



The variation of the non-protein nitrogen content by region and season and their impact on the analysis of milk proteins in Morocco.

M. Chrif¹, A. El Hourch¹, S. Chouni², S. Aitbenyouf², A. El abidi²

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 Battouta, 10090 Rabat, Morocco.

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M Chrif
maro41@hotmail.com
+212667144584

Abstract

This work concerns a statistical study whose objective is to study the variation in non-protein nitrogen content on samples from cow milk collection centers (cooperative collection centers) and farms in the Doukkala region (DKK), Chaouia, Fkih Ben Salh (FBS), El-Kalaa, Meknes and Gharb, and the possibility of eliminating the non-protein nitrogen determination phase to calculate the protein milk. Samples were collected throughout the year 2015. Analysis results show that there is a relationship (factor) between the total nitrogen (NT) and protein nitrogen (NP) parameters and also show that the effect Of the milk origin and the period does not significantly affect non-protein nitrogen (NPN) in milk.

1. Introduction

Proteins are one of the essential components of milk. In the last few years, an increase in cheese consumption occurred, so the determination of protein content of milk is an important factor for the price paid for by the industry [1].

Morocco is transitioning from extensive pastoralist livestock and dairy production to more intensified production due to increasing demands for dietary protein by a growing human population [2].

Protein contains about 95% of the total nitrogen present [3]. The NPN content of milk represents only 5 to 6% of the total N in milk. However, the significance of this milk N fraction to energy and N metabolism in the dairy cow has not been well characterized. The single largest contributor to the NPN fraction of milk NPN is urea [4]. The objective of this work is optimization of the costs related to the control of the protein content of milk. We are studying the possibility and utility of eliminating the non-protein nitrogen determination phase. The NPN fraction of milk and other foods can be isolated using different techniques. Trichloroacetic acid (TCA) was used by Rowland (1938) to separate the protein and NPN fraction of milk [5]. The proteins was determined by the Kjeldahl method using the known formula $[(TN - NPN) * 6.38]$ [6].

2. Material and Methods

2.1. Method of analysis

A large number of methods have been used for the quantitative determination of milk proteins. The reference method for the determination of the protein content for payment is based on the measurement of total nitrogen by Kjeldahl analysis. Nitrogen is multiplied by a factor, usually 6.38, to express the results as total protein (also called "crude" protein) [7, 8].

2.2. Study area and sampling

The tests began in January 2015 and ended at the end of December 2015. Milk samples of mixture "Cistern" by region were collected, with two monthly withdrawals over different period. Milk tank are collected during the

collection of milk by the slag and represent the mixed milk of different farms on average. This collection rounds have been selected to represent the variability of the milk products and therefore include cooperative farms and collection centers in different regions of Morocco [9] (Leading regions in terms of milk production): Doukkala (DKK), Chaouia, Fkih Ben Salh (FBS), El-Kalaa, Meknes and Gharb in different milk production seasons (winter, spring, summer and autumn) (Figure 1).

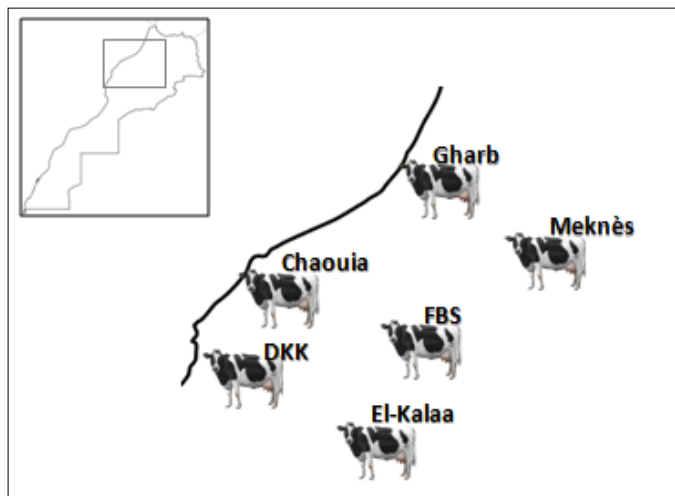


Figure 1: Map of the six regions studied.

