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THE RELATION OF THE NUTRITIONAL COMPOSITION OF TMR AND THE UREA CONCENTRATION IN MILK FOR EVALUATION NUTRITION AND EFFICIENCY OF PROTEIN UTILIZATION IN LACTATING DAIRY COWS

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Abstract

In order to diagnose of protein nutrition of dairy cows in the first lactation phase, were used the analysis milk urea nitrogen (MUN) as the average per production group and individual cows in the group. The aim of this study was to evaluate the nutritional and production factors that affect the MUN and the relationships between MUN, the efficiency of nitrogen utilisation from feed into milk, and the ecological burden via excretion of urea nitrogen in urine. The evaluation of the production parameters of dairy cows together on 30 farms in total in 3 150 dairy cows was carried out according to the individual evaluations of the production carried out by the Plemenárska služba SR š.p. The analysed relations evaluated by linear, or by multiple stepwise regression confirmed the crude protein (CP) as the best single marker for the estimation of MUN and included the proportion of the total tolerance of 0.693 expressed regression equation [MUN (mg/dl) = -13.2 + 0.16 x CP (g/kg dry matter)]. For mixed models, the rate of variation expressed by this relationship increased to 0.720 (P<0.0001) for nutrient concentration and 0.783 for nutrient intake (P<0.0001). The relationship between MUN and the evaluated nutrients was closer when nutrient concentration in TMR was used, rather than daily intake of nutrients. MUN had a positive relationship to CP and neutral detergent fibre (NDF) and the negative relationship was confirmed with NEL and non-fibrous carbohydrates (NFC) and NFC/CP, NEL/CP and NDF/CP in TMR.
Keywords: protein nutrition, milk urea nitrogen, efficiency of N utilisation

Introduction

Increasing of milk production on farms in today's places great emphasis on the nutritional requirements and mainly on the level of protein nutrition in high-yielding dairy cows through the supply of protein and amino acids without environmental burden. Effective utilization of nitrogen (EUN) is the ratio between the content of nitrogen in milk and the amount of nitrogen received from TMR. Efficiency of protein utilization is often in the range of 25 to 35% (*Sinclair et al.*, 2014), but a study (*Higgs et al.*, 2012) shows an increase in utilization efficiency of nitrogen of herds in the range of 30-31% with proper formulation of feed ration. The efficiency utilization of nitrogen from TMR is most limited by the proportion and degradability of proteins and the amino

acid composition of digestible proteins at the level of small intestine (*Misciatelli et al.*, 2003). An important aspect of the metabolic transformation of proteins is the synthesis of microbial proteins and the utilization of ammoniacal nitrogen in the rumen. The amount of ammonia in the rumen is dependent by the intake of crude protein (CP) in daily ration, rate of rumen degradation of proteins and synthesis of microbial proteins (*McDonald et al.*, 2011). Rumen protein degradability is a complex influenced by several factors and is related to the content of CP, protein solubility, protein structure as well as microbial proteolytic activity, composition of structural and non-structural carbohydrates (*Bach et al.*, 2005). Synchronizing supply of carbohydrates and protein in TMR for rumen metabolism increases microbial protein yield and reduces excess N at the rumen level, thereby reducing serum urea as well as reduced N excretion (*Burgos et al.*, 2007). Concentration of serum urea is affected by intake of rumen degradable and undegradable protein, intake of fermentable carbohydrates, liver function and urinary excretion (*Guliński et al.*, 2016). Analysis of levels of protein and urea in milk are important indicators for evaluation of the intake and efficiency transformation of CP. Level of milk urea is a suitable indicator for assessing phase nutrition in terms of monitoring the protein-energy ratio of nutrients in feed rations. Given the highly significant relationship between levels of rumen ammonia, levels of blood urea and levels of milk urea, as well as the unassuming method of determining and obtaining samples, such a method for evaluation of the level of phase nutrition in a herd is very advantageous.

The aim of the study was to analyse the nutritional factors (nutrients content of TMR), which influence the milk production in relation to the MUN content per production group. Analysed values of studies in field condition of farms after statistical processing was used to formulate the regression relationships applicable to the control level of protein nutrition based on the analysed values of MUN to evaluate the content of CP in the TMR, and to evaluate the efficiency of N transformation for milk production.

