*Short Communication:*

# Optimized Chemical Analysis of Cow's Milk Proteins: Evaluation of New Measuring Devices

**Marouane Chrif[1*], Abderrahim El Hourch[1], and Abdellah El Abidi[2]**

[1]*Laboratory of Electrochemistry and Analytical Chemistry, Faculty of Sciences, University Mohammed V, Avenue Ibn Battouta 1014, Rabat, Morocco*

[2]*Laboratory of Physical Chemistry, Department of Hydrology and Toxicology, National Institute of Hygiene, 27 Avenue Ibn Batouta, 10090 Rabat, Morocco*

* ***Corresponding author:***

*tel: +212-603054344*
*email: maro41@hotmail.com*

*Received: February 5, 2021*
*Accepted: June 10, 2022*

**DOI:** 10.22146/ijc.63900

***Abstract:*** *The demands of quality and choice in the dairy industry require analysis of extended performance. Ancient milk protein measuring devices take a long time and provide slow and inaccurate results. This work is part of the reliable analysis of cow's milk proteins and defines the laws linking the two parameters, total nitrogen (NT) and protein nitrogen (NP). We are studying to prove a fast and effective method for measuring the non-protein nitrogen (NPN) composition of milk that allows the direct calculation of NP from the NT value, whose objective is to adapt the calibration of the Milko Scan FT2 cow's milk protein analysis since NPN has a direct impact on protein analysis, payment of milk, and on the manufacture of milk products. The study showed that there is a compatibility between these two parameters and gave an idea of the percentage (5.9%) of NPN in milk. New analytical solutions such as the latest generation of the Kjeldahl K-375/376 and the new Milko Scan FT2 meet these needs. Data processing is done using XLSTAT, which is free statistical analysis software.*

***Keywords:*** *protein; milk; total nitrogen; non-protein nitrogen; fat*

## ■ INTRODUCTION

The determination of the non-protein nitrogen (NPN) content in milk is critical. Its results can cause major problems for industrial and economic decisions since they directly affect the analysis of milk proteins and the calibration of routine equipment, which plays an important role in milk payment and manufacturing of dairy products [1]. Thus, the NPN content of milk has an almost neglected nutritional value in the form of free amino acids [2]. Therefore, its dosage requires very high accuracy. The determination by the old official method (Kjeldahl K-350, Büchi) does not always give exact values (the volumetric determination at the time of titration and manual passage of the mineralized samples) [3]. Moreover, the contribution of new milk nitrogen analysis technologies allows the measurement to be controlled and the analysis to be reliable [4].

However, the use of high-tech equipment (Kjeldahl K-375/376, Büchi) minimizes to the maximum the human intervention during the analysis by a potentiometric assay at the time of titration and with an automatic passage of the mineralized samples [5]. In the dairy industry, the most important analysis at the financial level is the protein dosage being in milk [6]. These industries use routine methods (Milko Scan brand FOSS), which make it possible to lighten the routine work in the laboratory by eliminating the digestion, distillation, and titration procedures obtained by the official method (Kjeldahl), which require a lot of time [7]. In fact, most studies to date have been based on the results of protein analyses using Milko Scan S50 (FOSS) [8], consisting of a microprocessor with infrared control for the determination of milk proteins and which is also used for the determination of fat and the defatted dry extract. The Milko Scan S50 has drawbacks since, at the time of

the presence of milk wetting, it does not give reliable results [9].

Moreover, a new technique of routine devices such as the Milko Scan FT2, FOSS, ensures new functionality for dairies since it analyses the main parameters of milk in a single operation and allows the measurement of the lowering of the freezing point by a conductance which makes it possible to detect effectively wetting of raw milk [10].

## ■ EXPERIMENTAL SECTION

### Materials

The study was carried out on 84 samples of 500 mL taken from different cow's milk collection centers in Morocco over various periods from August 2019 to January 2020. Among these samples, 56 were made up of several cooperatives, and the other 28 were made up of cow's milk from farms.

### Instrumentation

The analysis of cow's milk proteins was carried out using the machine newly used in the dairy industry: the Milko Scan FT2 (FOSS). The Milko Scan FT2 uses an FTIR interferometer that scans the medium infrared spectrum, providing all the spectral information necessary for determining fat, proteins, and defatted dry extract [11]. Several quality checks of the analyzes to verify their reliability, accuracy, and precision were carried out on the FT2 device [12]. A calibration step according to the recommendations of the company FOSS on 20 samples for which the dosages were carried out in double and in parallel with the reference method using the latest generation of the device with an automatic sample changer Kjeldahl, K-375/376 (Büchi) and with the Milko Scan FT2 [13].

