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Original Paper

The effect of protein nutrition on production, the metabolic transformation of nitrogen and ecological load in dairy farming

Iveta Maskaľová*, Vladimír Vajda, Petra Timkovičová Lacková

University of Veterinary Medicine and Pharmacy in Košice Department of Animal Nutrition and Husbandry, Slovak Republic

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The aim of the study is to evaluate the relation of protein nutrition to transformation of nitrogen (N) from feed to milk and its urinary excretion in dairy cows under the farm conditions. In breeding conditions of dairy farms in the groups of dairy cows in the 1st phase of lactation, with low content of CP (139 ±11.8 g/kg), optimum (161.8 ±6.3 g/kg) and high content of CP (179.7 ±8.5 g/kg) in dry matter of total mix ration (TMR), was confirmed the effect of increased the level of ammonia (NH_3) in the rumen (17.4 ±1.7; 19.6 ±2.3; 22.8 ±3.4 mg/100 ml). The same tendency with higher statistical significance ($P < 0.001$) was confirmed to the increased concentration of urea in the blood (22.8 ±3.7; 27.8 ±4.8; 35.1 ±6.6 mg/dl) and to the concentration of urea in the milk (18.2 ±2.6; 24.8 ±3.9; 33.3 ±6.7mg/dl). In the groups with increasing content of CP in the TMR, the ecological load of the environment increases, that was confirmed by a significant increase ($P < 0.001$) of excreted N in the urine (139.4 ±19.8; 189.5 ±30.4; 254.7 ±51.3 g N/day) and an increase ($P < 0.001$) in the emission of ammonia into the environment (68.1 ±6.1; 83.7 ±9.4; 103.8 ±15.9 g/day). The highest efficiency of N utilization at the level of 31.1 ±1.4% was confirmed at a CP content of 150–170 g/kg dry matter of TMR. The evaluation of direct regression dependence between the analysed CP content in TMR and analysed parameters of N transformation in individual farms was confirmed a significant relationship in CP and NH_3 content ($r = 0.641$), CP and urea in the blood ($r = 0.689$), resp. CP and excretion of N in urine ($r = 0.600$). Direct relation was confirmed by the analysed NH_3 content in the rumen and the urea content in the blood ($r = 0.795$), and urea in the milk ($r = 0.786$), as well as the urea content in the blood in relation to the urea in the milk ($r = 0.767$). The obtained results confirm the suitability of the use of the monitored markers to assess the level of protein nutrition and the environmental load at the farm.

Keywords: nitrogen, transformation, urea, milk, urine

1 Introduction

Increasing milk production on farms in current conditions places great emphasis on meeting nutritional requirements, especially protein concentration in feed ration for high-producing dairy cows. At the level of dairy farms, it remains a challenge to determine the optimal amount of protein for production groups of cows in terms of increasing milk and milk protein production for effective utilization of nitrogen (EUN) (Hristov et al., 2011) by optimizing of fermentable carbohydrates and utilization of metabolizable proteins (Schwab, 2010). In addition to urinary N loss, protein overfeeding in dairy cow's increases energy requirements (Milano et al., 2000) and negatively affects reproductive parameters (Ferguson and Sklan, 2005). Increasing the N content in feed ration reduces EUN and increases excretion of N mainly in the form of urinary urea nitrogen (Huhtanen and Hristov, 2009).

The effectiveness of nitrogen transformation for production of milk protein rarely exceeds 30%, where more than 70% of received crude protein representing 30% loss in faces and 40% loss in urine, mostly in the form of urea (Yan et al., 2006). The levels of urea in blood resp. blood urea nitrogen (BUN) and urea in milk resp. milk urea nitrogen (MUN) are affected by the metabolic transformation of nitrogen in the rumen and whole-body transformation of metabolizable amino acids. With an

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*Corresponding Author: Iveta Maskaľová, University of Veterinary Medicine and Pharmacy in Košice, Department of Animal Nutrition and Husbandry, Komenského 73, 041 81 Košice, Slovak Republic; e-mail: iveta.maskalova@uvlf.sk ORCID: https://orcid.org/0000-0002-5253-9791 average daily production of 1 kg of milk protein (157 g N per day), 4 g N (ranging from 2 to 9 g/d) is usually excreted in the form of milk urea, which is almost 40 times less urea N compared to N of milk protein (Wattiaux and Ranathunga, 2016). Rumen-hepatic N recycling is part of nutritional models to avoid excessive excretion of nitrogen in the urine (Lapierre and Lobley, 2001) and ammonia release into the environment at excessive protein supply in the feed ration (Hristov et al., 2011).

