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Short communication: Variations in major mineral contents of Mediterranean buffalo milk and application of Fourier-transform infrared spectroscopy for their prediction

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ABSTRACT

The aims of this study were (1) to assess variability in the major mineral components of buffalo milk, (2) to estimate the effect of certain environmental sources of variation on the major minerals during lactation, and (3) to investigate the possibility of using Fourier-transform infrared (FTIR) spectroscopy as an indirect, noninvasive tool for routine prediction of the mineral content of buffalo milk. A total of 173 buffaloes reared in 5 herds were sampled once during the morning milking. Milk samples were analyzed for Ca, P, K, and Mg contents within 3 h of sample collection using inductively coupled plasma optical emission spectrometry. A Milkoscan FT2 (Foss, Hillerød, Denmark) was used to acquire milk spectra over the spectral range from 5,000 to 900 wavenumber/cm. Prediction models were built using a partial least square approach, and cross-validation was used to assess the prediction accuracy of FTIR. Prediction models were validated using a 4-fold random cross-validation, thus dividing the calibration-test set in 4 folds, using one of them to check the results (prediction models) and the remaining 3 to develop the calibration models. Buffalo milk minerals averaged 162, 117, 86, and 14.4 mg/dL of milk for Ca, P, K, and Mg, respectively. Herd and days in milk were the most important sources of variation in the traits investigated. Parity slightly affected only Ca content. Coefficients of determination of cross-validation between the FTIR-predicted and the measured values were 0.71, 0.70, and 0.72 for Ca, Mg, and P, respectively, whereas prediction accuracy was lower for K (0.55). Our findings reveal FTIR to be an unsuitable tool when milk mineral content needs to be predicted with high accuracy. Predictions may play a role as indicator traits in selective breeding (if the additive genetic correlation between FTIR predictions and measures of milk minerals is high enough) or in monitoring the milk of buffalo populations for dairy industry purposes.

Key words: buffalo milk, mineral content, Fourier-transform infrared spectroscopy

Short Communication

Buffalo (*Bubalus bubalis*) milk production is customary in many parts of the world, representing about 13% of worldwide global milk production, in second place after cow milk (FAOSTAT, 2014). Minerals are fundamental for human health, as they are required for many physiological functions such as tissue growth, blood clotting, muscle contraction, and nerve function, but they also play an important role in milk coagulation as they influence casein micelle structure and aggregation, rennet coagulation time, curd structure, and, ultimately, cheese yield (Lucey and Fox, 1993; Ariota et al., 2007). However, the concentrations of many minerals are altered as a result of mastitis (Ahmad et al., 2007; Eshratkhah et al., 2012). The mineral content of milk, as well as lactose, is subject to little variation during lactation and could be used as an indicator of infection of the mammary gland (El Zubeir et al., 2005). Therefore, determination of the milk mineral profile could be a means of evaluating the nutritional and technological quality of milk and as a tool for early diagnosis of subclinical mastitis (Managuli et al., 2014).The most common method for determining mineral content in milk for research purposes is inductively coupled plasma atomic emission spectrometry, although this method is too expensive and too time-consuming for routine analysis of milk samples on a wide scale. The current tool used to measure the major milk components during regular milk recording is Fourier-transform infrared (**FTIR**) spectroscopy; regarding the milk mineral profile, however, very little information exists in the literature regarding the use of FTIR spectroscopy, and most relates to bovine milk (Soyeurt et al., 2009). Re-

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cently, Bonfatti et al. (2015) investigated the ability of FTIR spectroscopy to predict the protein composition of buffalo milk, but so far no studies have investigated the possibility of predicting major mineral contents. The aims of our study were (1) to assess variability in the major mineral components of buffalo milk, (2) to estimate the effect of certain environmental sources of variation on the major minerals during lactation, and (3) to investigate the possibility of using FTIR spectroscopy for indirect prediction of the mineral content of individual buffalo milk samples.