2.3. Collection of milk samples and laboratory analyzes

The sampling of the milk took place immediately after the morning milking and concerned exclusively the milk of this milking. Two samples are taken at each passage at a rate of 0.5 L of milk of mixture, for the physical and chemical analysis.

The sample is refrigerated to avoid the effect of the ambient temperature during transport to the laboratory in sterilized vials and kept in coolers containing refrigerant block at temperature between 1°C and 4°C. The temperature is checked with a portable thermometer in a bottle filled with water which is stored with the samples in the coolers [10].

The protein content $PN = ((TN - NPN) \times 6.38)$, determined within 24 hours, was measured by the Kjeldahl method (NF EN ISO 8668-1).

The total nitrogen was analyzed by direct introduction of 0.2 g of the milk sample into the matras (vial). For analysis of non-protein nitrogen, 10 g of the sample was weighed, 40 g of 15% trichloroacetic acid (CCl_3COOH) was added, filtered with filter paper (the filtrate should be transparent and free of particulate matter), and then 20 g of the filtrate was transferred to the matras (vial). The mineralization was done at 420 °C with 25 ml of sulfuric acid (95-97%) and two pellets of the Kjeldahl catalyst (Cu-TiO₂). The distillation was done with NaOH (33%) (the digest takes a blue color) and the distillate was collected in a boric acid solution 4% at pH = 4.65. The titration was done by potentiometer with a hydrochloric acid solution (0.1N for TN) and (0.01N for NPN).

3. Results and discussion

3.1. Total nitrogen and non protein nitrogen by season and region

The study of deviations between extreme values (Table 1) shows a variability in the total nitrogen content in the summer and spring season, which is 44.7 and 51.4 mg TN/100 g respectively and slight variability that varies between 22.7 and 31.7 mg TN/100 g for the fall and winter season.

The difference between the extreme values for the regions studied shows a variability in total nitrogen content of 38.2 and 54.4 mg TN/100 g belongs to the blended milk from the Chaouia region and FBS respectively.

Whereas during the entire study period, the non-protein nitrogen content varied little from 5 to 7.7 mg of non-protein N/100 g for all mixing milk from the 6 regions and varied not significantly from 3.6 to 4.7 mg non-protein N/100 g in all seasons.

These results also show that the non-protein nitrogen content, whatever the factor (season or region), varies between 25.4 mg and 33.4 mg NPN /100 g, which appears to be very low. These values are very close to those found by Roger [11] which are in the range of 16 mg to 32 mg NPN/100 g in normal milk provided by healthy animals. Although the NPN content found by Haimei and Ying [12] which is 23 and 42 mg NPN/100 g was a little larger than in this study.

Table 1: Comparison of the variability between the total nitrogen content and non-protein nitrogen content of mixing milk, between regions and seasons.

Factor of variation	Difference between extreme values (mg N/100 g of milk)						2/1 (p.100)
	TN (1)			NPN (2)			
	min value	max value	difference	min value	max value	difference	
4 seasons							
Winter	501.4	533.1	31.7	29.8	33.4	3.6	11.4
Spring	467.6	519.0	51.4	25.7	29.6	3.9	7.6
Summer	476.0	520.7	44.7	25.4	29.8	4.4	9.8
Autumn	511.3	534.0	22.7	28.2	32.9	4.7	20.7
12 months							
DKK	490.4	534.0	43.6	27.9	32.9	5.0	11.5
Chaouia	495.5	533.7	38.2	26.5	33.4	6.9	18.1
FBS	476.0	530.4	54.4	27.3	32.6	5.3	9.7
El-kalaa	467.6	521.5	53.9	25.7	32.3	6.6	12.2
Meknes	480.3	528.5	48.2	25.4	33.1	7.7	15.9
Gharb	482.3	521.0	38.7	25.9	32.9	7.0	18.3
Mixing milk	467.6	534.0	66.4	25.4	33.4	8.0	12.0

Table 2: Non-protein fractions of mixed milk compared between regions and seasons.