Urea is the final metabolite of nitrogen excretion, which includes – ammoniacal nitrogen absorbed from the rumen derived from rumen degradable protein of feed ration, – metabolic nitrogen formed by deamination of part of the absorbed amino acids (AA), which are not included in proteosynthesis due to imbalance of essential AAs. Monitoring MUN for the estimation of the efficiency of N utilization and excretion of N in urine can be effectively used as a tool for nutritional and environmental management by control of protein nutrition.

The aim of the work was to evaluate the different level of protein nutrition and the metabolic transformation of nitrogen, by analysis of NH_3 in the rumen, urea in blood and milk as metabolic indicators of protein transformation in dairy cows with the possibility of use for evaluation of protein nutrition of dairy cows. Based on the content of the analysed milk urea, estimate the excretion of N in the urine for the evaluation of the effective utilization of N and the environmental load in relation to the protein content in the feed ration and at the overfeeding of proteins.

2 Material and methods

The evaluations were carried out under farms conditions in trials within 26 herds with an average annual production of milk between 8,500 and 9,500 kg per cow. The selected farms were predominantly from the lowland region of Slovakia. All cows were clinically healthy and free from internal and external parasites. A total in 216 dairy cows of Holstein breed in the 1st stage of lactation was evaluated for the effect of protein nutrition on production, the efficiency of metabolic transformation of N and excretion of N as a marker of environmental load. Daily feed rations on evaluated farms were predominantly based on corn and alfalfa silage, supplemented with a different species of carbohydrates (cereal grains and cereal grain by-products) and protein supplements (soybean and rapeseed meal) fed as total mix ration (TMR).

2.1 Samples of prepared TMR

Samples of prepared TMR in the monitored farms were taken from the feeding trough on the control day and were analysed for dry matter (DM), crude protein (CP), acid and neutral detergent fibre (ADF, NDF), contents of starch and ether extract (EE) according to conventional methods according to the Commission Regulation (EC) no. 691/2013 (2013) at the Department of Animal Nutrition and Husbandry, UVLF Košice. DM was determined by weight upon drying the sample at 105 °C under prescribed conditions. The CP content was determined according to the Kjeldahl methods using a 2300 Kjeltec Analyzer Unit (Foss Tecator AB, Hoganas, Sweden). Fat (as ether extract) was determined by the device Det-Gras (JP SELECTA, Spain). ADF, NDF were determined using Dosi-Fiber Analyzer (JP SELECTA, Spain) and the content of starch was determined polarimetrically. Netto energy for lactation (NEL) and non-fiber carbohydrates (NFC) were calculated using regression equations by NRC (2001).

2.2 Determination of rumen metabolites

Determination of rumen metabolites by analysis of the amount and proportion of volatile fatty acids (VFA), pH values, NH_3 in rumen contents samples 4–6 hours after morning feeding with the addition of thymol by stomach canulas. Samples of rumen fluid were strained through 3-layer of gauze. VFA in the rumen content were determined in a two-capillary isotachophoretic analyser EA100 (VIL*LA LA*BECO, Slovak Republic). The pH of the rumen content was determined potentiometrically with portable electronic pH-meter (JP SELECTA, Spain). The level of NH_3 in the samples of rumen content was determined by a Kjeldahl-N using a 2300 Kjeltec Analyser Unit (Foss Tecator AB, Hoganas, Sweden).

2.3 Determination of metabolites of intermediary metabolism

Determination of metabolites of intermediary metabolism for the assessment of protein metabolism was determined photometrically with a diagnostic kit using an automatic biochemical analyser "Ellipse" (AMS Italy). Blood samples were centrifuged at 3,000 rpm for 20 min; the obtained sera were stored at – 20 °C until analysis.

2.4 Analysis of production parameters

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).

2.5 Estimation of utilisation and environmental load

Efficiency of Nitrogen Utilization (EUN) for production group of dairy cows was estimated according to the analysed content of MUN and the amount of milk produced by using regression equation by Huhtanen et al. (2015) from meta-analytical assessments of the balance experiments.

Calculation of N excretion in urine was determined according to the analysed content of MUN using the model of regression equation by Kauffman and St-Pierre (2001), as results of the meta-analysis of balance trials to the monitoring excretion of nitrogen in the urine.