Materials and methods

The evaluations were carried out on feed trials within 30 herds with a controlled nutritional level system and with an average annual production of between 8,500 and 9,500 kg per cow. In dairy cows (n = 3,150) at the peak of lactation intensively monitored the daily intake of feed in the group, the nutritional composition of TMR and evaluated the level of protein intake, the efficiency of utilizing N for milk protein. Daily rations of dairy cows were predominantly based on corn and alfalfa silage, supplemented with concentrated feed (dry ground corn or higher moisture corn, cereal grain by-products) and protein supplements (soybean meal and rapeseed meal) were fed as TMR *ad libitum* system. The cows were regularly monitored and evaluated for TMR intake, which was obtained from the difference of delivered and refusal feed weight after 24 hours of the feeding program for the production group. Samples of prepared TMR in the monitored farms were taken from the feed manger on the control day and were analyzed for dry matter (DM), crude protein (CP), acid and neutral detergent fibre (ADF, NDF), starch and ether extract (EE) contents according to conventional methods according to the Commission Regulation (EC) no. 691/2013. Non-fibrous carbohydrates (NFC) was calculated by difference (100− (CP + (NDF–NDF bound protein) + ash + ether extract)) and netto energy of lactation (NEL) by regression equations (NRC 2001).

Analysis of production parameters on the control day on individually collected milk samples was evaluated for milk production levels in dairy cows, milk components and milk urea. Milk samples were analysed the total protein content, fat, lactose and urea concentration by near infrared spectrophotometric assay using MilkoScan FT+ and BENTLEY FTS at the Central

Analytical Laboratory of Milk with accreditation under registration number 096/5878/2015/2. The analysed urea in milk (MU) was converted to urea nitrogen in milk (MUN) using the equation by Oudah (2009).

Statistical processing of results. The average values, descriptive statistics, and variability of the examined markers, as well as the influence of factors on these properties, were studied through XLSTAT2018. Statistical analysis of the relationships between MUN and nutritional and production measurements were evaluated by linear regression analysis. A simple regression analysis of relationships was done to identify those factors that explain the change in concentration of MUN at the herd level. Factors explaining more than 50% of the variability of MUN were combined into multiple regression analysis. For the evaluation of the dependencies between MUN and nutritional and production variables in multiple stepwise regression models a level of statistical significance was determined ($P < 0.05$). Based on the criteria R^2, the root mean square error (RMSE) and the Akaike Information Criterion (AIC), it was decided which factors contribute to the clarification of the analysed relationship.

Results and discussion

Dietary nutrients of daily rations, intake of feed and milk production in dairy cows

The average concentration of nutrients in TMR, daily intake of nutrients and the production characteristics of dairy cows at the evaluated farms are presented in *Table 1*. The parameters of milk production and composition of TMR showed significant differences in chemical dietary composition against actual production and composition of milk on evaluated farms. With comparable nutritional and production parameters from meta-analysis, *Spek et al.* (2013) it was confirmed that the MUN content was at 13.1 ± 3.6 mg/dl for North America. For Northwestern Europe, the MUN content averaged 12.5 ± 5.1 mg/dl, with lower production (25.5 ± 4.5 kg) and the half content of starch (13.2 ± 8.4 %) in TMR. The levels of urea N in milk between 8 and 12 mg/dl indicate optimal intake efficiency of nitrogen from the feed ration (*Nousiainen et al.*, 2004, *Spek et al.*, 2013). For high-producing dairy cows with an annual milk yield of 12,000 kg, the recommended MUN is an average of over 14.5 mg/dl (*Kohn,* 2007) or 10 - 16 mg/dl (*Wattiuax and Ranathunga,* 2016).

Table 1: **Nutritional composition of TMR and composition of milk in evaluated farms**

		Average	SD	Minimum	Maximum
Nutrients in TMR (% of Dry Matter)					
Crude protein		16.20	0.8	14.42	17.85
RDP* % CP		10.55	0.5	9.31	10.92
RUP* % CP		5.78	0.6	5.05	6.63
NEL MJ/kg DM		6.76	0.2	6.38	7.13
NDF		34.44	2.6	28.04	38.75
ADF		20.69	1.6	16.07	24.68
Starch		25.01	3.3	16.93	29.78
NFC		37.99	3.4	31.65	48.95
NEL/CP		0.42	0.03	0.37	0.48
Starch/CP		1.55	0.3	1.02	2.07
NFC/CP		2.35	0.3	1.89	3.17
Daily intake of nutrients (kg/day)					
Dry matter		22.35	1.3	20.76	24.90
CP intake		3.62	0.2	3.20	4.02
NEL intake (MJ/day)		150.89	10.6	134.09	171.83
NDF intake		7.68	0.7	6.07	8.69
ADF intake		4.62	0.5	3.42	5.40
Starch intake		5.60	0.9	3.66	7.03
Production and composition of milk					
Milk yield	kg/d	35.40	6.5	23.20	47.90
Milk protein	%	3.15	0.2	2.77	3.46
Milk fat	%	3.62	0.4	2.95	4.55
Yield of milk protein	kg/d	1.11	0.2	0.77	1.46
Yield of milk fat	kg/d	1.26	0.20	1.00	1.67
Milk urea	mg/dl	27.40	3.58	19.74	35.66
Milk urea nitrogen	mg/dl	12.79	1.67	9.21	16.64

