

## **Improving nitrogen use efficiency of dairy cows in relation to urea in milk – a review**

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Dietary protein intake is the most important factor determining milk production, milk composition, milk nitrogen efficiency, urinary nitrogen losses, urea content in milk and consequently, ammonia emissions from dairy cow manure. According to the nutrition requirements two main protein sources are available for cows: rumen degradable protein, provided to the animal through ruminally synthesized microbial protein, and rumen undegradable protein that escapes ruminal degradation (but is digested and absorbed in the small intestine). The presence of urea in the milk of cows is caused by metabolic changes in the gastrointestinal tract, resulting in the excess of microbially undigested ammonia in the body. Rumen degradable feed proteins are degraded by rumen microorganisms via amino acids into ammonia and branched chain fatty acids. The bacterial population uses ammonia in order to grow. The extent, to which ammonia is used to synthesize microbial protein, is largely dependent upon the availability of energy generated by the fermentation of carbohydrates. On average, 20 grams of bacterial protein are synthesized per 100 grams of organic matter fermented in the rumen. Bacterial protein synthesis may range from less than 400 g/day to about 1500 g/day, depending primarily on the digestibility of the diet. A highly toxic chemical compound generated during those processes, i.e. ammonia, is detoxified in the liver and converted to urea. The main reason for elevated levels of urea in milk is connected with excess protein contents in feed rations, and energy and protein imbalance. The increase in the percentage of total protein from 13% to 18% DM per ration is accompanied by an increase in the urea level by about 80 mg to over 150 mg in 1 liter of milk. Additional factors affecting the level of urea in milk include the following: frequency of feed administration, number of milkings and length of the interval between milkings, cow's body weight, water intake volume, the level of Na and K ration supplementation, as well as rumen pH. Recent research indicated that an addition of natural plant-origin biologically active compounds, such as tannins, saponins and essential oils, reduce ammonia production and finally urea content in milk. Information on the concentrations of milk urea and nitrogen in dairy cows allows to assess energy balance and protein rations supplied, which in turn can help to reduce both feed costs and nitrogen emission (N) to the environment.

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The role of dietary protein in the nutrition of modern dairy cows may be summarized as the effects on milk yield and milk composition, environmental effects, potential effects on reproduction efficiency and effects on feed costs. The NRC [2001] definition for metabolizable protein is as follows: digestible protein (digestible total amino acids) that is provided to the animal through ruminally synthesized microbial protein and feed protein that escapes ruminal degradation (but is digested and absorbed in the small intestine).

Milk urea nitrogen (MUN) is a tool used in modern dairy farming to monitor the effectiveness of protein utilization in the gastrointestinal tract of dairy cows. Rumen microorganisms break down feed protein to ammonia. When its production is carried out in the optimal amount of fermentable carbohydrates, rumen microbial organisms can capture the ammonia and use its nitrogen for amino acid and protein synthesis. However, in practice that particularly concerns high-yielding cows an excess of ammonia occurs in the rumen, where it is absorbed through the rumen wall, penetrates to the blood stream and goes to the liver where hepatocytes convert it to urea in the urea cycle.

Therefore, the level of urea in milk conveys detailed information on the volume of nitrogen, which is not utilized for growth or synthesis of milk proteins and is present in the feed consumed by animals every day. When administered feed rations contain too much protein, excess nitrogen is excreted to body fluids, i.e. plasma (PUN – plasma urea nitrogen), milk and urine (UN - urine nitrogen). Hence, the level of urea in milk is a very useful indicator, informing modern dairy farmers about situations, in which cows do not fully utilize the ingested feed protein and excrete excess nitrogen, or when they are fed rations low in protein. The practical use of information on milk urea level facilitates evaluation of the energy and protein balance of rations, reduction of feed costs and potential losses of nitrogen from cattle farms.

Research clearly shows that there is a direct relationship between the level of protein in dairy cattle feed rations and the amount of nitrogen excreted in body fluids of cows. Approximately 75-85% of the excessive protein provided in feed rations are excreted from the organism [Spek 2013]. The level of nitrogen excreted in the feces of animals is generally constant and cannot be significantly altered. In contrast, the level of nitrogen in the urine of cows can be effectively controlled by balancing protein and energy needs of animals. It is estimated that about 50% of the excess urea is removed immediately from the body in the form of urine, in which it amounts to 70-80% urine volume. The main components of bovine urine containing nitrogen include urea (70%), allantoin (8%), hippuric acid (6%), creatinine (4%), creatine (3%) and ammonia (3%). Moreover, dairy cows remove approx. 2.5-3.0% of the total amount of urea in the body with milk [Spek 2013].

The level of urea in milk varies considerably and depends on a variety of factors. In practice, some urea is always found in milk. Demonstration of the lack of urea in output registers indicates errors in its determination. The content of urea in milk is generally expressed in mg per dl or mg per 1 l of milk. It is estimated that in

approximately 95% milk samples of PHF cows, the range of urea contents falls within 50 to 200 mg per 1 liter. Average urea concentration in the case of well-balanced feed rations ranges from 100 to 120 mg in 1 l. These upper limits are markedly increased with an excess of protein or too low carbohydrate levels in rations. It is generally accepted both in Poland and worldwide that the range of 150-250 mg of urea in 1 l, at the protein content in milk of 3.2-3.6%, is optimal in terms of protein and energy balance in feed rations for dairy cows.