Emission of ammonia in our study was estimated using the model of regression equation by Burgos et al. (2010) according to the analysed content of MUN.

2.6 Statistical analysis

Statistical processing of the achieved results was performed using mathematical and statistical methods using the GraphPad Prism4 program. The effect of different protein nutrition on the farms was evaluated by repeated one-way ANOVA. The significance of different individual values between the sample collections of rumen content and blood was evaluated by Tukey´s Multiple comparison test at significant levels of $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, respectively. Each parameter was presented with its mean (x), standard deviation (SD), or descriptive statistics.

Ethical statement

All procedures concerning the animals were performed in compliance with the national guidelines for animal care

3 Results and discussion

3.1 Nutrient content of ration, feed intake and milk production in groups of dairy cows

Evaluation of nutritional composition and milk production on an evaluated of 26 farms and 216 individual dairy cows in the first lactation phase, daily feed intake in the group and the nutritional composition of TMR were precisely monitored. Days in milk (DIM) of evaluated dairy cows in the 1st phase of lactation was 88 ±17 on average with a minimum 60 and a maximum 130 in the holding. The average concentration of nutrients in TMR 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.

Table 1 Nutritional composition of TMR and composition of milk

	Average	SD	Min	Max
Nutrients in TMR (% of dry matter)				
Crude protein	16.59	1.5	12.57	19.61
NEL	6.64	0.2	6.20	7.13
NDF	34.48	3.6	28.04	41.82
ADF	21.29	1.8	18.33	24.98
Starch	23.35	3.5	16.93	30.21
NFC	36.52	3.3	29.45	43.88
NEL/CP	0.40	0.04	0.34	0.50
Starch/CP	1.43	0.3	1.02	1.91
NFC/CP	2.22	0.3	1.73	2.79
Production and composition of milk				
Milk yield (kg/d)	35.20	7.1	23.00	52.90
Milk protein (%)	3.12	0.2	2.70	3.55
Milk fat (%)	3.66	0.4	2.91	4.91
Yield of milk protein (kg/d)	1.09	0.2	0.69	1.48
Yield of milk fat (kg/d)	1.27	0.3	0.76	1.78
Milk urea (mg/dl)	27.20	7.1	15.53	43.50
Milk urea nitrogen (mg/dl)	12.69	3.3	7.25	20.30

3.2 *Evaluations of effect different CP content on production and protein transformation in dairy cows*

The analysed content of CP in the evaluated farms was on average 165.9 ±15.0 g/kg DM of TMR with significant fluctuations of values from 125.7 to 196.1 g/kg DM. The evaluation of the effect of the level of protein nutrition in dairy cows in the 1st phase of lactation on the transformation of proteins through selected indicators is summarized in Table 2, where farms are divided according to CP content with low content of CP <150 g/kg (3 farms), optimal 150–170 g/kg (13 farms) and high content of CP >170 g/kg DM (10 farms). The different content of protein in TMR showed significant changes between monitored farms, mainly between farms with low content of CP and high content of CP (Table 2, $P < 0.001$).

In the monitored farms, the analysed content of NH_3-N was depended on the intake of protein and energy in TMR and ranged from 12.75 to 22.0 mg/dl with an average value of 16.8 ±2.6 mg/dl. Excessive protein intake is the cause of increased NH_3, resp. NH_3-N of production in the rumen. Microbial proteins are 80% composed of NH_3 resp. NH_3-N of ammoniacal N (Bach et al., 2005), where the minimum level of NH_3-N required for their synthesis is 9.2 mg/dl (Reynal and Broderick, 2005).

An important marker in the evaluation of protein transformation is the concentration of urea in the blood serum. The analysed serum urea averaged 30.2 ±7.1 mg/dl with ranges of values from 20.53 to 47.0 mg/100 ml. The concentration of blood urea and milk urea (Table 2) was statistically significant on farms with higher content of CP ($P < 0.001$), as well as on farms with optimal content of CP ($P < 0.01$), compared to milk and blood urea concentration on farms with low CP content. Synthesis of urea ranges from 40 to 70% depending on content of CP and the ratio of carbohydrate to protein in TMR (Recktenwald, 2010).

