DE GRUYTER
OPEN

Proc.Latv.Univ.Agr.,2014,32(327)
DOI: 10.2478/plua-2014-0011

Crude Protein and Non-protein Nitrogen Content in Dairy Cow Milk

Diana Ruska*, Daina Jonkus
Institute of Agrobiotechnology, Latvia University of Agriculture, Liela str. 2, Jelgava, LV 3001, Latvia

Abstract. Milk composition is of prime economic importance for farmers. Milk total proteins are composed of casein, whey proteins and non-protein nitrogen. The objective of this work was to establish milk crude protein, non-protein nitrogen (NPN) and urea content in dairy cow milk produced in different farms in Latvia. Cow milk samples (n=30) were collected in September 2012 from four different farms breeding diverse cow breeds. Average crude protein, casein and urea content in milk varied significantly among farms. NPN content in cow milk varies among farms – from 0.194% to 0.232%. Average crude protein and casein content was significantly higher ($p<0.05$) for Latvia Brown breed cows, while NPN content did not differ significantly among breeds. Regression between NPN and urea content in milk was $R^2 = 0.458$. Correlation between NPN and urea content was significant ($r = 0.677$). This study allowed establishing that crude protein and NPN content in milk varied significantly ($p<0.05$) in farms with differing dairy cow housing and feeding technologies.
Key words: crude protein, casein, NPN, urea.

Introduction

Milk of dairy cows is biological solution containing approximately 12.8% of dry matter. Milk dry matter consists of proteins, carbohydrates, fats, minerals and vitamins. Depending on animal breed, genotype, phase in lactation, nutritional value of feed, milking technology, animal health status, environmental conditions, animal age, interval between milking, as well as other factors milk composition may change (Coballero et al., 2003; Roginski et al., 2003).

Recently increasingly higher interest has been devoted to researches on milk proteins, since they are the main component used to produce curd and cheese. Analysis of milk samples with standard Kjeldahl method helps in finding out volume of total nitrogen (N) in milk, and multiplying the acquired result by 6.38 we may acquire crude protein content (*CP – crude protein = N × 6.38*) in milk (ISO, 2001). Proteins in cow milk comprise 95 – 97% of the crude protein content, and non-protein nitrogen (NPN) accounts for 3 – 5%. Proteins in milk are represented by casein and whey proteins. Average proportions of proteins in crude protein are the following: 75 – 85% casein, 18 – 20% whey proteins. Casein content in proteins accounts for 85 – 90% (Hui, 1993). Non-protein nitrogen (NPN) gets into milk from animal blood after protein metabolism. One of the largest (~50%) and most stable NPN parts is formed by milk urea. Apart from milk urea, NPN consists also of free amino acids, creatine, uric acid, peptides, organic acids and phospholipids (DePeter and Ferguson, 1992; DePeters and Cant, 1992; Твердохлеб и Раманаускас, 2006).

Nutrition experts point out that evaluation of food quality from nutritional point of view requires finding out quality of proteins, as human body, unlike one of ruminants, may use only proteins formed from amino acids. Thus, the content of crude protein in milk should be taken into account when balancing diet; otherwise, expected results may not be achieved (Moughan, 2012).

Lately interest towards environmental pollution has been growing. In Europe, several regulatory enactments are controlling possible environmental pollution that may arise when performing agricultural activities. In the Netherlands, farms are monitored based on urea content in milk. This allows to specify possible pollution sources and notify farms about preventive actions (Bijgaart, 2003). The optimum amount of urea in milk set in Europe is 15 – 30 mg dL^{-1}.

Data of the National Research Council (in USA) show that nitrogen amount in feed of dairy cows is exceeded on average by 6.6%; thus, nitrogen content in urine rises by 16% and by 2.7% in manure. With an aim to calculate amount of nitrogen used, the milk urea content is used, since it is easy to find it out and it does not require collecting and testing special (urine or faeces) samples (Jonker *et al.,* 2002; Broderick and Huhtanen, 2013). Researches show that milk urea characterises content of urea in both blood and urine. Milk urea content reflects losses of crude protein for dairy cow, especially excess in digestive tract; therefore, this indicator may be used to assess environmental pollution and digestive efficiency (Meijier *et al.,* 1996; Broderick and Clayton, 1997;

* Corresponding Author's email:
delta@e-apollo.lv

Hof *et al.,* 1997a; Hof *et al.,* 1997b; Sederevičius *et al.,* 2008; Burgos *et al.,* 2010).

