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Non-nutritional factors of milk urea concentration in Holstein cows from large dairy farms in Croatia

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Summary

Milk urea nitrogen concentration is a reliable indicator of protein-energy balance in dairy cows. Concentration of milk urea nitrogen is mostly affected by nutritional factors, but also its concentration can be influenced by some non-nutritional factors. The aim of this research was to determine the effect of season, parity and stage of lactation on concentration of milk urea nitrogen, as well as its association with daily milk yield, milk fat and protein content and somatic cell count. For that purpose, milk control data were collected for 5061 Holstein dairy cows from four dairy farms during five-year period (between January 1999 and December 2005). When milk urea nitrogen was associated with season, the higher concentration was found in the summer and autumn period, while significantly lower concentration was found in the winter and spring period. Milk urea nitrogen was the lowest in first lactation (27.34 mg/dL) and significantly increased with parities. The highest milk urea nitrogen concentration was recorded during mid-lactation stage (100-200 days), while the lowest concentration was found during late lactation stage (>200 days). Daily milk yield increased notably until above 35.00 mg/dL concentration of milk urea nitrogen, and above that level daily milk yield decreased. Milk fat and protein, content and somatic cell count had negative relationship with milk urea nitrogen concentration. The highest value of milk protein (3.41 %) was recorded when milk urea nitrogen ranged from 15 to 25 mg/dL, while milk fat percentage was the highest (4.06 %) when milk urea nitrogen ranged from 15 to 20 mg/dL. Cows with milk urea nitrogen concentration <15 mg/dL had the highest mean somatic cell count (333x10^3/mL). Results of this study show significant influence of analyzed non-nutritional factors on milk urea nitrogen concentration. These results may be useful in improving the accuracy of models for controlling protein-energy balance in Holstein dairy cows.

Key words: milk urea nitrogen, season, parity, stage of lactation, daily milk yield, Holstein

Introduction

Urea as metabolic product is a result of digestion of nitrogen component in the food. In dairy cows, urea can be found as a component in blood, milk, saliva and urine. The ammoniac appears as a product of protein or non-protein nitrogen components digestion by the micro-organisms in the rumen-reticular area. The rumen micro flora uses ammoniac, energy and minerals from food for microbial proteins production. The excess of ammoniac in the rumen appears when microorganisms are not supplied with sufficient quantity of energy or when the protein component in the diet is in surplus. The excess of ammoniac enters into bloodstream and is transported into the liver, where it is transferred to urea by metabolic processes (Bendelja et al., 2009). Urea is transported by bloodstream to the kidneys and excreted from the organism, or is transported

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to some other parts of organism like udder, were it easily passes through the cell membrane increasing urea concentration in milk.

Urea is the normal component of cow's milk as a part of non-protein nitrogen (NPN) component in milk (Ferguson, 1999). Marenjak et al. (2004) reported that normal concentration of milk urea nitrogen (MUN) in cow's milk ranges between 10 to 30 mg/100 mL (from 1.7 to 4.5 mmol/L). Protein and energy relation, crude protein concentration in the diet and concentration of rumen digestive or non-digestive protein influence the MUN concentration (Oltner and Wiktorsson, 1983; Macleod et al., 1984; DePeters and Ferguson, 1992). High and positive correlation between urea in blood and urea in cow's milk was reported by several authors (Refsdal, 1983; Butler et al., 1996; Broderick and Clayton, 1997; Rodriguez et al., 1997; Campanile et al., 1998). Also, farmers use MUN as a practical method for diet optimization in dairy cows. Particularly, protein component in the diet is more expensive than energy component that is produced by farmers themselves (maize forage, maize corn, barley, oats...). Usage of higher amount of protein components in the diet than required has negative influence on reproduction traits of cows, as well as on environment protection (Broderick and Clayton, 1997).