### Nitrogen metabolism in the cows' rumen

Proteins provide the amino acids needed for the maintenance of vital functions, growth, milk production and reproduction [Jóźwik *et al.* 2012]. Cows can utilize different nitrogen sources because of their rare ability to synthesize amino acids and protein from non-protein nitrogen sources. This ability is associated with the presence of microorganisms in the rumen. Feed proteins are degraded by microorganisms in the rumen via amino acids into ammonia and branched chain fatty acids. The bacterial population uses ammonia in order to grow. The extent, to which ammonia is used

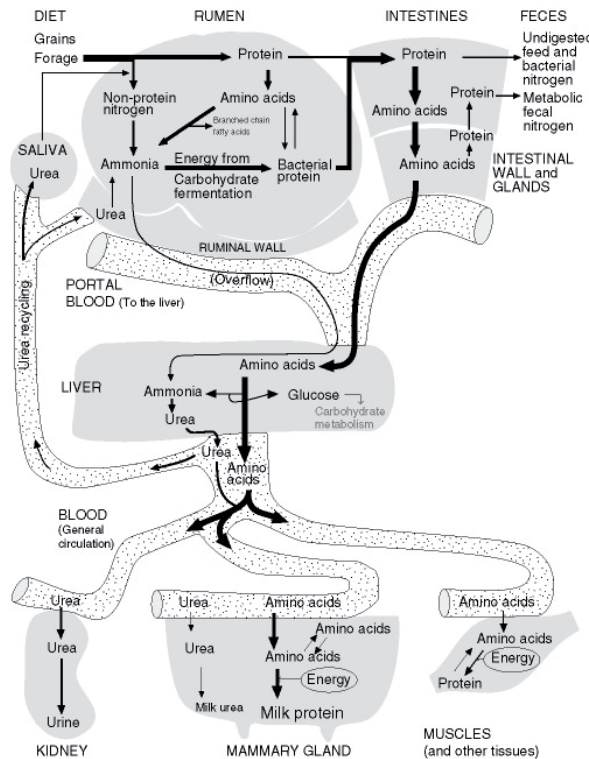


Fig. 1. Overview of protein metabolism in dairy cows [Wattiaux 2014].

to synthesize microbial protein, is largely dependent upon the availability of energy generated by the fermentation of carbohydrates. On average, 20 grams of bacterial protein is synthesized per 100 grams of organic matter fermented in the rumen. Bacterial protein synthesis may range from less than 400 g/day to about 1500 g/day depending primarily on diet digestibility. The percentage of protein in bacteria ranges from 38 to 55%. Non-protein nitrogen from the feed and urea recycled into the rumen through saliva or the rumen wall also contribute to the pool of ammonia in the rumen. In addition, ruminants possess a mechanism to spare nitrogen. When a diet is low in nitrogen, large amounts of urea (typically excreted in the urine) return to the rumen, where it can be used again by the microbes. If ammonia levels in the rumen are too low, there will be a shortage of nitrogen available to bacteria and feed digestibility will be reduced. An overview of protein metabolism in dairy cows is presented in Figure 1.

### Factors affecting urea level in cow's milk

#### The level of protein in feed rations

From the practical point of view the first question in this chapter is as follows: how much can metabolizable protein in a diet be decreased before production is affected? In some cases dietary crude protein (CP) as low as 12% did not affect milk production in dairy cows, although nutrient digestibility and microbial protein synthesis in the rumen were depressed (Aschemann *et al.*, 2012). In the investigations of Colmenero and Broderick (2006) in the USA fed diets varied in their crude protein contents from 13.5 to 19.4%. Milk urea nitrogen (MUN) is a characteristic signal, indicating potential imperfections in the cattle feeding system and feed rations. The increase in protein in the diet of animals, i.e. the main source of nitrogen for cattle, results in a significant increase of entirely excreted urine nitrogen (UN, g N/d) in relation to the nitrogen excreted in the feces, leading to increased MUN (N mg/dl) [Broderick

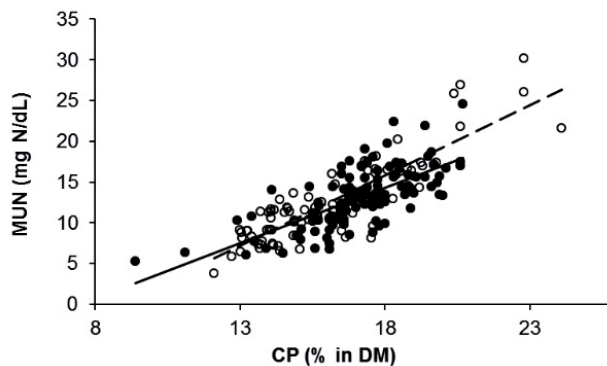


Fig. 2. The correlation between protein level in feed and urea concentration in cow's milk [Spek 2013].

2003, Jonker *et al.* 1999, Kebreab *et al.* 2002]. According to Jonker *et al.* [1999] and Kebreab *et al.* [2002], an increase from 13 to 18% in the proportion of protein in DM of the ration led to a higher concentration of urea in milk from 70 to more than 150 mg per liter (Fig. 2).