The relative proportion of structural and structural carbohydrates in the feed ration, together with the protein content, affects the recycling of urea from the blood to the

Table 2 Metabolism and transformation of protein according to CP content in TMR

	Average	CP <150 g/kg	CP 150–170 g/kg	CP >170 g/kg
Daily ration		n 3	n 13	n 10
Crude protein (g/kg DM)	165.9 ±15.0	139.0 ±11.8^{a,b}	161.8 ±6.3^{a}	179.7 ±8.5^{b}
Starch (g/kg DM)	235.2 ±35.3	217.6 ±14.2	242.1 ±44.3	230.8 ±22.9
NDF (g/kg DM)	348.8 ±36.0	394.8 ±20.5	343.1 ±37.9	341.1 ±28.0
Rumen		n 49	n 89	n 78
NH_3 (mg/100ml)	20.5 ±3.2	17.4 ±1.7	19.6 ±2.3	22.8 ±3.4
NH_3-N (mg/100ml)	16.8 ±2.6	14.3 ±1.4	16.1 ±1.9	18.7 ±2.8
pH	6.48 ±0.36	6.47 ±0.2	6.55 ±0.4	6.39 ±0.4
$\sum$VFA (mmol/l)	122.7 ±21.0	118.7 ±20.9	118.5 ±19.6	129.9 ±23.1
Blood		n 49	n 89	n 78
Total proteins (g/l)	81.3 ±2.4	80.5 ±5.1	80.9 ±2.0	81.7 ±2.0
Urea (mg/dl)	30.2 ±7.1	22.8 ±3.7^{i}	27.8 ±4.8	35.1 ±6.6^{i}
BUN (mg/dl)	14.2 ±3.3	10.7 ±1.7	13.0 ±2.3	16.5 ±3.1
AST (μkat/l)	1.70 ±0.2	1.69 ±0.2	1.68 ±0.2	1.72 ±0.1
Production and composition of milk		n 49	n 89	n 78
Milk yield (kg/d)	35.3 ±7.3	29.7 ±5.9	37.9 ±7.8	33.2 ±4.7
Milk protein (%)	3.09 ±0.2	3.21 ±0.2	3.04 ±0.2	3.22 ±0.2
Milk urea (mg/dl)	26.4 ±6.6	18.2 ±2.6^{c}	24.8 ±3.9	33.3 ±6.7^{c}
Milk urea nitrogen (mg/dl)	12.69 ±3.3	8.5 ±1.2^{d}	11.7 ±1.82	15.7 ±3.12^{d}
EUN (%)	30.3 ±1.7	29.8 ±1.5	31.1 ±1.4	29.0 ±1.6
Environmental load		n 49	n 89	n 78
Excretion of urine N (g N/day)	201.3 ±50.7	139.4 ±19.8^{e,f}	189.5 ±30.4^{e}	254.7 ±51.3^{f}
Emission of NH_3 (g/day)	87.3 ±15.7	68.1 ±6.1^{g,h}	83.7 ±9.4^{g}	103.8 ±15.9^{h}

b, c, d, f, h, i: $P < 0.001$; a,e,g: $P < 0.01$

rumen fluid through the rumen wall. 30–60% of the urea formed is recycled to the rumen and converted to NH_3 to support microbial protein synthesis (Van Amburgh et al., 2015), allowing rumen microorganisms to overcome low supply of nitrogen in TMR (CP <12%). Products of rumen fermentation (VFA NH_3 and CO_2) affect the pH in the rumen. Studies (Abdoun et al., 2005; Abdoun et al., 2010) confirmed the effect of reduced rumen pH and CO_2 content as well as butyric acid on the rate of transport of ammonia and urea through the rumen wall. Increased intake of proteins, mainly ruminally degradable protein, increases concentration of NH_3 in the rumen, which is directly correlated with the recycling of blood urea to the rumen. This relationship affects the transfer of urea into milk and the excretion of N in urine.

In the monitored dairy farms divided according to the content of CP in TMR (Table 2), during the analysis markers of protein metabolic transformation, a significant relationship at the analysed average values of urea in blood and milk was confirmed. An equally significant difference was found in the evaluated indicators of the environmental load – excreted nitrogen in the urine and NH_3 emission into the environment. The excretion of nitrogen in urine, as well as NH_3 emissions, were statistically significantly increased ($P < 0.001$ resp. $P < 0.01$) on farms with high and optimal content of CP in TMR compared to low CP content. These indicators are used as a biomarker of efficiency of N utilization for milk production (Huhtanen et al., 2015), but also to assess physiological dependences between intake and excretion of N in urine (Nousiainen et al., 2004, Burgos et al., 2007) and for the emission of ammonia on dairy farms (Van Duinkerken et al., 2011). To optimize protein nutrition, such an amount of CP in the TMR is needed, that on the one hand, prevents production losses at low CP intake and, on the other hand, does not lead to metabolic load and N losses in urine when the content of CP in TMR increases.