Factor of variation	mg d'N/100 g of milk		2/1 (p.100)
	TN (1)	NPN (2)	
4 seasons			
Winter	522.3 ±6.30	32.0 ±0.9	6.1
Spring	492.1 ±13.4	27.6 ±1.1	5.6
Summer	496.1 ±12.2	27.3 ±1.2	5.5
Autumn	522.9 ±7.30	31.5 ±1.3	6.0
12 months			
DKK	514.5 ±15.5	30.4 ±1.8	5.9
Chaouia	517.8 ±14.3	30.1 ±2.5	5.8
FBS	506.9 ±19.2	29.9 ±2.0	5.9
El-kalaa	498.5 ±19.4	28.7 ±2.6	5.8
Meknes	507.0 ±17.5	29.2 ±2.8	5.8
Gharb	505.3 ±13.6	29.4 ±2.6	5.8
Mixing milk	508.4 ±18.0	29.6 ±2.0	5.8

The difference in total nitrogen averages (higher and lower average) (Table 2) shows that there is a difference in total nitrogen content between regions, which is 19.3 mg TN/100 g and a difference of 30.8 mg TN/100 g according to the season. Non-protein nitrogen does not vary greatly in any region and season, from 27.3 mg to 32 mg NPN/100 g. The average non-protein nitrogen obtained in the study is 29.6 mg NPN/100 g, which is higher than that found by Ruska [13], which is 20.4 mg NPN/100 g and somewhat lower than that found by Haimei and Ying [12] which is 32.5 mg NPN/100 g. The rate of non-protein nitrogen ranges from 5.5 to 6.1 % in all seasons and from 5.8 to 5.9 % for all regions. Whereas the mix milk level from all regions and in all seasons is 5.8 %. Thus, it is clearly observed that the rate of non-protein nitrogen is slightly varied from 5.5 to 6.1 % relative to total nitrogen and is higher than that found by Haimei and Ying [12] which is 5 %.

Figures 2 and 3 show that the total and non-protein nitrogen content decreases progressively in the same way, regardless of the sampling region from the first week of April and then increases significantly from the first week of September for all regions studied. It is clearly observed that the medium and the season have a significant impact on the total nitrogen content. On the other hand, they do not have a large influence on the variation in the proportion of non-protein nitrogen since it varies within narrow limits.

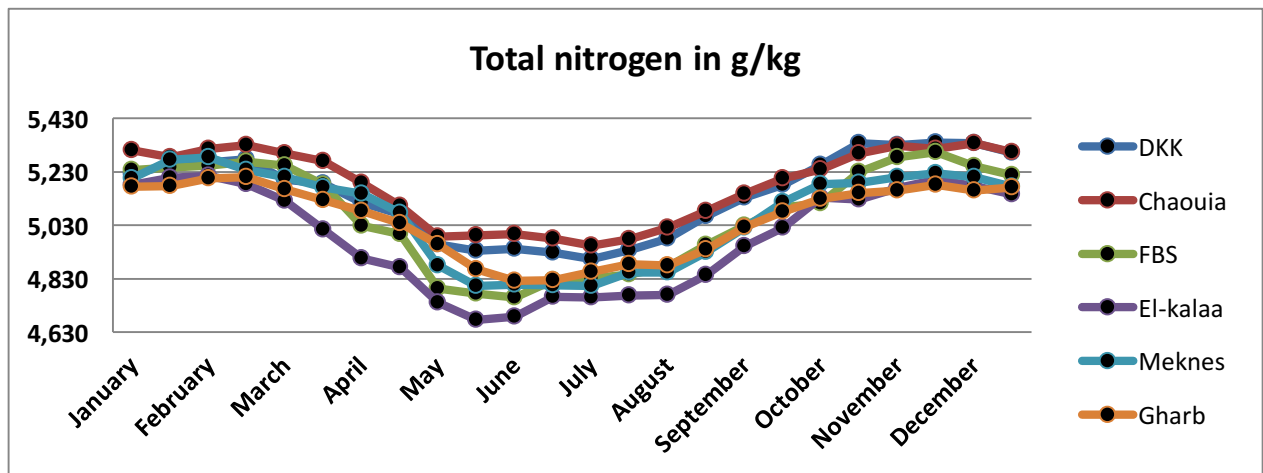


Figure 2: Influence of season and region on total nitrogen.

