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Prediction of bovine milk true protein content by mid-infrared spectroscopy

Estimativa do teor de proteína verdadeira do leite bovino por espectroscopia na região do infravermelho médio

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- NOTE -

ABSTRACT

The aim of this study was to estimate the concentration of milk true protein (TP) by mid-infrared absorbance method (MIR) in samples from bulk tank of dairy herds, and to determine the correlation between the results of TP of milk determined by Kjeldahl and MIR. Forty nine dairy herds were selected (17 Holstein, 6 Jersey and 26 Girolando) for monthly collections of samples from bulk tanks during the period of one year (284 samples). Fat, lactose, crude protein and total solids were firstly determined by MIR, and then analyzed for total and true protein by Kjeldahl method. The regression equation to estimate TP contents based on MIR crude protein determination was as follows: TP=0.0021+(1.0104xCP), where: TP is the content of true protein, CP is the crude protein content determined by the MIR method, and 0.0155 is the model error term.

Key words: *dairy cow, milk composition, protein determination.*

RESUMO

O objetivo deste estudo foi estimar o teor de proteína verdadeira (PV) do leite por meio de metodologia de espectroscopia na região do infravermelho médio (IVM), em amostras de tanque de rebanhos leiteiros comerciais, e determinar a correlação entre os resultados de proteína verdadeira do leite determinados pelo método de Kjeldahl e por IVM. Foram selecionados 49 rebanhos leiteiros (17 da raça Holandesa, seis da raça Jersey e 26 da raça Girolando) para coletas mensais de amostras de leite de tanque durante o período de um ano, totalizando 284 amostras analisadas. As amostras de leite foram analisadas inicialmente em relação aos teores de gordura, lactose, proteína bruta e sólidos totais por IVM, sendo em seguida analisadas quanto ao teor de PB e PV pelo método de Kjeldahl. A equação de regressão para estimativa do teor de PV com base nos teores de proteína bruta foi a seguinte: PV=0,0021+(1,0104xPB); em que: PV é o teor de proteína verdadeira estimado; PB é o teor de proteína bruta pelo método IVM e 0,0155 é o erro apresentado pelo modelo.

Palavras-chave: *vaca leiteira, composição do leite, determinação de proteína.*

The reference methodology for crude protein determination in milk is the Kjeldahl method, which measures the total nitrogen (TN) content in milk. By this method, the nitrogen released from protein and other components is converted to ammonia nitrogen by acid digestion. Once quantified the TN amount, milk concentration of crude protein (CP) is obtained by multiplying TN by 6.38 (TOUFEILI, 2007). Thus, the CP content determined by this method refers to nitrogen arising from TP and other sources of non-protein N (NPN) in milk. Since about 95-97% of CP is TP, the difference is represented by non-protein components such as ammonia, urea, creatine, creatinine, uric acid, orotic, hippuric, amino acids and other nitrogen compounds (FOX; MCSWEENEY, 2003). The concentration of milk casein comprises about 75-85% of CP and 85-95% of TP, while the whey proteins consist of 10-15% of TP (ROUCH et al., 2006).

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Currently, the protein content of milk is analyzed through automated analyzers (SILVEIRA et al., 2004), which are based on the principle of mid-infrared (MIR) energy absorbance of protein specific chemical group, amides, at the approximately wavelength of 6.7μm (LYNCH et al., 2006) peptide bonds to estimate the concentration of CP (BARBANO & LYNCH, 2006). The use of automated analyzers allows quick determination of milk composition at low cost in comparison with the reference method (KAYLEGIAN et al., 2006). MIR absorbance technique is an indirect method, which requires calibration of the equipment with reference values obtained by the reference methodology (ETZION et al., 2004).

As the portion of NPN in milk has no peptide bond in its structure, these analyzers, in theory, would perform better if true protein content was used for the equipment calibration (BARBANO & LYNCH, 2006). Thus, MIR is currently calibrated according to total nitrogen (TP + NPN) levels in the reference sample, which in turn are determined by reference method. However, since the NPN:NT ratio is not constant and varies according to the NNP, the concentration of CP in milk samples analyzed by MIR may not be accurately estimated.

Milk composition of raw milk is being increasingly used by dairy industry in milk payment systems to their producers (MACHADO, 2008), and one of the main criteria is the CP content in milk. There is a lack of studies in the literature that estimate the content of milk TP in commercial herds, as well as assessments on the use of MIR absorbance methodology for milk TP analysis.

The aim of this study was to estimate the true protein content of milk by the mid-infrared absorbance method in samples from dairy herds, and to determine the correlation between milk TP determined by Kjeldahl and by MIR methods.

Forty nine dairy herds were randomly selected in the State of São Paulo for monthly sampling of bulk tank milk during one year (284 samples). The average daily milk production of selected herds was 290±223 liters. All dairy herds had monthly monitoring of the milk composition and somatic cell count milk (SCC), and were distributed by breed (Holstein: 17, Jersey: six, Girolando: 26).