* RDP were analysed by in situ methods (Ørskov a McDonald 1979), analysed TMR n=15

MUN in relation to nutritional effects in the simple regression model

Summary statistical data describing the relationship of linear regression analyses between MUN concentration and concentration of nutrients in TMR are summarized in *Table 2*. The evaluation of relation between the nutrient content in TMR and MUN in milk by single linear regression analysis confirmed the most statistically significant positive relationship (*P <0.0001*) between average MUN values and content of CP for the production group (R^2 = 0.693). The analyzed protein degradability of TMR confirmed the same a positive relation of MUN to RDP with less statistical significance (R^2 = 0.510, P = 0.03). These results are comparative respectively with the same tendency has been confirmed by several authors (*Godden et.al*, 2001; *Broderick and Huhtanen*, 2013). In this evaluation, the more significant relation CP to MUN than RDP and useful is more practical in breeding conditions for controlling the level of CP in feed rations and rapid identification of protein overfeeding on farms according to the analysis of MUN. In contrast, the

negative correlation was confirmed between the MUN values and the analyzed content of starch, NFC and NEL at the statistical significance (*P<0.05*). The higher level of regression dependence was confirmed in experimental conditions with the coefficient of determination in range of R^2 = 0.78 (*Nousiainen et al*., 2004) and 0.84 (*Broderick and Clayton* 1997). These observations confirm that concentration of CP in TMR is the most important nutritional factor affecting the concentration of MUN, which is closely associated with excretion of nitrogen in urine and can be applied in commercial herds as a biomarker of the protein nutrition in dairy cows (*Nousiainen et al*., 2004). The most significant influence of CP in TMR on the concentration of MUN (R^2 = 0.93) was confirmed by *Broderick and Huhtanen* (2013) with a less pronounced impact of energy concentration and protein degradability in TMR. From the nutritional effects within the evaluated set, we confirmed the weaker relationship between MUN and the proportional ratio CP/NEL (R^2 = 0.642), where the decrease of the coefficient of determination at the CP/NEL ratio in TMR compared to content of CP (R^2 = 0.693) was caused by low negative regression (R^2 = 0.174) at content of NEL in relation to MUN. Similarly, Broderick and Clayton (1997) have reported closer relations between MUNs with dietary CP content as ratio CP to NEL.

Table 2: **Linear regression between MUN in milk and nutrients content of TMR**

Concentration of nutrients in TMR (% dry matter)				
Items	**Slope**	**SE**	**R^2**	**P**
CP	1.600	0.201	0.693	<0.0001
RDP*	0.1031	0.037	0.510	0.030
NEL	-3.223	1.329	0.174	0.022
NDF	0.181	0.105	0.096	0.096
ADF	0.136	0.182	0.019	0.462
Starch	-0.247	0.072	0.294	0.002
NFC	-0.233	0.072	0.273	0.003
Starch/CP	-4.208	0.816	0.487	<0.0001
NFC/CP	-3.895	0.698	0.527	<0.0001
NEL/CP	-44.05	6.119	0.649	<0.0001

The evaluation of ratio of NEL/CP, NFC/CP, starch/CP in TMR in relation to MUN confirmed the negative statistical significance (*P <0.0001*) with coefficient of determination (R^2 = 0.649; R^2 = 0.527 resp. R^2 = 0.487). About 70% of typical diets for lactating dairy cows consist of carbohydrates, up to 37% are structural carbohydrates (NDF), 30% starch, and 3% simple sugars (*Eastridge* 2019), which provide the optimal supply of energy for the synthesis of microbial protein. Concentrations of MUN were most closely related to dietary CP content, but were less affected by dietary content of carbohydrates and ruminal protein degradability. The content of CP in TMR was the best single analysed marker for the MUN estimation in average for the production group, and represented 69.3 % of the total variance determined by the regression relationship [MUN (mg/dl) = -13.2 + 0.16 × content CP (g/kg DM)]. With a high correlation of MUN values with the content of CP in TMR, an increase of the content of CP by 1 percent in TMR resulted in an increase in MUN of 1.6 mg/dl in the evaluation of the average level of the group. This tendency was very close to the 1.6 or 1.7 mg/dl MUN, confirmed by *Powell et al*. (2014) and *Nousiainen et al*. (2004) resp. under experimental conditions. The same was confirmed by *Spek et al*. (2013) in the meta-analytical evaluation of relation MUN and concentration of CP in experiments on