Gonzalez and Vazquez [2002] analyzed the effect of supplementation of cattle rations with crude protein/soybean meal on the level of urea in the milk. Thirty-two Holstein-Friesian cows were tested by dividing them into the 4 following groups: no protein supplementation, only pasture; 14% protein ration DM; 17% protein ration DM; and 20% protein ration DM. Average urea levels in milk for particular groups of cows amounted to 244, 295, 317 and 364 mg/kg. The results were statistically

**Table 1.** The effect of food ration supplementation with protein on the level of urea in milk of HF cows [Gonzalez and Vazquez 2002]

Feed ration	14% total protein	17% total protein	20% total protein	Pasture	Mean	±SD
Milk yield (l/d)	18.0 <sup>a</sup>	19.2 <sup>a</sup>	19.4 <sup>a</sup>	15.3 <sup>b</sup>	18.0	2.00
Milk protein %	3.03 <sup>b</sup>	3.20 <sup>a</sup>	2.95 <sup>b</sup>	2.93 <sup>b</sup>	3.03	0.15
Milk fat %	3.22	3.36	3.35	3.28	3.30	0.30
Urea in milk (mg/l)	295 <sup>b</sup>	317 <sup>b</sup>	364 <sup>a</sup>	244 <sup>c</sup>	305	43

<sup>ab</sup>Means in rows marked with different letters differ significantly at  $P \leq 0.01$ .

significant at  $P \leq 0.05$  (Tab. 1).

Borkowska *et al.* [2002] analyzed milk samples from 485 cows kept in 5 herds in the Lublin Voivodeship and found that regardless of the level of herd production, milk samples from the summer feeding period contained significantly ( $P \leq 0.01$ ) more urea compared to the winter season (300 mg vs. 169 mg/l). The authors explained these results with a larger supply of feed protein in the summer production period. The results of a study of Guliński *et al.* [2008] also indicated a significant effect of the season of milk production and the associated high level of protein in the feed. In their work, milk produced in the summer contained the highest level of urea – 225 mg/l,

**Table 2.** The effect of feed ration supplementation with sodium on selected indicators of production and composition of milk and blood plasma in dairy cows [Spek 2013]

Indicator	Sodium intake g/day			
	69	198	292	417
Intake DM (kg/day)	21.0	21.4	21.6	21.6
Water intake (kg/day)	61.7 <sup>a</sup>	82.1 <sup>b</sup>	90.9 <sup>c</sup>	115.7 <sup>d</sup>
Urine volume (kg/day)	18.2 <sup>a</sup>	30.6 <sup>b</sup>	46.6 <sup>c</sup>	67.7 <sup>d</sup>
Creatinine level in urine (mmol/day)	123 <sup>a</sup>	114 <sup>a</sup>	134 <sup>a</sup>	128 <sup>a</sup>
Creatinine level in plasma (mmol/day)	56.0 <sup>a</sup>	53.0 <sup>b</sup>	50.3 <sup>c</sup>	47.8 <sup>d</sup>
Plasma urea (mg N/l)	152 <sup>a</sup>	129 <sup>b</sup>	137 <sup>c</sup>	118 <sup>d</sup>
Milk urea (mg N/l)	125 <sup>a</sup>	112 <sup>b</sup>	108 <sup>c</sup>	99 <sup>d</sup>

<sup>ab...</sup>Means in rows marked with different letters differ significantly at  $P \leq 0.01$ .

while the lowest was measured in milk produced in the winter season – 172 mg/l.

According to Bannink *et al.* [2003], rations rich in minerals, such as sodium (Na) and potassium (K), caused an increased water consumption and urine output, thereby reducing the level of urea in milk and plasma. A study of Campeneere *et al.* [2006] showed that cows fed silage (rich in Na and K) “produced” 43% more urine than their peers fed maize silage. A study of Van Duinkerken *et al.* [2005] demonstrated that the amount of urine produced by cows fed silage was 60% higher in comparison to the control. In a study of Spek [2013], the increase of sodium intake per day from 69 to 419 g was accompanied by an increase in water intake (from 61.7 to 115.7 kg), leading to an increase in the volume of urine produced by cows (from 18.2 to 67.7 kg/day) and a reduction of urea content in milk (from 152 to 118 mg/l) (Tab. 2).

The above results indicate a negative correlation between milk urea concentration and the volume of urine produced by animals. Administration of feeds rich in Na and K leads to increased urine output and, consequently, contributes to reducing the level of urea in milk.