Milk samples were sent to the Laboratório Clínica do Leite – Departamento de Zootecnia da ESALQ/USP (Piracicaba – SP) in plastic bottles and preserved with bronopol (2 - bromo-2-nitro-1 ,3-propanediol) in a concentration of 8mg 40mL^{-1} of milk for analysis of composition (fat, lactose, protein, total solids and urea). Analysis of milk composition were performed electronically by mid-infrared absorbance method, using 2000 Bentley® (BENTLEY INSTRUMENTS INC. Chasca, MN, USA), in Laboratório Clínica do Leite – Departamento de Zootecnia da ESALQ/USP (Piracicaba – SP).

After the analysis of composition by mid-infrared absorbance method, the samples were frozen (-20ºC) for CP and TP determination by Kjeldahl method as described by the ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (2000) (AOAC, method number 33.2.11, 991.20). The nitrogen content was then multiplied by a factor (6.38), so that the results were expressed as protein equivalent (TOUFEILI, 2007).

For non-protein nitrogen determination (NPN), a 15% trichloroacetic acid solution was added to milk sample for clotting of all milk proteins. After coagulation, proteins were removed by filtration, and filtrate was then submitted to nitrogen concentration analysis by Kjeldahl method (AOAC method number 33.2.12, 991.21). TP was obtained by difference according to TP=CP-NPN.

Statistical analysis was performed using the Statistical Analysis System (SAS Institute Inc., 2001). For the estimation of the prediction equations of the true protein content, it was performed a linear regression analysis using PROC REG. The regression model used was as follows: $y= \alpha+\beta x$, where y = milk true protein; α intercept; β is the linear coefficient associated with the crude protein content, x = the percentage of crude protein.

Results of protein content analysis by MIR method and Kjeldahl (standard) were not statistically different (P>0.05). According to TP results obtained by the Kjeldahl methodology, it was possible to develop a prediction equation to estimate TP from CP. The proposed equation had a coefficient of determination of r^2=0.94), indicating high correlation between calculated and measured levels by the standard method. The correlation between the contents of true protein calculated by the equation and the levels measured by the standard method (Kjeldahl) is presented in Figure 1. Statistical analysis indicated that the TP concentrations estimated by the equation are highly correlated (P<0.0001) with those found by the standard method. The proposed equation was as follows: TP=0.0021+(1.0104xCP), where: TP is the content of true protein, CP is the crude protein content as measured by MIR and 0.0155 is the error of the model.

The content of non-protein nitrogen had a significant effect on true protein concentration. Thus, a second equation was proposed (r^2=0.827) considering non-protein nitrogen level: TP=0.01175+(1.0869xCP)-(1.2895xNPN); where TP is the true protein concentration, CP is the crude protein content by MIR; NPN is the non-protein nitrogen content and 0.687 is the error model .

GRAPPIN (1992) affirmed that milk NPN is a factor that must be taken into account in the analysis

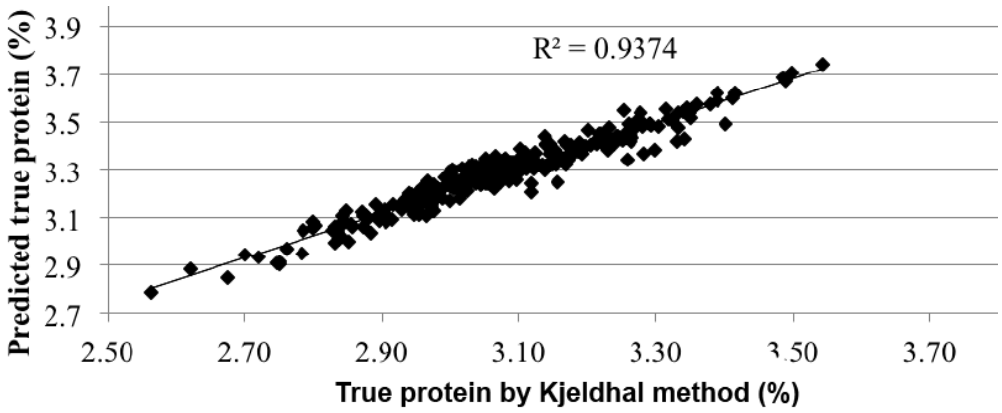

Figure 1 - Correlation between the calculated true protein content and the concentration measured by the standard method (Kjeldahl).

and expression of milk protein concentration. This author reports that several studies in France showed the disadvantage of using the crude protein content in payment systems for protein due to large variations that this milk component is submitted, as a result of the non-protein nitrogen fraction, which has little or no nutritional or commercial value.

Using the crude protein instead of true protein as a reference for equipment calibration for milk analysis, the dairy industry accepts certain inaccuracies in milk protein determinations. This procedure penalizes both, producers and industry. In one hand, producers are being underpaid on protein content basis due to the underestimations of true protein content of milk they supply, and industry undergoes inaccurate yield prediction of milk true protein-based based products.

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