concentrated types of feed ration in North America and bulk types of feed ration in Northern Europe which showed that a 1 % increase of CP increased the concentration of MUN by 1.36 mg/dl or 1.73 mg/dl. On the other hand, *Aquilar et al*. (2012) identified an increase of MUN by 1.04 or 1.24 mg/dl per percentage increase of CP in the production of 40 or 30 kg of milk.

MUN in relation to nutritional effects in the model of multiple regression

In the statistical evaluation in the model of multiple regression the evaluated set was added by the stepwise method with independent variables at the significance level *P <0.05* and eliminated with variables at the significance level *P>0.1*. The evaluation of data according to the analyzed average of MUN values in the production groups (*Table 3*) in relation to the nutrient concentration in TMR confirmed high correlation (*P <0.0001*) to CP and NDF with 72.0 % level of variability on the concentration of MUN. The same tendencies with comparable results in experimental conditions were confirmed by *Nosiainen* (2004), where the expressed CP/ME ratio on fluctuations of MUN was most affected by different CP contents ($R^2 = 0.876$) in the feed ration. The ranges of fluctuation of energy (NEL 6.7 ± 0.2 MJ / kg DM) and starch ($25.0 \pm 3.3\%$ DM) in TMR at the monitored farms were small and their inclusion in the models of multiple regressions together with CP had a minimal effect on the fluctuation of MUN. Concentration of energy in our analyses were comparable to *Nousiainen* (2004), *Broderick and Clayton* (1997), and the findings confirm that content of CP in TMR has a major effect on MUN concentration. In a practical application for the interpretation of the analysed MUNs the confirmed lower impact of the concentration of energy on the fluctuation of MUN is important, if it is included in the models of multiple regressions together with the content of CP in the TMR.
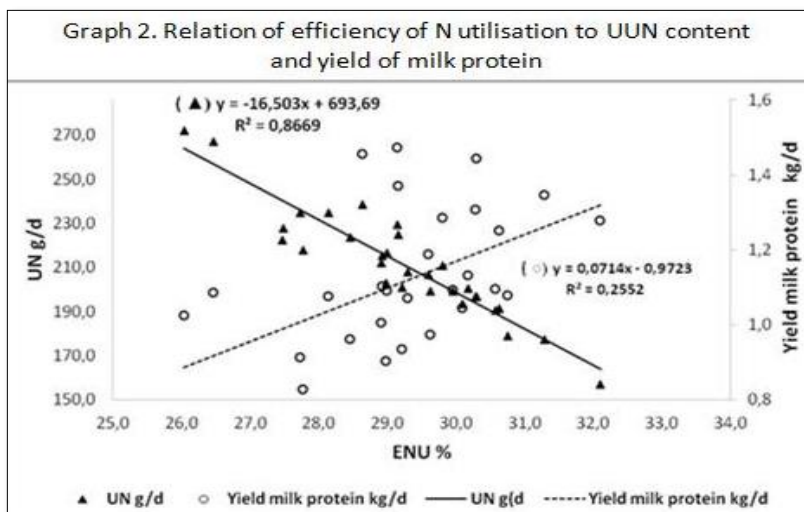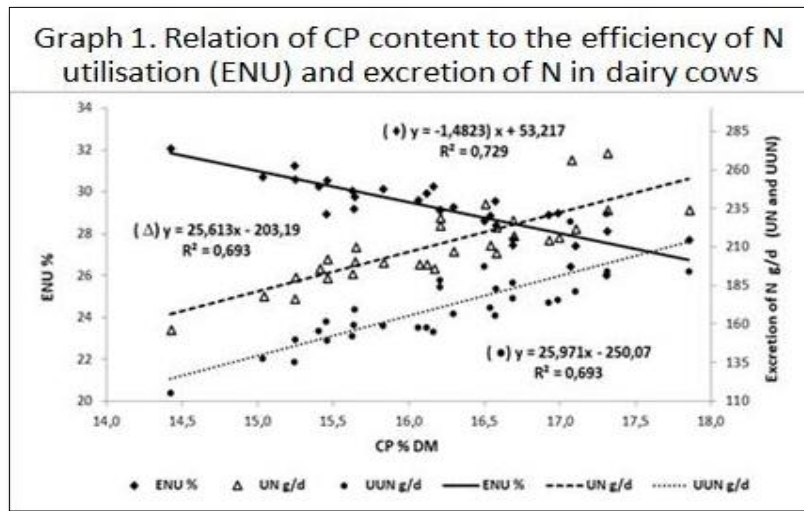
Table 3: **Multiple regression of nutritional effect on MUN concentration**