The analysis of differences in daily urea concentration in milk reported in many studies demonstrated that the main reason is the time interval between feed ingestion by animals and the time of milk sampling for analysis. The time of food intake during the day affects diurnal fluctuations in the level of ammonia in the rumen, PUN, MUN, and the correlations between PUN and MUN. Microbial protein degradation occurs after feed ingestion in the rumen, leading to an increase in ammonia concentration in the rumen. Next, ammonia is transported with the blood to the liver, where it is detoxified and converted to urea, and thus its concentration dramatically increases in body fluids. According to Spek [2013], the highest urea level in milk of cows was

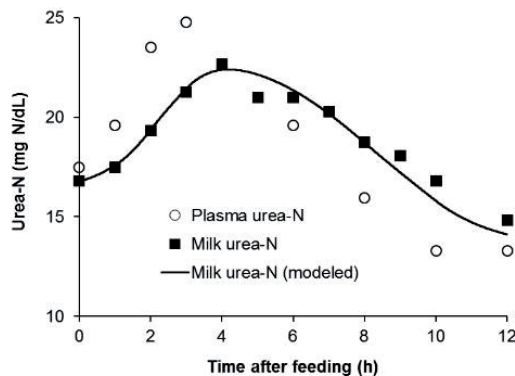


Fig. 3. Urea concentration in cow’s milk in consecutive hours after feeding [Spek 2013].

reached at 4-6 hours after feed ingestion (Fig. 3).

Broderick and Clayton [1997] compared urea levels in milk and urine of cows during daytime (04:00 –16:00 h) and during the night (16:00 – 04:00 h). The average daily MUN (16.0 mg N/dl) was 33% higher than at night (12.0 mg N/dl), while urine

urea nitrogen (UUN) was higher during the night. These differences between urea levels during the day and night meant that the ratio of urea in milk and urine (UUN:MUN) was 4.6 and 7.7 for the day and night, respectively. Diurnal variations in urea levels in milk and urine of cows indicate that the relationship between urea concentrations in cow's milk and urine is not constant. The moment of milk sample collection likely plays the main role in determining the ratio of MUN and UUN in the bodies of cows.

#### Milk yield

One of the key factors connected with milk urea concentration in cows is their performance level. Results reported by many authors clearly indicate that the level of milk productivity is one of the key factors influencing the increase in urea level [Guliński *et al.* 2008, Rzewuska and Strabel, 2013b, Sawa *et al.* 2010]. Figure 4 present the optimal urea concentration in cows with different lactation yields [Jonker

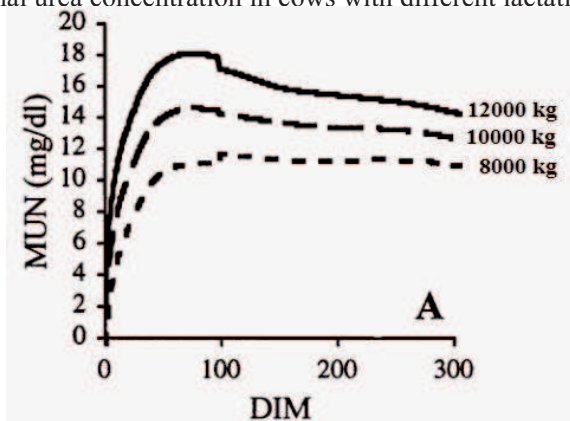


Fig. 4. Optimum urea concentration in milk of cows with different lactation productivity levels [Jonker *et al.* 1999].

*et al.* 1999].

Results of a study by Rzewuska and Strabel [2013b] also indicated a significant impact of milk yield levels of cows on urea concentration. Those authors analysed 900,962 milk samples from primiparous cows included in milk recording in Poland in the years 2001-2009 and found that urea content per 1 liter of milk was 206.7, 212.4 and 236.2 mg for cows of low (<20 kg), medium (20-25 kg) and high (>25 kg) daily production levels, respectively. Also Jonker *et al.* (1998) estimated MUN throughout lactation for cows producing 8000, 10000, or 12000 kg milk per lactation. The trend in MUN was similar to the results given by Rzewuska and Strabel [2013b], even though milk production levels in the Polish population were lower when compared with the US populations. This correlation may have resulted in high-producing herds from the energy deficit as a result of problems in satisfying energy requirements. Another reason for higher MUN concentrations in herds with higher milk production levels could be connected with a positive correlation between MUN and milk yield [Jonker *et al.* 1998].



### The effect of body weight

Body weight of cows is positively correlated with PUN and MUN levels [Kauffman and St-Pierre 2001]. The logical reason for this is that large animals have a greater volume of urea when compared to small animals. Therefore, the decrease in plasma urea nitrogen (PUN) in a “large” animal requires more urea excretion in urine and milk compared to smaller animals. Kauffman and St-Pierre [2001] found a linear relationship between MUN and UUN that were different for cows of Jersey and Holstein-Friesian breeds (Tab. 3).

**Table 3.** The effect of body weight on the level of urea nitrogen in urine of cows ( /d) (the authors’ analysis)

Body weight	Milk urea level (mg/dl)	The equation	The level of urea in urine (g/dl)
400	10		103.6
400	20		207.2
400	30		310.8
650	10	0.0259 x body weight (kg) x MUN (mg/dl)	168.3
650	20		336.7
650	30		505.1

HF cows excreted 17.6 g N/dl per 1 mg of urea in milk, while in Jersey cows it was 11.8 g N/dl. In addition, there are known and described coefficients for cow’s weight and milk urea level that are taken into account in the prediction of urinary nitrogen in the urea of cows. The coefficient for body weight in a study of Kauffman and St-Pierre [2001] was  $b = 0.0259$ , in that of Wattiaux and Karg [2004] it was  $b = 0.0283$ , and  $b=0.0247$  in a study of Zhai *et al.* [2007]. Positive regression coefficients indicate that increasing body weight in cows by 1 kg is associated with raising the milk urea level by  $\approx 0.026$  g/l. Kohna *et al.* [2002] were also of an opinion that body weight is a fixed factor influencing urea level in body fluids of animals. Therefore, according to those authors the level of nitrogen in the urine of cows can be predicted taking into account the size of cows, and using the following regression equation calculation:  $UN = 0.026 \times \text{body weight of cows (kg)} \times \text{the level of urea in milk (mg/dl)}$ .