Research aims at finding out content of cure protein and non-nitrogen protein in cow milk.

Materials and Methods

Farms included in the research are located in various places of Latvia and represent different animal housing and feeding technologies. Two large holdings (B and D, 503 and 164 cows, respectively) have an indoor freestall housing system. Feeding in these farms was organized in groups according to lactation stage. Moreover, it was balanced and with total mixed ration in all years without pasture period. Small farms (A and C, 28 and 20 cows, respectively) have tie stall housing system, their cows are not grouped and graze in summer.

Samples, for which NPN content was studied, were taken once (in September 2012) from all farms. 5 samples were taken from farms A and C, while from farms B and D – 10 samples from each. Milk samples were taken from cows representing various breeds (LB, n=14; HM, n=10; XP, n=6) and lactation 1st (n=6); 2nd (n=7); 3rd (n=7); 4th (n=10), while all cows were in the same lactation phase – from 100th to 200th lactation day.

Milk composition was analysed in an accredited laboratory for milk quality control. Infrared spectroscopy method helped in finding out the content of milk crude protein, fat and lactose in compliance with ISO 9622:1999. Content of casein and urea were measured in line with the methods validated in the laboratory – MET-006 and MET-003. Milk samples to estimate NPN were preserved with 10mg of $K_2Cr_2O_7$ 100mL^{-1} and sent to the central milk laboratory *QLIP* in the Netherlands, where they were analysed with a method validated in the laboratory in compliance with ISO 8968-4 *Milk nitrogen content, non-protein nitrogen content*.

With an aim to research influence left by environmental and selected physiological factors as well as cow breed on changes in milk composition the multifactor linear model was used; it included the factors fixed:

$$y_{ijklmnsr} = \mu + S_i + \check{S}_k + e_{ik} \tag{1}$$

S – farms (i=4);
Š – breed (k=3).

Data on milk quantities yielded from dairy cows, cow breed, lactation and day in lactation were acquired from monthly herd recording data available in Agricultural Data Centre database.

Content of crude protein, casein and urea nitrogen (that in the laboratory was measured as per cent of crude protein, casein and urea nitrogen volume (kg and g)) was recalculated in compliance with ICAR guidelines (ICAR, 2011).

Statistical processing of the data was carried out with *MS for SPSS* (SPSS Inc. Chicago, Illinois, USA) and *MS Office* programme *Excel*.

Results and Discussion

Average milk yield in the research group comprised 28.8 kg. Milk yield amplitude was rather wide – from 15.0 kg to 47.8 kg (Table 1). Average crude protein content in group accounted for 3.43% and casein content comprised 2.65%, i.e., 77.3% from crude protein content in milk.

Average urea content in milk constituted 28.3 mg dL^{-1}, thus, meeting the limits suggested. Non-protein nitrogen content comprised 0.204%. The NPN obtained within the study is higher than one in American researches (Raden and Powell, 2009) – 0.19%. While NPN content found in research conducted in the Netherlands was lower (Heck *et al.,* 2009) than in this study (0.182%).

Table 1

Average milk productivity traits and NPN content in research group

Traits	$\bar{x} \pm s_{\bar{x}}$	Min.	Max.
Milk yield, kg	28.8±1.03	15.0	47.8
Crude protein content, %	3.43±0.039	2.88	4.19
Casein content, %	2.65±0.028	2.18	3.23
Milk urea content, mg dL^{-1}	28.3±1.25	12.6	52.9
NPN, %	0.204±0.0032	0.162	0.255

Table 2

Average milk yield, content of crude protein, casein, non-protein nitrogen, and urea depending on breed

Traits	Breed		
	LB (n=14)	HM (n=10)	XP (n=6)
Milk yield, kg	22.7±1.04[a]	33.0±1.23	36.1±1.59
Crude protein content, %	3.59±0.049[a]	3.33±0.058	3.24±0.075
Casein content, %	2.78±0.035[a]	2.57±0.042	2.51±0.054
Milk urea content, mg dL^{-1}	30.5±1.79 [a]	24.6±2.12[b]	29.2±2.74[a,b]
NPN, %	0.210±0.005	0.197±0.006	0.201±0.007

[a;b] – productivity traits with unequal letter differed significantly among breeds ($p<0.05$).