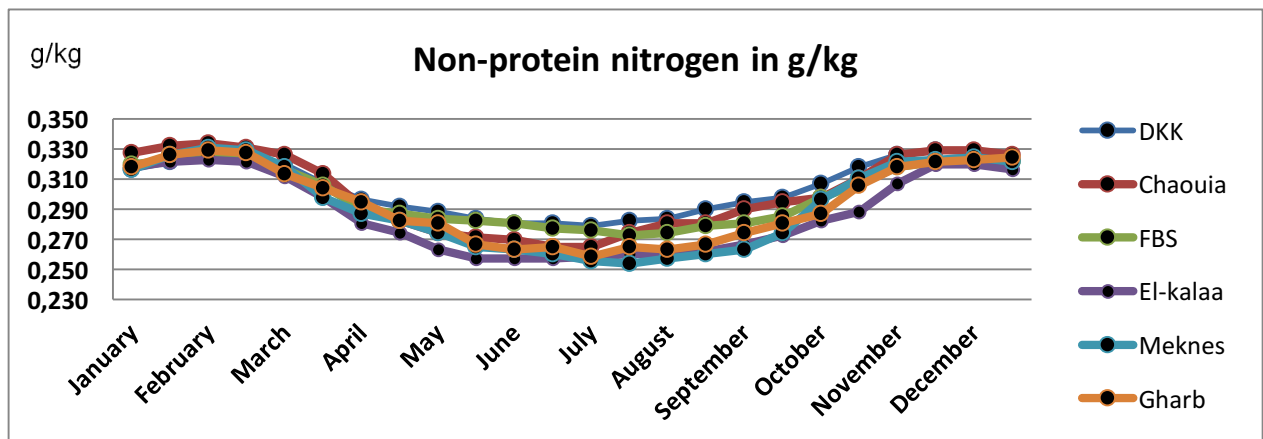


Figure 3: Influence of season and region on non protein nitrogen.

3.2. Average protein content of the mixed milk

The protein content of the mixed milk between the regions (Figure 4) appears to be stable on all the milk collected and varies in a range of 29.97 g/kg to 31.12 g/kg with an average of 30.55 g/kg lower than those found by Nistor [14] 32.5 g/kg with a maximum of 45 g/kg and a minimum of 20 g/kg.