### Procedure

The samples were divided into two 250 mL portions, stored at 4 °C, and analyzed on the collection day. They are sent to the laboratory under a cold regime using a stacked ice pack cooler. Before analysis on the Milko Scan FT2 and Kjeldahl K-375/376, the milk samples were heated to 40 °C for 20 min and stirred several times to ensure homogeneous distribution of these components [14].

### Statistical Analysis

The elimination of the NPN determination phase requires the definition of a law linking the two parameters NP and NT, for any sample of cow's milk. To do this, we conduct a statistical study on analytical data. We want to know the effect of NPN on two types of methods of analysis of milk proteins since it intervenes directly in the Kjeldahl method to analyze milk proteins and indirectly for the calibration of Milko Scan FT2 (FOSS).

#### *Simple linear regression*

Our goal is to evaluate whether the protein content determined by the Kjeldahl method using simple linear regression varies according to the protein content obtained by the FT2 analyzer and whether a linear relationship has meaning. Thus, the relationship between two continuous quantitative variables is modeled, and the simple linear regression model is presented in Eq. (1) [15].

$$y = \beta 0 + \beta 1x + \varepsilon \quad (1)$$

where, y is the variable to be explained (with values in R); x is the explanatory variable (with values in R); ε is the random error term for the model; β0 and β1 are two parameters to be estimated.

#### *Analysis of variance or ANOVA*

One-way analysis of variance (ANOVA) was performed with milk productions over different periods of lactation from August 2019 to February 2020, using the general linear models (GLM) procedure of the statistical analysis software package (XLSTAT) [16]. In effect, our aim is to study how the protein content varies according to the total nitrogen content (qualitative variable taking the date value), the non-protein nitrogen of cow's milk, and whether a linear relationship makes sense.

## ■ RESULTS AND DISCUSSION

### Milko Scan FT2 Calibration

Creating programs for different raw milk constituents on the Milko Scan requires a wide range to accept all kinds of samples with fat and protein contents out of bounds [17]. The results obtained from the raw milk samples are summarized in Table 1.

**Table 1.** Summary statistics (quantitative data) and goodness of fit (Fat FT2)

| | |
|---|---|
| Observations | 20.000 |
| $R^2$ | 0.998 |
| Adjusted $R^2$ | 0.997 |
| MSE | 0.003 |
| RMSE | 0.058 |
| MAPE | 0.117 |
| DW | 1.409 |
| Cp | 2.000 |
| AIC | -111.715 |
| SBC | -109.724 |
| PC | 0.003 |

A simple bias adjustment -0.058% (0.058 g/100 mL) for fat and 0.06% (0.06 g/100 mg) for proteins allows for adjusting the values of Milko Scan FT2 on those obtained by the reference method with a very good correlation of 0.97 and 0.95 respectively (Fig. 1.). The calibration of the Milko Scan FT2 adapts well to the analysis of cow's milk after a simple bias adjustment. Thus, the repeatability between the 2 determinations was less than 0.05%, therefore perfect. Consequently, the Milko Scan FT2 can be used as a tool for rapid analysis of the chemical composition of cow's milk.

## Analysis of Variance or ANOVA

The analysis of the covariance determined the degree of homogeneity between the different analyses of the cow's milk protein. This has also made it possible to make several combinations of constituents with more or less similar results [16]. This last step is to determine the law that defines the variation of protein nitrogen as a function of NT obtained by the Milko Scan FT2 (FOSS) as a function of the NT obtained by the Kjeldahl reference method. Therefore, we are interested in the study of the linkage degree that may exist between these two NT and NPN variables for each milk sample.

We present the results found for raw milk coming from different regions at different times.

### *Regression of the variable NP by the Kjeldahl method*

In our case (Table 2), 98% of the protein variability is explained by NT. The rest of the variability is due to effects (other explanatory variables) that are not considered. Table 3, which shows the results of ANOVA of NP obtained by the Kjeldahl reference method, shows that the Fisher's F test (probability associated with F) is,

**Table 2.** The goodness of fit statistics (NP Kjeldahl)

| | |
|---|---|
| Observations | 84.000 |
| Sum of weights | 84.000 |
| DF | 77.000 |
| $R^2$ | 0.987 |
| Adjusted $R^2$ | 0.986 |
| MSE | 0.017 |
| RMSE | 0.132 |
| MAPE | 0.291 |
| DW | 2.352 |
| Cp | 7.000 |
| AIC | -333.913 |
| SBC | -316.897 |
| PC | 0.015 |