Researches performed prior in other countries affirm changes in non-protein nitrogen content depending on holding, breed, lactation, day in lactation and season (Ng-Kwai-Hang *et al*., 1985); therefore, the author of this paper has evaluated results of this research considering all the factors above.

The results obtained indicated (Table 2) that the volume of milk yielded from LB cows is significantly smaller, while crude protein and casein content in milk is significantly higher. Non-protein nitrogen and urea content does not differ significantly among breeds. Also, studies of other researchers do not show that cow breed causes changes in content of non-protein nitrogen and cure protein (Carroll *et al.,* 2006).

Research group results may be compared with data acquired in the Netherlands. The cow breed dominating in Holland is HM. Urea and non-protein nitrogen content results analysed in the Doctoral Paper were obtained in September, and for breed HM those were 24.6 mg dL^{-1} and 0.197%, meeting the data acquired in the Netherlands where urea and non-protein nitrogen parameters obtained in August comprised 26.0 mg dL^{-1} and 0.190% (Heck *et al.,* 2009).

With an aim to evaluate farm influence on milk productivity traits and non-protein nitrogen content in the research group, the results found are compiled in Table 3. Holding factor had significant influence on milk yield, crude protein, casein and urea content. Also, farm C cows engaged in the research had significantly higher urea and non-protein nitrogen content in milk (45.6 mg dL^{-1} and 0.232%), while milk yield in holding C was the lowest.

Significant differences were observed among all holdings. Farm D indicated the highest milk yield – 35.8 kg. Also, crude protein content varied remarkably among the farms. The highest crude protein and casein content was recorded in farm B

Table 3

Average milk yield, content of crude protein, casein, non-protein nitrogen and urea in farms

Traits	Farms			
	A (n=10)	B (n=20)	C (n=10)	D (n=20)
Milk yield, kg	30.6±1.79[a]	25.2±1.27[b]	20.4±1.79[c]	35.8±1.27[d]
Crude protein content, %	3.30±0.084 [a]	3.61±0.060[b]	3.51±0.084[a,b]	3.29±0.060[c,a]
Casein content, %	2.56±0.060[a,c]	2.79±0.043[b]	2.71±0.060[c,b]	2.53±0.043[a]
Milk urea content, mg dL^{-1}	21.3±1.76[a]	24.5±1.24[a,c]	45.6±1.76[b]	26.9±1.24[c]
NPN, %	0.194±0.007	0.201±0.005	0.232±0.007[a]	0.198±0.005

[a;b;c;d] – productivity traits with unequal letter differed significantly among farms ($p<0.05$).

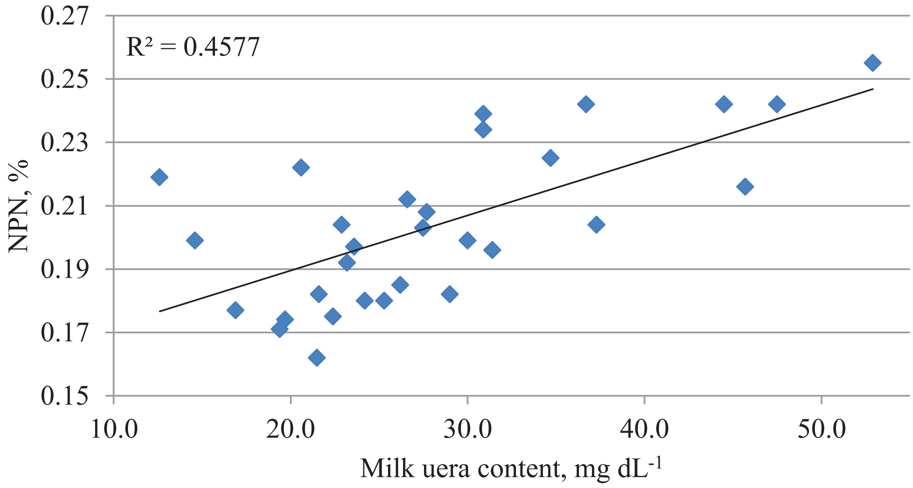

Fig. 1. Regression between NPN and urea content in milk.

