness of MIRS to predict individual milk proteins and FAA as well as to estimate the association between these MIRS-predicted traits and other phenotypic characteristics of milk including RCT, curd firming time (\mathbf{k}_{20}), curd firmness (\mathbf{a}_{30}), heat coagulation time (HCT), and

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