### Genetic factors

Gołębiewski *et al.* [2011] compared milk urea levels of PHF and Montabaliarde cows kept in six herds under the milk performance testing. The results of observations showed that milk produced by these breeds had an identical average urea level of 251 mg/l. In turn, Pytlewski *et al.* [2011] evaluated the importance of crossbreeding with HF in the genotypes of Black and White cows in Poland for milk urea level. Those authors showed that milk produced by animals with different proportions of HF genes in their genotypes, i.e.  $\leq 75\%$ , 75.1-87.5, and  $> 87.5\%$ , contained 162, 168 and 173 mg of urea in 1 liter, respectively. Doska *et al.* [2012] analyzed the level of urea in Brazil in 16,013 cows of the following four breeds: Holstein, Jersey, Brown Swiss and HF x



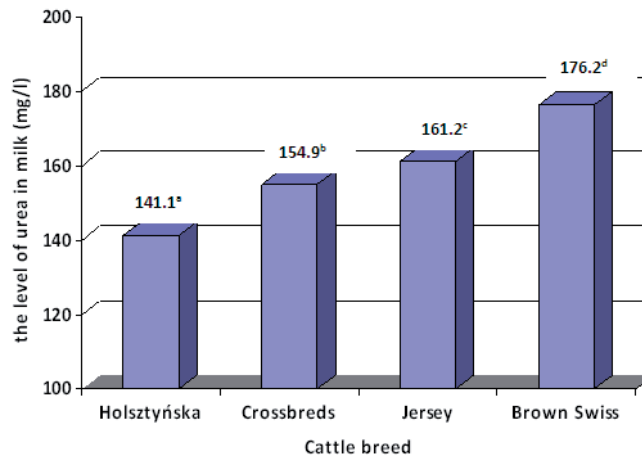


Fig. 5. Urea concentration in milk of cows of different breeds [Doska *et al.* 2012]. Means marked with different letters differ significantly at  $P \leq 0.01$ .

Girolando crossbreds. Their results indicated a significant influence of the cattle breed on milk urea concentration (Fig. 5).

Some of the higher milk urea levels were indicated in a study of Baset *et al.* [2010] in low productive cows of local breeds in Bangladesh. These authors evaluated milk characteristics of two groups of cows, i.e. a local breed and crossbred (local breed x HF), classified according to the intensity of nutrition. The mean daily milk yield amounted to 6.76 kg and 3.67 kg, respectively; average body weight of the two groups was 350.9 and 215.5 kg, respectively; and milk urea concentration amounted to 388.6 and 285.5 mg per 1 l, respectively.

Heritability of this trait has been estimated by many authors. Heritability coefficients for milk urea concentration estimated in the following studies were:  $h^2 = 0.13 - 0.14$  [Bastin *et al.* 2009, König *et al.* 2008],  $h^2 = 0.15 - 0.22$  [Mitchell *et al.* 2005] and  $h^2 = 0.44 - 0.59$  [Wood *et al.* 2003]. Rzewuska and Strabel [2013a] reported that the average coefficient of heritability for MUN was 0.22 (1st lactation) and 0.21 (2nd and 3rd lactations). Genetic correlation coefficients between MUN and other milk traits were significantly different. Correlations of protein content and SCC were low and negative (from -0.24 to -0.11, and from -0.14 to -0.09). The weakest correlations were found for MUN and fat and lactose content (from -0.10 to 0.10). The highest genetic correlations were recorded for MUN and milk yield in lactation (0.20 to 0.42). The authors concluded that selection towards SCC and milk production conducted in Poland can lead to an increase in urea content in cow's milk.

In conclusion, the cited authors observed that the heritability of milk urea concentration is moderate, but the breeding value for this trait is loosely connected with the effectiveness of urea nitrogen removal from the body of animals.

### **The frequency of feeding, number of milkings, level of water and pH of the rumen**

Shabi *et al.* [1998] observed that increasing feeding frequency from two to four times per day was accompanied by a decrease in plasma urea levels by 4.4 mg/dl. Administration of total mixed rations (TMR) that combine roughage and concentrated feeds is the most appropriate way to proceed in terms of balanced urea levels in cow's milk. However, a large number of farmers still supply feeds in the traditional way, i.e. roughage, concentrate and mineral feed separately, or they apply grazing systems. This procedure affects the temporary increase in protein degradation in the rumen, as well as the volume and duration of ammonia concentration in the rumen and blood. As a result, this type of feeding leads to rapid fluctuations in urea levels in milk, plasma and urine of cows. In turn, Nilsen *et al.* [2005] observed an increase in milk urea concentration in cows milked at 6-hour intervals (3 x day) as compared to cows milked at 12-hour intervals (2 x per day). The level of urea amounted to 113 and 99 mg/l of milk, respectively. The conclusion is that increasing the number of feedings during the day decreases urea concentration, while increasing the number of milkings elevates milk urea levels.