Milk urea nitrogen concentration depends on various genetic, productive, nutritional and non-nutritional factors. Only several researchers investigated the effect of non-nutritional factors on MUN concentration (Eicher et al., 1999; Godden et al., 2001; Rajala-Schultz and Saville, 2003; Arunvipas et al., 2003; Johnson and Young, 2003; Hojman et al., 2004; Yoon et al., 2004., Jilek et al., 2005; Abdouli et al., 2008). Variability of milk urea concentration affected by production and non-nutritional factors is 13.3 % to 37 % (Arunvipas et al., 2003; Hojman et al., 2004).

The aim of this research was to determine the effect of non-nutritional factors, such as season, parity, stage of lactation and days in milk on urea nitrogen concentration in milk of Holstein cows kept on large dairy farms in Croatia.

Material and methods

The study included 5061 Holstein cows from four dairy farms in Slavonia region. Open free-stall housing and feeding by total mixed ration (*ad libitum*) were applied in all investigated farms. Milk recording control was performed by BT method (HSC, 2004). Monthly herd milk tests beginning January 2005 and ending December 2005, that included milk urea nitrogen concentration (MUN), were combined into a dataset that included date of test (season), daily milk yield, milk fat content, milk protein content, somatic cell count (SCC), MUN, parity and days in milk (DIM). Milk samples were collected at the same time, from 6 to 8 a.m. (morning milking) and from 6 to 8 p.m. (evening milking) in the bottles (cca 40 mL) with preservatives (0.3 mL azidiol), cooled at +4 °C and transported to Central laboratory for milk control in Krizevci. Milk urea nitrogen concentration and milk chemical composition (contents of milk fat and protein) was determined by infra-red spectrophotometric method (HRN EN ISO 9622:2001) by MilkoScan FT 6000 and MilkoScan 4400. Somatic cell count was determined by fluorooptoelectronic method (HRN EN ISO 13366-2:2007) by Fossomatic FC 5000 and Somacount 500. Milk chemical composition (contents of milk fat and protein), milk urea N concentrations <1 or greater than 50 mg/dL, milk fat <1.5 % or greater than 6 %, milk protein <1.5 % or greater than 5 % were excluded from the analysis to remove outliers and because there were too few values. Also, cows with lactation period longer than 600 days were excluded from further analysis. The complete dataset consisted of 68 154 individual cow-month records.

According to the season, milk samples were divided into four groups: winter (December - February), spring (March - May), summer (Juny - August) and autumn (September - November). Days in milk were grouped into categories in two different ways. In the first, DIM were grouped into 30-d increments, with those greater than 420 d grouped into one category (>420 days). In the second set, DIM were grouped into three lactation stages: early (<100 days), mid lactation (100-200 days) and late lactation (>200 days). According to parity, investigated animals were grouped in three categories (first, second and third+). Milk urea nitrogen was grouped into six categories by increments of 5 mg/dL, started with those less than 15 mg/dL and finishing with concentration greater than 35 mg/dL. SCC values were not normally distributed and were $\log_{10}$ transformed before analysis. Statistical data analysis was carried out using SAS program (SAS, 2001). Analysis included: Least Square Means (LSM), means ($\bar{x}$), Minimal (Min.) and Maximal (Max.) values, Standard Error (S.E.), Standard derivation (SD) for individual parameters. The relations between MUN concentration, DIM and parity were analyzed using the PROC GLM of SAS (2001), while sampling month within year was added to the model as a covariate. Relation between MUN and production variables was analyzed using PROC GLM procedure of SAS (2001), while parity, DIM and month within test year were added to the model as covariates. Pearson's Correlations coefficient between MUN, daily milk yield and chemical composition were analyzed using PROC CORR procedure (SAS, 2001).

Results and discussion

Overall, average cow-level MUN concentration (27.47 mg/dL) was higher than values reported in studies of Carlson et al. (1995), Johnson and Young (2003), Rajala-Schultz and Saville (2003) and Yoon et al. (2004), but lower than values reported by Godden et al. (2001) and Abdouli et al. (2008) for Holstein dairy cows. High MUN concentration (Table 1) might be caused by the fact that, in this study, cows were fed with diets rich in protein components or by some non-nutritional factors (days in milk, season, parity of lactation, level of milk production and composition).