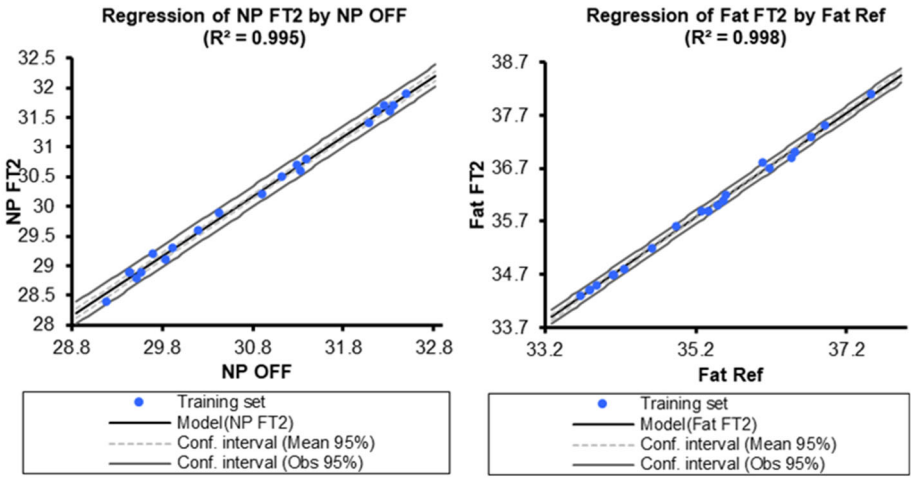


**Fig 1.** Regression of NP and Fat (reference method and FT2)

**Table 3.** Analysis of variance (NP Kjeldahl)

| Source | DF | Sum of squares | Mean squares | F | Pr > F |
|---|---|---|---|---|---|
| Model | 6 | 105.177 | 17.530 | 1010 | < 0.0001 |
| Error | 77 | 1.335 | 0.017 | | |
| Corrected Total | 83 | 106.512 | | | |

Computed against model Y=Mean(Y)

in this case, less than 0.0001, which means that we take the risk of being wrong by less than 0.01% by concluding that the explanatory variable provides a quantity of significant information to the model.

Moreover, Fig. 2 is a graphical representation of the analytical data for analyses of total nitrogen and cow's milk protein. The first graph shows the data, the regression line, and the two confidence intervals (the closest to the curve is the interval around the estimator mean, and the second is the interval around the point estimate, also called the prediction interval). We thus clearly see a linear trend, but with a strong variability around the left. We can also see that the observations that are outside the interval (-1.96, 1.96) are also outside the second confidence interval.

However, on the first graph, the point cloud, corresponding to the representation of NP as a function of NT, is modeled by a regression line with a correlation coefficient of 0.987. We observed perfect compatibility between the points represented and the regression line. The reduced centered residue histogram visually identifies the presence of values outside the range (-2, 2). Taking into account the protein analysis results obtained by the Milko Scan FT2 (FOSS) after calibration by the Kjeldahl reference method, there is repeatability between the results of the two methods by eliminating the phase relating to the determination of the NPN (NPN ranges from 5.8 to 6.1% relative to NT).

The milk samples used are supposed to be a mixture of cows' milk related to the term and different lactation ages obtained by the collection operation; it should have a stable and schematically similar composition to that of milk from different periods regions, and other factors. Our results prove that this is