Water intake affects urea concentration both in the blood plasma (PUN) and milk (MUN) [Burgos *et al.* 2001]. A study of Burgos *et al.* [2001] demonstrated that dairy cows receiving only 50% of their water requirements had a 1.58-fold higher milk urea concentration compared to their peers supplied water ad libitum. According to those authors, an increased water consumption lowered plasma urea (PUN) and consequently milk urea (MUN), while limited access to water increased milk urea levels.

Remond *et al.* [1993], Abdoun *et al.* [2010] and Abdoun *et al.* [2005] indicated the types of carbohydrates in the diet that may affect the rate of fermentation and the short chain fatty acid profile, as well as ammonia concentration in the rumen, and consequently the dynamics of urea concentration in the blood plasma (PUN) and milk (MU). Administration of fast-fermenting feeds had an effect on the flow rate of urea from the blood to the rumen fluid through the rumen wall [Kennedy *et al.* 1981]. The results of scientific observations showed that pH of the rumen fluid, and CO<sub>2</sub> and butyric acid concentrations are correlated with the rate of ammonia and urea transport across the rumen wall [Abdoun *et al.* 2010, Abdoun *et al.* 2005, Remond *et al.* 1993, Norton *et al.* 1982]. Lowering the pH of the rumen leads to an increase in urea level in cow's milk.

### **Urea in milk and fertility of cows**

Butler *et al.* [1996] conducted an experiment that is currently well-known and widely cited in research papers that assessed the importance of milk urea for cow's fertility. Those authors showed that urea had a significant impact on the percentage of pregnant cows after the first insemination procedure (Fig. 6). The fertility percentage was by 21.4 higher in the group of cows with a milk urea level  $\leq 190$  mg per 1 l of milk, when compared to their peers, which milk contained  $>190$  mg of urea in one liter of milk.

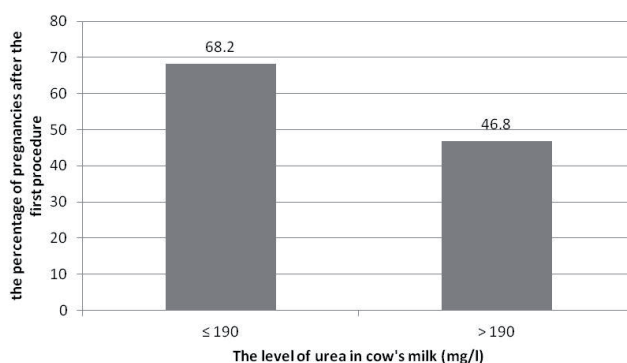


Fig. 6. The influence of urea levels in milk on the percentage of pregnant cows after the insemination procedure [Butler *et al.* 1996].

The fertility of cows in the opinion of Skrzypek *et al.* [2005] is also substantially related to milk urea concentration. Those authors obtained the most favorable reproduction rates in cows, in which urea concentration from milkings preceding the first insemination procedure was low (<150 mg/l) or medium (201-250 mg/l). Differences in the number of insemination procedures per documented pregnancy and the calving interval between the two groups of cows and their peers, which had more than 300 mg of urea in milk, proved to be considerable and statistically significant. Rajala-Schulz *et al.* [2001] considered the importance of milk urea level for cows' reproductive performance. They demonstrated that in cows, in which milk urea level was below 10.0 mg/dl, pregnancy was confirmed 2.4 times more often when compared to animals with milk urea levels above 15.4 mg/dl. The results reported by those authors indicated that increasing MUN concentration had a negative effect on traits associated with fertility and a lower probability of successful fertilization. Studies of Sawa *et al.* [2011] and Jankowska *et al.* [2010] clearly suggested a deterioration of the reproduction rate in dairy cows with increasing milk urea concentrations. The correlation coefficients calculated in a study of Sawa *et al.* [2011] between the level of urea in milk and the length of the calving interval, rest period and service period were positive and amounted to  $r=0.05$ ,  $r=0.07$  and  $r=0.01$ , respectively. In conclusion, the authors pointed out that lowering the level of urea could also lead to a significant improvement in reproductive performance of dairy cattle herds.

### **The potential applicability of information on milk levels in reducing nitrogen contamination produced by cattle**

Cows consuming 419 g N daily have similar milk production levels as those consuming 516 g N daily (Tab. 5). Nevertheless, 74% of the consumed additional N is excreted as urine urea nitrogen (UUN), which could be released to the environment as ammonia emissions. Urea in the large part is removed in the form of urine urea.

According to the findings reported by Spek [2013], as much as 90% total volume of urea found in the body of animals is excreted.