Additionally, concentration of MUN was significantly higher ($P<0.001$) in milk samples collected at a.m. milking (29.93 mg/dL) in comparison with p.m. milking (28.34 mg/dL). Godden et al. (2001) reported generally lower concentration of MUN in samples collected at a.m. milking. Differences between a.m. and p.m. MUN concentration may be influenced by different interval between a.m. and p.m. milkings (Gantner et al., 2009) or by differences in feeding-to-milking intervals (Godden et al., 2001). Mean daily milk yield per cow was lower in comparison with earlier studies on this topic (Godden et al., 2001; Johnson and Young, 2003), but higher than values reported in some other studies (Yoon et al., 2004; Jilek et al., 2005; Abdouli et al., 2008). Mean values for milk fat and protein contents determined in this study were higher than average values for total Holstein population in Croatia (milk fat: 3.94 %; protein: 3.26 %) given by Croatian Agricultural Agency (HPA, 2008). Also, means milk fat and protein contents were higher than those reported by other authors (Godden et al., 2001; Johnson and Young, 2003; Rajala-Schultz and Saville, 2003; Yoon et al., 2004; Abdouli et al., 2008). Mean SCC value (229 x 10^3/mL) was lower then reported by Yohnson and Young (2003), Rajala-Schultz and Saville (2003) and Yoon et al. (2004). Mean DIM was 198 and was similar to DIM for Holstein cows in the USA (Johnson and Young, 2003).

Table 1. Descriptive statistics of analyzed variables (n = 68154 cow-months record)
Tablica 1. Opisna statistika za istraživane pokazatelje (n = 68.154 kontrola mliječnosti)

Parameter/Pokazatelj	$\bar{x}$	S.E.	SD	Min.	Max.
Daily milk yield (kg/day) Proizvodnja mlijeka (kg/dan)	25.62	0.03	8.42	0.92	75.16
MUN (mg/dL) Urea u mlijeku (mg/dL)	27.47	0.03	6.76	0.10	50.00
Milk fat (%) Mliječna mast (%)	4.03	0.01	0.80	1.50	6.00
Milk protein (%) Mliječne bjelančevine (%)	3.40	0.01	0.43	1.92	5.00
Somatic cell count (10^3/mL) Broj somatskih stanica (10^3/mL)	229.189	2.22	578.435	1.00	9999.00

Table 2. Coefficient of correlations between days in milk (DIM), daily milk yield, milk composition components, somatic cell count and milk urea nitrogen concentration (MUN)
Tablica 2. Koeficijenti korelacija između dana laktacije, dnevne proizvodnje mlijeka, sastava mlijeka, broja somatskih stanica i uree u mlijeku

Parameter/Pokazatelj	Daily milk yield Dnevna proizvodnja mlijeka	Milk fat Mliječna mast	Protein Bjelančevine	MUN Urea	SCC BSS
DIM/Dani laktacije	-0.57**	0.26**	0.64**	-0.07***	0.19**
Daily milk yield Dnevna proizvodnja mlijeka		-0.31**	-0.52**	0.13**	-0.26**
Fat/Mast			0.44***	-0.09**	0.11**
Protein/Bjelančevine				-0.06**	0.21**
MUN/Ureja					-0.10**

**P<0.01; ***P<0.001

Coefficients of correlations of the milk production traits and the MUN concentration are presented in Table 2.

Days in milk were positively correlated (P<0.01) with protein, milk fat content and SCC, but negatively correlated with daily milk yield and MUN (P<0.001). Daily milk yield was negatively correlated (P<0.01) with milk fat, protein content and SCC, but positively correlated with MUN. Similar results were reported in previous researches (Oltner et al., 1985; Yoon et al., 2004). Positive correlation between daily milk yield and MUN was expected because cows with higher milk production were fed diets richer in protein component. Finally, negative correlations (P<0.01) were found between MUN and milk fat, protein and SCC. However, Yoon et al. (2004) reported positive correlations between MUN, milk fat, and SCC and negative correlation between MUN and protein. A small, but significant positive relationship between SCC and MUN was reported by Ng-Kwai-Hang et al. (1985), but Hojman et al. (2005) reported negative relationship between SCC and MUN.