(3.61% and 2.79%, respectively); whereas farm D indicated the lowest crude protein and casein content (3.29% and 2.53%, correspondingly). The farm factor had a notable influence on milk yield, content of crude protein, casein and urea in milk. The highest urea and NPN content was recorded in farm C, while milk yield in this farm was the lowest.

Analysis of seasonal factors influencing milk productivity traits shows that other scientists have discovered that the lowest crude protein and true protein content in milk may be observed in summer months, while the content of non-protein nitrogen and urea during these months is significantly higher (Heck *et al.,* 2009). Also, other studies have observed that crude protein and casein content in milk is lower when the volume of non-protein nitrogen (containing urea) increases (Carlsson *et al.,* 1995; Ferguson *et al.*, 1997; Godden *et al.,* 2001).

Regression analysis of urea and NPN content indicates positive correlation. This fact allows using milk urea content to forecast NPN proportion – indicating rise in NPN share (as milk urea content will increase as well), and thus pointing out also the volume of unnecessary nitrogen-containing substances in milk (Fig. 1).

Results of the research show that when assessing crude protein content in milk for setting payment or breeding value it is advisable to use casein or protein indicators instead of milk crude protein content, as those are indicators giving better information to milk processers about possible production outcome from the particular milk purchased (Šustova *et al*., 2007).

With an aim to find out influence left by NPN on milk productivity indicators, the correlation between NPN content and yield, crude protein, casein and urea content was calculated.

Significant, positive, and close (r = 0.677) correlation was found between content of NPN and urea, whereas NPN content and yield indicated weak, negative, and non-significant ($r = -0.099$) correlation. NPN content correlation with crude protein and casein content was only slightly positive (weak) (r = 0.088 and 0.017, respectively) and insignificant.

The results acquired within the framework of the research indicate that NPN share is variable and is affected by several factors: in the meantime, it is part of milk not giving economical or nutritional value, and thus there is no need to determine NPN content in milk on daily basis. Close NPN content correlation with urea content allows assessing milk production efficiency by using only urea indicators, as it has been suggested also by other scientists. Moreover, this phenomenon suggests that you base the evaluation of milk productivity on infrared-spectrometry method (Ng-Kwai-Hang *et al*., 1985).

Conclusions

1. Content of crude protein, casein and urea in dairy cow milk varies notably among farms with different animal housing and feeding technologies.
2. As urea content in milk rises, the content of non-protein nitrogen increases (R^2 = 0.458), too.
3. There is a close and significant (r=0.677) correlation between the content of non-protein nitrogen and urea in milk.

References

1. Bijgaart, H. van den. (2003). Urea. New applications of mid–infra–red spectrometry. *Bulletin of the IDF*, No. 383, 5–15.