For this reason, milk urea nitrogen (MUN) in dairy farms should be considered as a very useful marker for monitoring total urea excreted from the animal organism with urine (UN – g N/d). A correlation between milk and urine urea in cattle is known

**Table 4.** Correlations between urine nitrogen (UN, g N/d), milk urea nitrogen (MUN, mg N/dl), plasma urea nitrogen (PUN, mg N/dl)

Source	Factors compared	Relationship	Ratio	
			factor A	factor B
Kaufman and St. Pierre [2001]	Holstein-Friesian and Jersey breeds	UN : MUN	11.8 Jersey	17.6 HF
Broderick and Clayton [1997]	Night and day	UN : MUN	4.6 Day	7.8 Night
Colmenero and Broderick [2006]	Low-protein (156 g kg/DM) and high-protein diets (176 g kg/DM)	UN : MUN	14.6 low-protein diet	16.5 high-protein diet
Utley et al. [1970]	0.6 x water <i>ad libitum</i> and 1.0 x water <i>ad libitum</i>	UN : PUN	3.1 for 0.6 x water <i>ad libitum</i>	4.4 for 1.0 x water <i>ad libitum</i>

**Table 5.** Differences between annual nutrient requirements of cattle in various age groups (NRC 2001)

Age groups	Feed component requirements				
	dry matter (kg)	energy (MJ/kg dry matter)	protein (%)	nitrogen (kg)	phosphorus (kg)
Milk cow (650 kg body weight, 7000 kg milk yield)	6500	11	15	170	30
Calf (0-6 months of age)	700	12	16	37	2
Heifer (6 months of age to the first calving)	2900	10.5	12.5	58	9

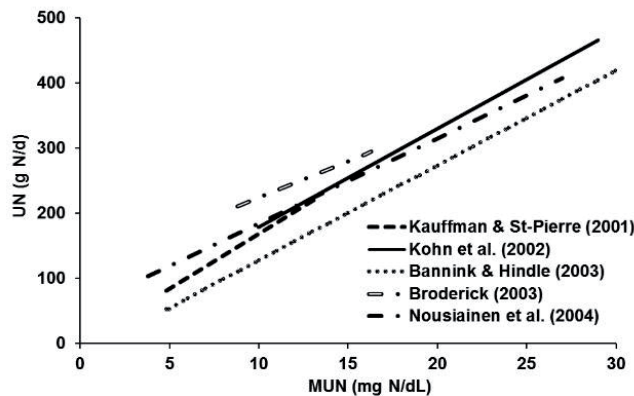


Fig. 7. Interdependence between the level of urea in milk (MUN, mg N/dl) and in urine (UN g/d) for a cow with a body mass of 650 kg, producing 30 kg of milk per day, according to various authors [Spek 2013]: Kohn et al. [2002]  $UN = 15.1 \times MUN + 27.8$ ; Kauffman and St-Pierre [2001]  $UN = 0.0259 \times \text{Body weight} \times MUN$ ; Broderick [2003]  $UN = 11.2 \times MUN + 111.6$ ; Bannink and Hindle [2003]  $UN = 14.5 \times MUN - 4.79 \times S.M. + 126.3$  and Nousiainen et al. [2004]  $UN = 13.1 \times 6.00 \times MUN + S.M.$

and widely described in specialized literature [Bannik and Hindle 2003, Bannik *et al.* 1999, Jonker *et al.* 1999, Nousiainen *et al.* 2004] (Fig. 7, Tab. 4).

First, it should be noted that the relationship between urea concentration in the cow's urine and milk is not constant. In a majority of scientific studies, correlation coefficients for these traits were high within a range of  $R_c=0.63-0.74$ . Although all studies showed a positive relationship between MUN and UN, they varied greatly in assessing the relationship between these traits. Jonker *et al.* [1999] provided the following empirical relationship between urine nitrogen (UN) and milk urea nitrogen (MUN):  $UN (g/d) = 12.54 \times MUN (mg/dl)$ . According to Broderick [2003], the level of 50 mg/l of urea in milk corresponded to 168 g/d of urea in urine and an increase in milk urea concentration to 150 mg/l was accompanied by an increase in the level of urea in the urine to 280 g/d. Bannik and Hindle [2003] claimed that the level of urea in urine can be estimated on the basis of the following regression equation:  $UN = 11.2 \times MUN + 111.6$ .

Reduction of nitrogen excreted by dairy cattle is desirable due to concerns for the global contribution of agriculture to environmental pollution with nitrogen compounds ( $N_2O$ ,  $NO$ , and  $NO_2$ ), in particular the volatilization of ammonia to the atmosphere and nitrate leaching to surface and groundwater [Draaijers *et al.* 1989, Howarth *et al.* 1996]. The primary source of ammonia is the urea nitrogen present in animal manure and urine, which is hydrolyzed to ammonia and carbon dioxide through urease activity of microorganisms present in feces. The negative effects of ammonia ( $NH_3$ ) in the environment are connected with the formation of acid rain, soil and surface water eutrophication, as well as the formation of fine particulate matter. Excessive N levels may especially lead to many problems with surface waters. Nitrogen causes excessive growth of algae that utilize large amounts of oxygen from the water. Deoxygenated water makes it difficult or impossible for fish and other aquatic organisms to survive. Contamination of drinking water supplies with nitrogen can also exert a negative impact on human health. In humans nitrates present in drinking water are converted to nitrites in the gastrointestinal tract. These compounds may replace oxygen in hemoglobin, forming a compound called methemoglobin. An elevated methemoglobin level limits access to oxygen in the blood, which may result in cyanosis or anoxemia. Children are very susceptible to methemoglobinemia, which is known as the "blue baby syndrome". Ingestion of nitrites or nitrates from drinking water may also lead to the formation of tumors.