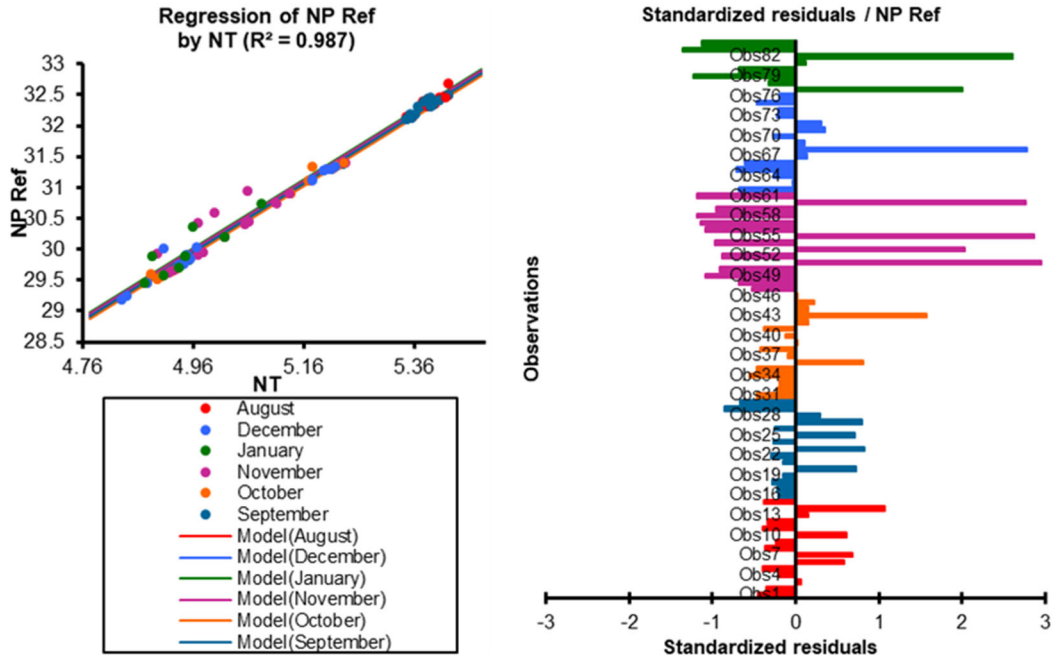


**Fig 2.** Graphical representation of NP as a function of NT

**Table 4.** Results obtained from cow milk mixture samples

| Content (g/100 mL) | Cow's milk (n = 84) | Farm samples (n = 56) | Cooperatives samples (n = 28) |
|---|---|---|---|
| Kjeldahl proteins | 3.13 (3.02–3.23) | 3.23 (3.21–3.25) | 3.02 (2.92–3.14) |
| FT2 Proteins | 3.12 (3.02–3.22) | 3.22 (3.20–3.26) | 3.02 (2.91–3.14) |
| Total nitrogen | 0.52 (0.50–0.54) | 0.54 (0.53–0.54) | 0.50 (0.48–0.52) |
| Non-protein nitrogen | 0.03 (0.02–0.03) | 0.03 (0.03–0.03) | 0.03 (0.02–0.03) |
| Fat by Gerber method | 3.54 (3.37–3.75) | 3.62 (3.54–3.75) | 3.43 (3.37–3.53) |
| FT2 Fat | 3.59 (3.43–3.81) | 3.68 (3.59–3.81) | 3.49 (3.43–3.59) |

not the case (Table 4). Thus, this result improves the study on NPN variation by Chrif and Haimei and Ying (5.5 to 6.1% and 5%, respectively) [18-19]; Also, there is significant variability in protein and fat between the different samples of cow's milk [20].

## ■ CONCLUSION

Using ANOVA and the simple linear regression test, we were able to combine samples with identical NPN contents. As for the linear adjustment, it made it possible to define the law linking the two parameters NT (obtained by the Kjeldahl reference method) and NP (obtained by the Milko Scan FT2 (FOSS) for each cow's milk sample. We noted that all series showed a linear trend with correlation coefficients very close to 1. This will allow saying that the method of analysis by the Milko Scan FT2 (FOSS) gives reliable results by comparing with the Kjeldahl reference method, and the compatibility between the two parameters NT and NP forming part of the different milk is well verified. As a result, this follow-up of the non-protein nitrogen analysis of different milk samples gave us a clearer idea of the percentage of NPN in milk, and this may help to make the measurement of proteins in milk more reliable. This can prove that when analyzing protein nitrogen in mixed milk, the analysis of the non-protein phase can be eliminated and replaced with 5.9% to optimize the cost of physical and chemical quality control.

## ■ AUTHOR CONTRIBUTIONS

M.C prepared and carried out the experiments and wrote the manuscript. A.E performed the data processing analysis and examined the manuscript. All authors agreed to the final version of this manuscript.

## ■ REFERENCES

[1] Yang, Y., Hettinga, K.A., Erasmus, S.W., Pustjens, A.M., and van Ruth, S.M., 2020, Opportunities for fraudsters: When would profitable milk adulterations go unnoticed by common, standardized FTIR measurements?, *Food Res. Int.*, 136, 109543.

[2] Ruska, D., and Jonkus, D., 2014, Crude protein and non-protein nitrogen content in dairy cow milk, *Proc. Latv. Univ. Agric.*, 32 (1), 36–40.

[3] Das, M., Nath, P.C., Sileshi, G.W., Pandey, R., Nath, A.J., and Das, A.K., 2021, Biomass models for estimating carbon storage in *Areca* palm plantations, *Environ. Sustainability Indic.*, 10, 100115.

[4] Hristov, A.N., Bannink, A., Crompton, L.A., Huhtanen, P., Kreuzer, M., McGee, M., Nozière, P., Reynolds, C.K., Bayat, A.R., Yáñez-Ruiz, D.R., Dijkstra, J., Kebreab, E., Schwarm, A., Shingfield, K.J., and Yu, Z., 2019, Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques, *J. Dairy Sci.*, 102 (7), 5811–5852.