In farms with a low content of CP (<150 g/kg DM of TMR), was analysed the content of CP in the average 139.0 ±11.8 g/kg DM with an average rumen NH_3-N content of 14.3 ±1.4 mg/100 ml, the concentration of blood urea nitrogen (BUN) 10.7 ±1.7 mg/dl and the concentration of milk urea nitrogen (MUN) 8.5 ±1.2 mg/dl. The efficiency of N utilization (EUN) in these farms was found on average 29.8 ±1.5% (28.2–31.2%), and with low CP content in TMR was confirmed the lowest excretion of N in urine (139.4 ±19.8 g N/day) and also lowest emission of ammonia (68.1 ±6.1 g/day) into the environment.

In farms with a high content of CP at a level >170 g/kg DM was found the highest content of NH_3-N, BUN and MUN, but also the highest excretion of N in urine (254.7 ±51.3 g N/day) and emission of ammonia (103.8 ±15.9 g/day) to the environment.

The efficiency of nitrogen utilization (EUN) from feed to milk was the highest in the group of dairy cows with an optimal average content of CP 161.8 ±6.3 g/kg DM. Inefficient utilization of N per milk protein is the reason why dairy cows in agriculture are considered to be an environmental problem and the source of ammonia emissions (Powell et al., 2014). The low efficiency of N utilization is directly related to the high content of CP in the feed ration (Aguilar et al., 2012). Correction of the daily intake of N reduces the levels of excreted N in the urine and leads to improved efficiency of nitrogen utilization per milk protein and optimal concentration of milk urea. Optimal values of milk urea in the range from 17 to 26 mg/dl resp. MUN 7.9–12.1 mg/dl (Ishler, 2017), confirm the optimal level of ammonia for the synthesis of microbial proteins and the efficient transformation of N to milk protein, where the amount of excreted urea in the urine does not cause an environmental load. These values are characteristic for the protein content in the feed ration at the level of approximately 16%. This reference is confirmed by the results from the monitored farms in our conditions with the content of CP from 150–170 g/kg DM of TMR with the highest EUN on average 31.1 ±1.4% (28.6–33.4%), with a MUN concentration of 11.7 ±1.82 and a BUN concentration of 13.0 ±2.3 mg/dl. In TMR with a lower compared to a higher CP content, no significant difference in milk and milk protein production was confirmed, but a significantly lower excretion of N in urine, that is comparable to the results of monitoring in our farms (Colmenero and Broderick, 2006).

The ecological load of the environment in the farms, expressed by the level of N excretion in the urine, was estimated according to the analysed content of MUN using the model of regression equation (Burgos et al., 2010) and it is presented in figure 1. The content of the CP in TMR significantly correlates ($r = 0.600$, $P < 0.01$) with

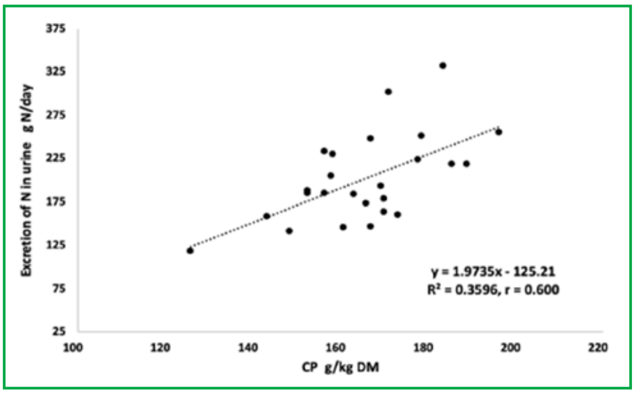

Figure 1 Regression relationship of CP content in TMR and excretion of N in urine

the excretion of N in urine, where each increase of CP content by 1% increases the excretion of N in urine by 19.7 g N per day. The content of CP in this range is in our conditions depending on the soil-climatic conditions, the production of protein forages, and their inclusion in the TMR. High intake of N causes the accumulation of NH_3 in the rumen and increases its absorption with higher production of urea in the liver (Reynolds and Kristensen, 2008), which increases the content of blood urea and milk urea and is directly related to increased excretion of N in urine and reduces utilization of N to milk protein (Recktenwald et al., 2014). This process is directly affected by the content of CP and the ratio of starch to CP and less by the content of starch in the TMR. The emission of ammonia is dependent on the excretion of nitrogen in the urine. Excreted N in urine is higher in the form of urinary urea nitrogen, which upon contact with urease is rapidly converted to ammonium and gaseous ammonia (Burgos et al., 2007), which is a potential source of environmental and water contamination.