Concentration of nutrients in TMR (% DM)			
Items	**Coefficient**	**SE**	**P**
Intercept	-16.799	3.473	<0.0001
CP	1.552	0.190	<0.0001
NDF	0.216	0.099	0.038
Precision of model			
Adjusted R^2			0.720
RMSE			0.803
AIC			-10.302
P			<0.0001

The evaluation of the N efficiency of feed into milk

The efficiency of N use was defined as the percentage of N-admitted, which is transformed into milk protein and calculated according to the production of milk protein and intake of CP in ration (*DePeters and Ferguson* 1992; *NRC* 2001) in the monitored farms was determined in average level of 30.1 ± 5.0 % with significant variations of values of farms from 21.1 % to 39.5 % and in relation to the analyzed content of CP in fed TMR is expressed by the regression dependence *ENU % = 90.5 - 3.73 × CP %* ($R^2 = 0.350$; P = 0.001). The equally calculated ENU % in the relationship with the analysed content of MUN gives a weaker negative regression dependence (*Graph 1*) expressed by the equation *ENU % = 48.25 – 1.43 × MUN mg/dl,* ($R^2 = 0.19$; P = 0.016).

On the basis of the evaluated set, the reduction of MUN content by 1 mg/dl in the range of 9-16 mg/dl is associated with an increase in the conversion of nitrogen to milk at 1.43 %.

Graph 1. Relation of CP content to the efficiency of N utilisation (ENU) and excretion of N in dairy cows

Graph 2. Relation of efficiency of N utilisation to UUN content and yield of milk protein

The efficiency of N utilisation (ENU) was determined according to analyzed MUN by calculation according to regression equations by *Nousiainen et al.* (2004), *Wattiaux and Ranathunga* (2016) and *Huhtanen et al.* (2015). The dynamics of the ENU changes, calculated on the analyzed MUN content expressed as the mean value of the regression equations of selected authors in the monitored farms (*Graph 1*) shows a negative correlation relation (*P <0.0001*, R^2 = 0.729) with respect to the analyzed CP content in TMR, where 1% increase of CP in the range of 14 to 18 % in TMR reduces the efficiency of the use of nitrogen from feed to milk by 1.48 %. The dynamics of ENU in relation to the production of protein milk (*Graph 2*) shows a positive correlation (P=0.004, R^2=0.26) where according to regression dependence, the increase of ENU by 1% corresponds to an increase in milk protein production by 70 g/day at the calculated ENU range from 26 to 32%.

The observed ENU in the studied farms according to the way of evaluation showed different values: - in the calculation of the metabolic transformation (N milk / N intake) the average was 30.1 ± 5.0 with a range of 21.1 - 39.5 %, and according to analysed content of MUN averaged 29.2 $\pm$ 1.4 % with lower fluctuation (26.0 - 32.1). The authors *Jonker et al*. (2002) and *Gourley et al*. (2012) found that the efficiency of utilisation in commercial conditions was 28%, with variations between 18 % and 35 %. *Pacheco* (2016) reports the average value of efficiency of N utilization at the level of 29 % with a range of fluctuations from 19 to 40 % among different farms, which are comparable to our results obtained from calculation according to the metabolic conversion.

Conclusion

Based on the obtained results of farm trials we can conclude that MUN concentrations are simple and non-invasive approaches to examine the protein status of rations fed to dairy cattle. Monitoring and evaluation of milk urea on farms provides an opportunity to formulate rations and adjust levels of protein to optimize effective utilisation of nitrogen in order to increase milk and milk protein production and to avoid the negative effects of urea excretion in urine. In summary, milk is a useful indicator of the animal nutritional (protein and energy specific) status.

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