[5] Serra, I., Capusoni, C., Molinari, F., Musso, L., Pellegrino, L., and Compagno, C., 2019, Marine microorganisms for biocatalysis: Selective hydrolysis of nitriles with a salt-resistant strain of *Meyerozyma guilliermondii*, *Mar. Biotechnol.*, 21 (2), 229–239.

[6] Sraïri, M.T., Chatellier, V., Corniaux, C., Faye, B., Aubron, C., Hostiou, N., and Lortal, S., 2019, Réflexions sur le développement du secteur laitier et sa durabilité dans différentes parties du monde, *INRA Prod. Anim.*, 32 (3), 339–358.

[7] Kim, H., Kang, S., Jung, B.M., Yi, H., Jung, J.A., and Chang, N., 2017, Breast milk fatty acid composition and fatty acid intake of lactating mothers in South Korea, *Br. J. Nutr.,* 117 (4), 556–561.

[8] Li, S., Ye, A., and Singh, H., 2019, Seasonal variations in composition, properties, and heat-induced changes in bovine milk in a seasonal calving system, *J. Dairy Sci.*, 102 (9), 7747–7759.

[9] Srisaikham, S., Jaijapo, W., Lounglawan, P., and Suksombat, W., 2018, Effect of feeding fermented fresh cassava pulp on lactoperoxidase activity and performance of Holstein Friesian crossbred lactating dairy cows, *PIM 1st International Conference 2018*, Amphoe Pak Kret, Thailand, 21 June 2018, 1–38.

[10] Souhassou, S., Bassbasi, M., Hirri, A., Kzaiber, F., and Oussama, A., 2018, Evaluation of Milkoscan FT2 milk analyzer for determining the freezing point of cow milk under different analytical conditions, *Int. J. Chem. Mater. Environ.* Res., 5 (2), 165–172.

[11] Baum, A., Dominiak, M., Vidal-Melgosa, S., Willats, W.G.T., Søndergaard, K.M., Hansen, P.W., Meyer, A.S., and Mikkelsen, J.D., 2017, Prediction of pectin yield and quality by FTIR and carbohydrate microarray analysis, *Food Bioprocess Technol.*, 10 (1), 143–154.

[12] Løkke, M.M., Engelbrecht, R., and Wiking, L., 2016, Covariance structures of fat and protein influence the estimation of IgG in bovine colostrum, *J. Dairy Res*., 83 (1), 58–66.

[13] Stocco, G., Cipolat-Gotet, C., Bonfatti, V., Schiavon, S., Bittante, G., and Cecchinato, A., 2016, Short communication: Variations in major mineral contents of Mediterranean buffalo milk and application of Fourier-transform infrared spectroscopy for their prediction, *J. Dairy Sci.*, 99 (11), 8680–8686.

[14] Kharitonov, V., Sherstneva, N., Kharitonov, D., Yurova, E., and Kurchenko, V., 2019, Changes in physico-chemical properties of milk under ultraviolet radiation, *Foods Raw Mater.*, 7 (1), 161–167.

[15] Nguyen, Q.T., Fouchereau, R., Frénod, E., Gerard, C., and Sincholle, V., 2020, Comparison of forecast models of production of dairy cows combining animal and diet parameters, *Comput. Electron. Agric.*, 170, 105258.

[16] Hurtado-Lugo, N., Rincon, A., and Ceron-Muñoz, M., 2020, Physicochemical and statistical analysis of a dairy production, *J. Phys.: Conf. Ser.*, 1587, 012026.

[17] Franzoi, M., Niero, G., Penasa, M., and De Marchi, M., 2019, Development of infrared prediction models for diffusible and micellar minerals in bovine milk, *Animals*, 9 (7), 430.

[18] Chrif, M., El Hourch, A., Chouni, S., Aitbenyouf, S., and El Abidi, A., 2018, The variation of the non-protein nitrogen content by region and season and their impact on the analysis of milk proteins in Morocco, *J. Mater. Environ. Sci.*, 9 (3), 811–816.

[19] Roveglia, C., Niero, G., Penasa, M., Finocchiaro, R., Marusi, M., Lopez-Villalobos, N., and Cassandro, M., 2019, Phenotypic analysis of milk composition, milk urea nitrogen and somatic cell score of Italian Jersey cattle breed, *Ital. J. Anim. Sci.*, 18 (1), 405–409.

[20] Chrif, M., El Hourch, A., Chouni, S., Bouyahya, A., and El Abidi, A., 2019, Changes of physicochemical composition of Moroccan bovine's milk along the years, *Mediterr. J. Chem*., 8 (6), 470–475.