2. Carlsson, B. J., Bergstrom, J., Pehrson, B. (1995). Variations with breed, age, season, yield, stage of lactation, and breed in the concentration of urea in bulk milk and individual cow's milk. *Acta Veterinaria Scandinavica*, Vol. 36, 245–254.
3. Carrol, S. M., DePeters, E. J., Taylor, S. J., Rosenberg, M., Perez–Monti, H., Capps, V. A. (2006). Milk composition of Holstein, Jersey and Brown Swiss cows in response to increasing levels of dietary fat. *Animal feed science and technology*, Vol. 131, 451–473.
4. Coballero, Truco L., Finglas, P. B. (2003). *Encyclopedia of Food Sciences and nutrition*. Elsever Science, Vol. 6, 3947–3949.
5. DePeters, E. J., Ferguson, J. D. (1992). Nonprotein nitrogen and protein distribution in the milk of cows. *Journal of Dairy Science*, Vol. 75, No. 11, 3192–3209.
6. DePeters, E. J., Cant, J. P. (1992). Nutritional factors influencing the nitrogen composition of bovine milk. *Journal of Dairy Science*, Vol. 75, 2043–2070.
7. Ferguson, J. D., Thomasen, N., Slesser, D., Burris, D. (1997). Pennsylvania DHIA milk urea testing. *Journal of Dairy Science*, Vol. 80, Suppl. 1, 161.
8. Godden, S. M., Lissemore, K. D., Kelton, D. F., Leslie, K. E., Walton, J. S., Lumsden, J. H. (2001). Factors associated with milk urea concentrations in Ontario dairy cows. *Journal of Dairy Science*, Vol. 84, 107–114.
9. Heck, H. J. F, Valenberg, J., Dijkstra, A. C. M., Hooijdonk. (2009). Seasonal variation in the Dutch bovine raw milk composition. *Journal of Dairy Science*, Vol. 92, 4745–4755.
10. Hof, G., Lenaers, P. J., Tamminga, S. (1997a). *Monitoring protein utilization by use of milk urea nitrogen*. Mid–South Ruminant Nutrition Conference, Dallas–Fort Worth Airport, 1–2 May (1997) 74–81. Retrieved 26 October, 2009, from http://txanc.org/wp–content/uploads/2011/08/milkurea.pdf,.
11. Hof, G., Vervoorn, M. D., Lenaers, P. J., Tamminga, S. (1997b). Milk urea nitrogen as a tool to monitor the protein nutrition of dairy cows. *Journal of Dairy Science*, Vol. 80, 3333–3340.
12. *Dairy Science and Technology Handbook*. Ed. Y. H. Hui. Vol. 1, 1993, 280–281.
13. International agreement of Recording practices (2011). ICAR (International Committee For Animal Recording. Retrieved 29 February, 2012, from http://www.icar.org/Documents/Rules%20and%20regulations/Guidelines/Guidelines_2011.pdf..
14. Jonker, J. S., Kohn, R. A., High, J. (2002). Dairy herd management practices that impact nitrogen utilization efficiency. *Journal of Dairy Science*, Vol. 85, No. 5, 1218–1226.
15. Meijier, R. G. M, Remmelink, G. J., Boxem, Tj. (1996) OEB – niveau in melkveerantsoenen (Rumen efflux protein in rations for dairy cattle) Praktijkonderzoek Rundvee, *Schapen en Paarden* (PR) , 116.
16. Moughan, P., (2012). *Dietary protein quality – new perspectives*. IDF World dairy summit 2012. Retrieved 20 August, 2013, from http://www.asuder.org.tr/asudpdfler/mevzuat/idfsunumlari/moughan_paul.pdf.
17. Ng–Kwai–Hang, K. F., Hayes, J. F., Moxley, J. E., Monardes, H. G. (1985). Percentages of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. *Journal of Dairy Science,* Vol. 68, 1257–1262.
18. Raden, P. M., Powell, R. L. (2009). Genetic evaluation for true protein. Retrieved 26 October, 2009, from http://aipl.arsusda.gov/reference/trueprot.htm.
19. Roginski, H., Fuauay, J. W., Fox, P. F. (2003). *Encyclopedia of Dairy Sciences*. Elsever Science, Vol. 3, 1805–1808.
20. Sederevičius, A., Kabasinskiene, A., Savickis, S., Svedaite, V., Makauskas, S. (2008). Milk urea nitrogen as an important indicator of dairy cow nutrition review. *Veterinarija ir zootechnika*, t. 44 (66).
21. Šustova, K., Ružičkova, J., Kuchtik, J. (2007). Application of FT near spectroscopy for determination of true protein and casein in milk. *Czech journal Animal Science,* Vol. 52, No 9, 284–291.
22. Твердохлеб, Г. В., Раманаускас, Р. И. (2006). *Химия и физика молока и молочных продуктов*. (*Chemistry and physics of milk and dairy products)* Москва : ДеЛи принт, 360.

Acknowledgements

Research was conducted with support from ESF project No 2009/0180/1DP/1.1.2.1.2/09/IPIA/VIAA/017, agreement No 04.4-08/EF2.PD.94.