3.3 *Regression relationships of the intake and metabolic transformation of nitrogen*

The statistical processing of the average values of the selected indicators in the monitored farms confirmed the significant dependence of these indicators for the evaluation of the N metabolic transformation, which is graphically expressed by the regression relationships in Figures 2 and 3. The content of protein (CP) of feed ration most significantly influenced the increasing markers values of the metabolic transformation of N.

The effect of the protein content in TMR on the average values of NH_3 in the rumen content (Figure 2a), in a considerable number of farms and individual animals, confirms a high regression relation (r = 0.641; P <0.01) where with increasing CP intake by 10 g in the feed ration increase the NH_3 content in the rumen by 1.35 mg/100 ml. The confirmed deviations of NH_3 values in the rumen of individual farms from the trend line can be influenced by the different levels of degradable proteins in the feed ration and the level of fermentable carbohydrates with a higher NH_3 content to support the synthesis of microbial proteins.

Evaluation of the relation of different protein levels in the ration to urea in the blood (Figure 2b), was confirmed a higher regression (r = 0.689; P <0.01) with a lower variance of values in the monitored farms, that is influenced by the rate of proteolysis of muscle fibers to support synthesis milk in the 1st phase of lactation or

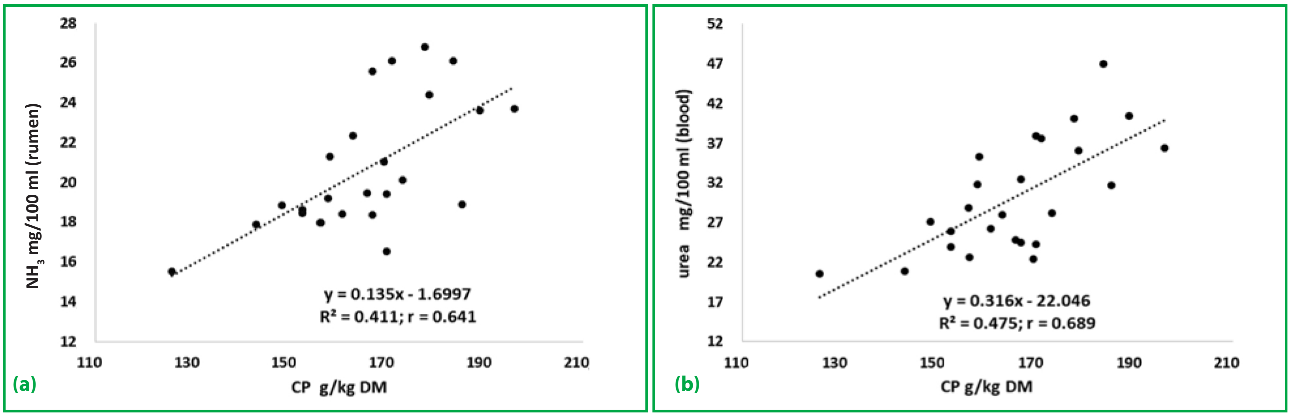

Figure 2 Regression relationship of CP content in TMR and rumen NH_3 concentration (a) and urea in blood (b)