It is estimated that the annual emission of ammonia in Poland amounts to approximately 386,000 tons, including cattle emission into the atmosphere of about 155,000 tons [Bieńkowski 2010]. A cow emits approximately 40 kg of ammonia to the atmosphere per year [Dammgen *et al.* 2009]. The amount of nitrogen excreted in the urine of cows (kg/year) is closely related to urea level in the milk. With an increase in its concentration from 100 to 300 mg/l, the amount of nitrogen excreted in the urine of cows increases from 45.8 to 137.3 kg/year (Tab. 6). Szarkowski *et al.* [2009] analyzed the feeding of high yielding dairy cows and also underlined the fact that the imbalance of energy and protein in feed rations leads to environmental pollution

**Table 6.** Annual amounts of nitrogen excreted in urine of cows depending on milk urea levels (the authors' analysis)

Milk urea level (mg/dl)	The amount of nitrogen in urine (g/d)	The amount of nitrogen excreted in urine (kg/year)
10	125.4	45.8
20	250.8	91.5
30	376.2	137.3

caused by excess nitrogen excretion. Because of the positive relationship between ammonia emission and urea concentration in milk, in 2002 in the Netherlands an agreement was made between the Dutch government and breeders of dairy cattle to reduce milk urea concentration to 200 mg of urea in 1 l. In return, Dutch farmers are not obliged to invest in systems maintaining low ammonia emissions [Van Duinkerken *et al.* 2011; Van Duinkerken *et al.* 2011].

In the last few years we have been observing an increasing interest among nutritionists in bioactive plant factors, the so-called phytofactors, which can modify rumen fermentation processes, improve protein metabolism [Jóźwik *et al.* 2010] and at the same time reduce ammonia production and emission to the atmosphere [Szumacher-Strabel and Cieślak 2010]. Cows fed diets containing less than 4% tannins on a DM basis showed a higher retention of nitrogen and lower plasma urea concentrations, because of the ability of tannins to protect feed protein from rumen microbial degradation. This effect is related to the ability of tannins to interfere with the membranes of rumen bacteria, binding enzymes or by deprivation of metal ions, such as iron. Recent research indicated that an addition of natural plant-origin biologically active compounds, e.g. tannins, saponins and essential oils, reduce ammonia production and finally milk urea content. A study of Cieślak *et al.* [2012, 2014] showed that tannin extracts have a potential to reduce rumen CH<sub>4</sub> production and ammonia concentration. Studies of Cardozo *et al.* [2004], Busqueta *et al.* [2005] and Benchaar *et al.* [2008] also suggested that essential oils are factors reducing cows' ammonia production and emission to the atmosphere. The application of bioactive phytochemicals (e.g. essential oils or saponins) typically results in reduced amounts of ammonia in the rumen as a consequence of decreased deamination and peptidolysis. Moreover, it was found that the use of these compounds may result in decreased counts and activity of ammonia-oxidizing bacteria, thus limiting the production of excess ammonia in the rumen. Moreover, we also need to consider other bioactive substances (e.g. carbohydrate fractions or phenolic acids), which when interacting with saponins or components of the feed ration may yield completely different results than in the case of pure forms being used [Szczechowiak *et al.* 2013].

## Summary

Dietary protein intake is the most important factor determining milk production, milk composition, milk nitrogen efficiency, urinary nitrogen losses, urea content in milk and consequently, ammonia emissions from dairy cow manure. According to the nutrition requirements, two main protein sources are available to cows: rumen degradable protein, provided to the animal through ruminally synthesized microbial proteins, and rumen undegradable protein that escapes ruminal degradation (but is digested and absorbed in the small intestine). The occurrence of urea in cow's milk is caused by metabolism in the gastrointestinal tract, resulting in the excess of ammonia undigested by microorganisms. This highly toxic chemical compound is detoxified in the liver and converted to urea. It is estimated that about 50% of excess urea is removed immediately from the body in the form of urine and accounts for 70-80% of the urine. Dairy cows remove about 2.5-3.0% of total urea in the body also with milk. The main reason for elevated milk urea levels is connected with excess protein in feed rations and their energy and protein imbalance. The increase in the proportion of total protein from 13% DM of the ration to 18% is accompanied by a rise in the level of urea from approximately 80 mg to over 150 mg per 1 liter of milk. Other factors affecting milk urea level include the frequency of feed administration, number of milkings and the interval between milkings, body weight, water intake, the level of Na and K supplementation in feed rations, as well as rumen pH. Reduction of nitrogen excretion by dairy cattle is desirable due to concerns for the global contribution of agriculture to environmental pollution with nitrogen compounds. In the last few years we have been observing an increasing interest among nutritionists in bioactive plant-origin factors, the so-called phytofactors, e.g. tannins, saponins and essential oils, which can modify rumen fermentation processes, improve protein metabolism and at the same time reduce ammonia production and emission to the atmosphere. Information on the concentration of milk urea nitrogen in dairy cows facilitates evaluation of the energy and protein balance of rations used, reduction of feed costs, while it may also promote reduction of nitrogen (N) emissions to the environment.

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