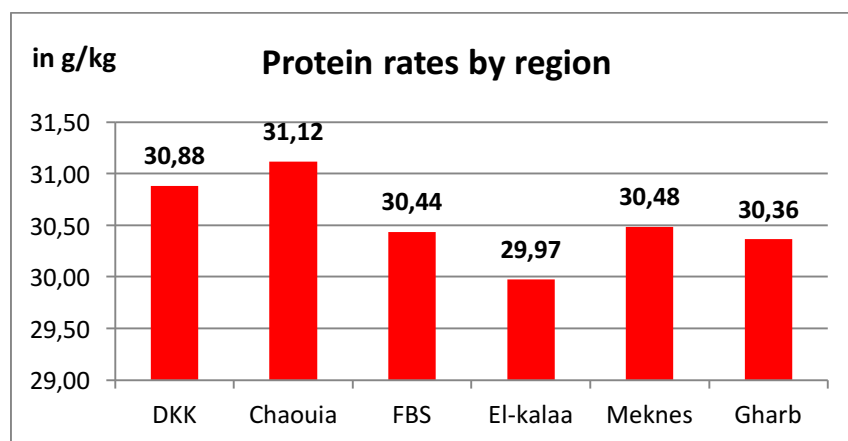
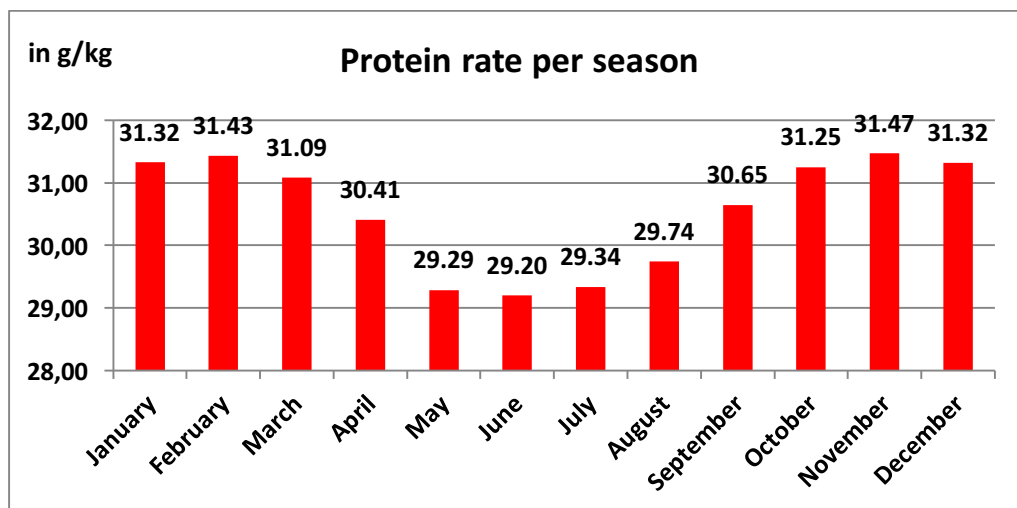


Figure 4: Protein content of the mixed milk by region

Table 3: Protein content of mixed milk compared between regions and seasons.

Factor of variation	min of PN g/gk	max of PN g/kg	difference	PN g/kg
4 seasons				
Winter	30.09	31.9	1.81	31.28 ±0.36
Spring	28.19	31.25	3.06	29.63 ±0.81
Summer	28.72	31.34	2.62	29.91 ±0.72
Autumn	30.72	32.02	1.30	31.35 ±0.42
12 months				
DKK	29.51	32.02	2.51	30.88 ±0.87
Chaouia	29.92	31.95	2.03	31.12 ±0.76
FBS	28.58	31.79	3.21	30.44 ±1.11
El-kalaa	28.19	31.21	3.02	29.97 ±1.08
Meknes	28.96	31.61	2.65	30.48 ±0.96
Gharb	29.09	31.15	2.06	30.36 ±0.72
Mixing milk	28.19	32.02	3.83	30.54 ±0.98

The protein content of milk mixing according to the seasons (Figure 5) appears to be somewhat stable on all milk collected and ranges from 29.20 g/kg to 31.47 g/kg with an average of 30.34 g/kg lower than those found by Nistor [14] 32.5 g/kg with a maximum of 45 g/kg and a minimum of 20 g/kg.

**Figure 5:** Protein content of the mixed milk by season.

The variation in the protein content is due to several factors that can influence milk quality (region, season and nature of producers: dairy cooperatives and farms) [15] and also the food factor. The influence of genetics on the protein content can also be characterized by considering the different breeds [16]. About 55 % of the variability in protein content is caused by genetics; The rest is environment-related [17]. Environmental factors are related to the production level, lactation stage, cow age, sanity and nutrition [16]. Thus, it is important to pay attention to selection criteria because of their potential effects on various physiological processes [18, 19].

Conclusion

Non protein nitrogen in the mixed milk varies within narrow limits with an average of 5.7 % under the influence of environmental factors and season. This could justify the fact that during analysis of the protein nitrogen in the milk mixture, the analysis of the non protein phase can be eliminated in order to optimize the costs relating to the control of the physico-chemical quality.

This result will reduce the cost and time of protein determination by Kjeldahl method, increase labor performance and identify the most important source of losses in dairy industry and to be able to master them to the highest amount.

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