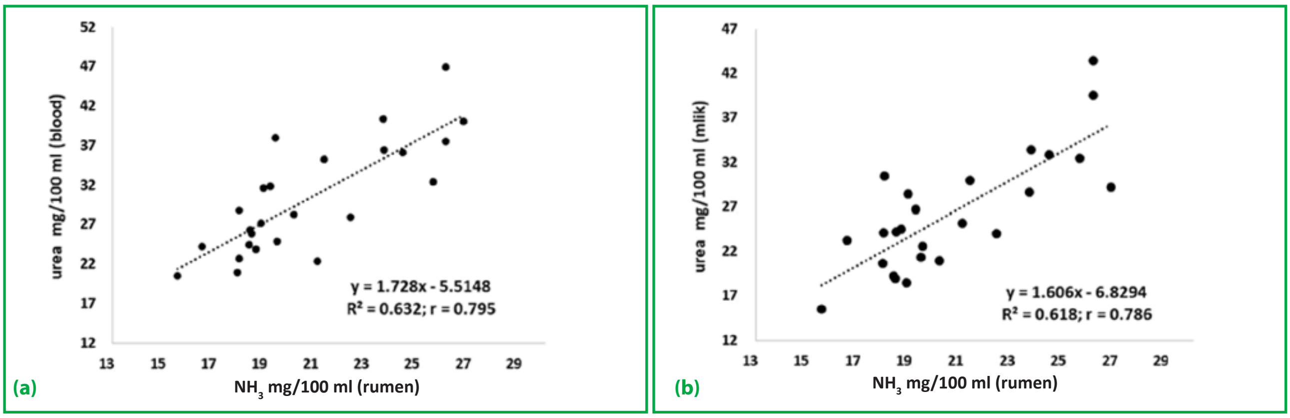

Figure 3 Regression relationship of content rumen of NH_3 and blood (a)and milk (b) urea concentration

the level of ammonia recycling. Increased supply of CP or rumen-degradable N increases the level of NH_3 in the rumen content and blood serum urea, increases the energy needs of metabolism and the functional load of the liver (Hammon et al., 2005).

The evaluation of the analysed values of rumen NH_3 content in relation to the average values of urea in blood (Figure 3a) and milk (Figure 3b) confirms a high regression with a correlation coefficient of $r = 0.795$ ($P < 0.01$), resp. $r = 0.786$ ($P < 0.01$). Every increase in the content of NH_3 in the rumen content by 1 mg/100 ml increases urea in blood by 1.73 mg/100 ml and urea in milk by 1.61 mg/100 ml.

The relationship between the metabolic level of the average values of urea in blood and urea in milk (Figure 4) gives a regression dependence with a correlation coefficient of $r = 0.767$ ($P < 0.01$) and confirms the analysed urea in milk as a suitable marker for evaluating the level of protein nutrition and the efficiency of N transformation, as well as environmental load. There is a narrow correlation between the concentration of milk urea and excretion of N in urine, whereas the concentrations of urea in urine were observed 32–38 times higher (Broderick and Clayton, 1997) than in the milk. The metabolic transformation of nitrogen from feed ration is most limited by the proportion of degradable proteins and the amino acid composition of digestible proteins at the level of the small intestine (Misciattelli et al., 2003). Energy limits microbial production more than the concentration of nitrogen, due to the cow's ability to regulate the rumen-hepatic transfer of N to the rumen (Reynolds and Kristensen, 2008). Positive relationships between the concentration of milk urea and the excretion of N in the urine were confirmed by Spek et al. (2013). The small size and neutral nature of the urea molecule ensure the maintenance of the balance between blood urea (BUN) and milk urea (MUN) by diffusion across the epithelium of the mammary gland (Jonker and Kohn, 2001).

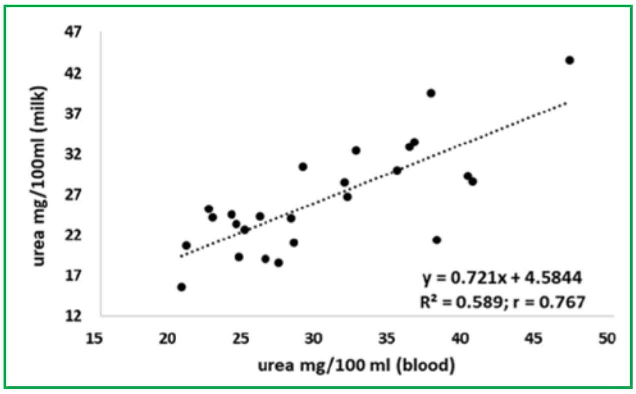

Figure 4 Regression relationships between blood and milk urea concentration

4 Conclusions

Monitoring of urea levels in milk and their evaluation for the production group in relation to the nutrients content of TMR and dry matter intake allows an objective evaluation of the protein nutrition of dairy cows. Change in content of milk urea is a risk factor for metabolic and reproductive disorders, and it is useful in evaluating herd health programs and ecological burden, by the evaluation of the efficiency of nitrogen utilization and excretion nitrogen in the urine.

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