A total of 173 Mediterranean buffaloes from 5 herds located in northern Italy were sampled once from January to May 2013. The number of animals sampled from each herd were 28, 29, 45, 39, and 32. Buffaloes were selected from each herd to represent the entire lactation and different parity order. Details of the sample procedure have been reported in Cipolat-Gotet et al. (2015). Milk samples were analyzed after dilution 1:100 (wt/wt) with ultrapure water. Inductively coupled plasma optical emission spectrometry (Ciros Vision EOP, SPECTRO Analytical Instruments GmbH, Kleve, Germany) was the reference method for determining Ca at 315.887 nm, P at 177.495 nm, K at 766.491 nm, and Mg at 280.270 nm. Instrument operating parameters were optimized for acid solution, and calibration standards were matched with Suprapur nitric acid 5% (Merck, Darmstadt, Germany). The elements to be determined were added from single element solutions (Inorganic Ventures, Christiansburg, VA). The concentration range of the calibration solutions was between 0 and 100 mg/L for each element. The accuracy and precision of this method were investigated by analyzing the certified reference material BCR – 063R "Skim milk powder" (Institute for Reference Materials and Measurements, Geel, Belgium). A MilkoScan FT2 (Foss, Hillerød, Denmark) was used to collect buffalo milk spectra over the spectral range from 5,000 to 900 wavenumber/cm. The transmittance spectra were converted in absorbance (A) spectra using the transformation A = log(1/T), where T is the transmittance. Two spectral acquisitions were carried out for each sample and the results were averaged before data analysis.

Sources of variation in the minerals (Ca, P, K, and Mg) were investigated using the SAS GLM procedure (SAS Inst. Inc., Cary, NC) according to the following linear model:

$$y_{ijkl} = \mu + \text{DIM}_i + \text{Parity}_j + \text{Herd} - \text{Date}_k + e_{ijkl},$$

where y_{ijkl} is the observed trait (milk concentrations of Ca, P, K, or Mg); μ is the overall mean of the model; DIM_i is the fixed effect of the ith class of DIM [$i = 1$ to 6: class 1, <30 d (n = 31); class 2, 30–60 d (n = 24); class 3, 61–120 d (n = 28); class 4, 121–180 d (n = 27); class 5, 181–240 d (n = 37); class 6, >240 d (n = 26)]; Parity_j is the fixed effect of the jth parity of the buffalo ($j = 1$ to 4 or more; with number of buffaloes equal to 43, 41, 34, and 55 for first, second, third, and fourth or more parity order, respectively); $\text{Herd} - \text{Date}_k$ is the fixed effect of the kth herd-date of sampling ($k = 1$ to 5); and e_{ijkl} is the random residual. Residuals were assumed to be independently and normally distributed with a mean equal to zero and variances of $\sigma^2_{e.}$ Polynomial contrasts ($P < 0.05$) were estimated between least squares means of traits for DIM effect: first order–second order comparisons measured linear and quadratic relationships, respectively.

The FTIR calibration models were built using partial least-square regression procedures implemented in the WinISI II software (Infrasoft International LLC, State College, PA). Prediction models were validated using a 4-fold random cross-validation, thus dividing the calibration-test set in 4 folds, using one of them to check the results (prediction models) and the remaining 3 to develop the calibration models. The process was repeated until all folds had been used for validation once according to Naes et al. (2002). The FTIR spectra were analyzed across the whole interval (from 5,000 to 900 wavenumber/cm) and without the 2 portions known to be characterized by very high phenotypic variability (Bittante and Cecchinato, 2013); that is, the transition region between the short-wave to mid-wave infrared (3,669–3,052 cm^{-1}) and the mid-wavelength infrared region from 1,698 to 1,586 wavenumber/cm. Several mathematical treatments of raw spectra were compared before regression analysis. Samples exhibiting a large spectral distance (i.e., a global Mahalanobis distance >3) from the population centroid and samples where the difference between the reference and the predicted values was much larger than the standard error of cross-validation were considered outliers and discarded from the calibration analysis.

Descriptive statistics of the major mineral contents of buffalo milk analyzed using inductively coupled plasma optical emission spectrometry instrument in the present study are summarized in Table 1, along with the most representative reference values reported in the literature, which are characterized by a very large variability. In our study, the Ca content averaged 162 mg/dL of milk, similar to that reported in the "National nutrient database for standard reference" of the USDA (Agricultural Research Service, 2016). A review carried out by the Food and Agriculture Organization (**FAO**;