Mean daily milk yield and MUN concentrations, by DIM categories, demonstrated curvilinear, but no parallel relationships (Figure 1), which were reported by Johnson and Young (2003). Milk urea nitrogen concentration was higher during period from 60 to 180 DIM compared to all other DIM categories. Similar results were reported in other studies (Carlsson et al., 1995; Eicher et al., 1999; Godden et al., 2001; Johnson and Young, 2003).

According to the data demonstrated in Figure 2, stage of lactation had significant effect on MUN concentration in Holstein cows. The highest MUN concentration was recorded during mid-lactation stage (100-200 days), while the lowest MUN concentration was found during late lactation stage (>200 days). Contrary to our results, Yoon et al. (2004) found the highest values of MUN during late lactation stage (>200 days) and lowest (15.07 mg/dL) during early lactation stage. These results should be interpreted with caution because feeding practice and management system is different in different countries.

The overall mean for the third and greater parity group was higher (P<0.01) than first and second parity, even though the difference was only 0.4 mg/dL and 0.27 mg/dL (Figure 3). Contrary to these results, Carlsson et al. (1995) and Johnson and Young (2003) found the highest MUN concentrations in the first lactation and the lowest in third and later lactation. According to this study, Godden et al. (2001) found a statistically significant, but numerically small difference in MUN due to the parity. The highest concentrations of MUN in later lactations (Figure 3) suggest the possibility of overfeeding cow's diets containing high amount of protein component, or a different amount or rumen degradable protein. However, because the overall differences in MUN concentrations between different parities are small, Johnson and Young (2003) concluded that the biological significance of previously mentioned observations is questionable.

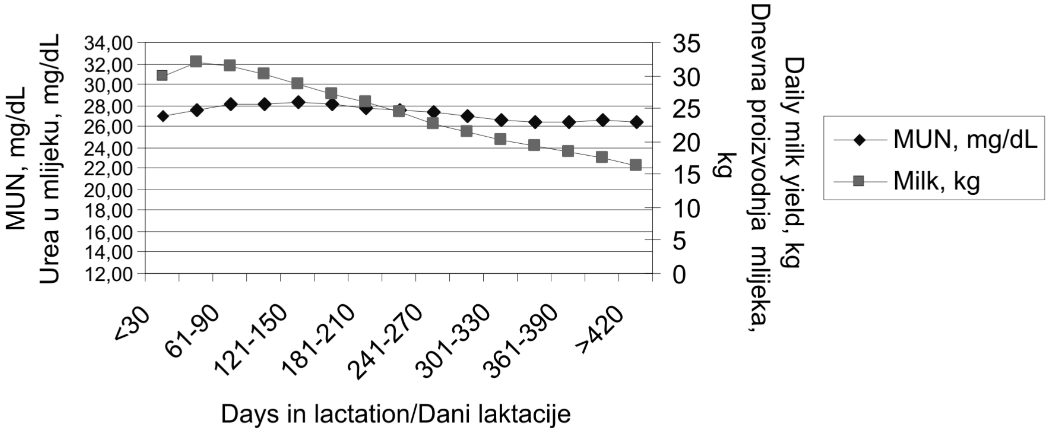
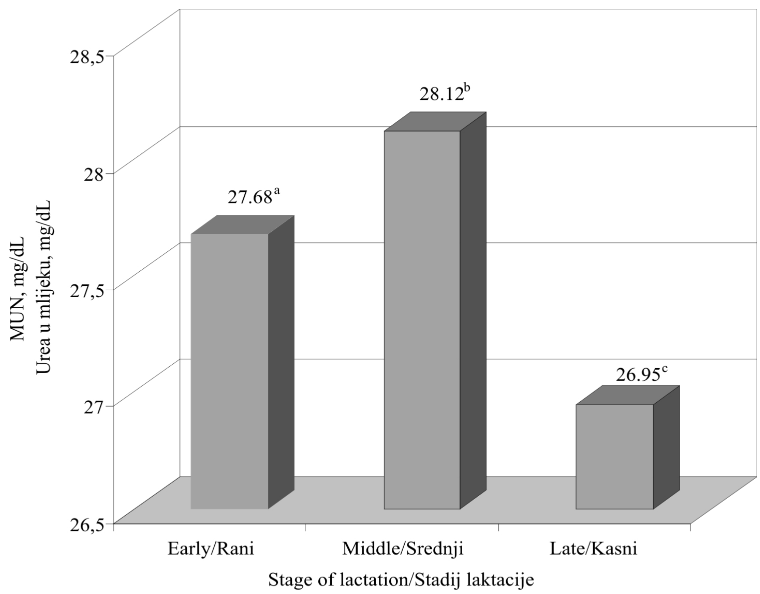


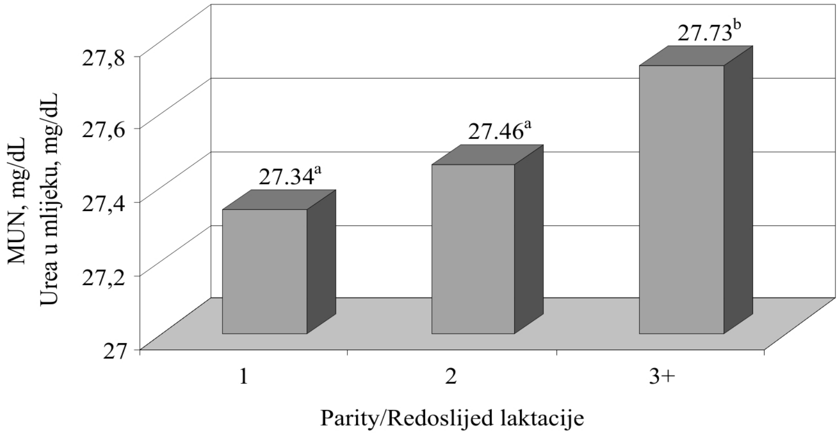
Figure 1. Means of milk urea nitrogen concentrations (MUN) and daily milk yield by 30-d days in lactation categories
Grafikon 1. Prosječni sadržaj uree u mlijeku i dnevna proizvodnja mlijeka tijekom laktacije u 30 dnevnim intervalima



[a,b,c]Means with different superscripts significantly differ (P<0.01)
[a,b,c]Vrijednosti označene različitim slovima značajno se razlikuju (P<0,01)

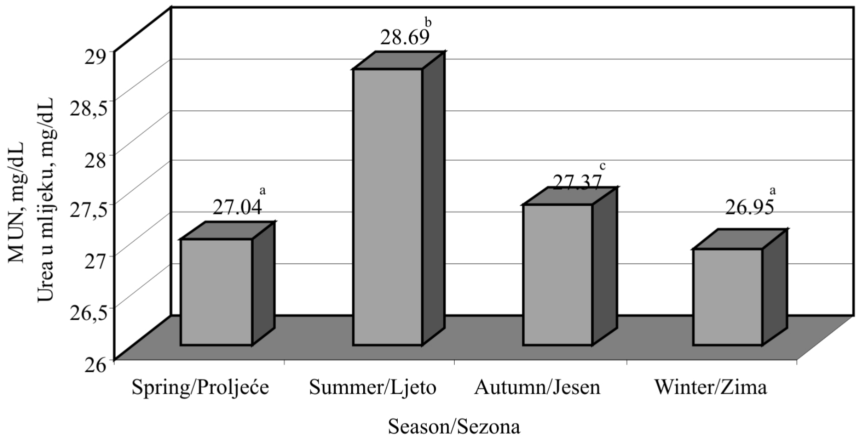
Figure 2. Means of milk urea nitrogen concentration (MUN) regarding stage of lactation
Grafikon 2. Prosječne vrijednosti uree u kravljem mlijeku s obzirom na stadij laktacije

Milk urea nitrogen was higher during the summer (Figure 4) and similar results have been reported by others (Carlsson et al., 1995; Godden et al., 2001). Rajala-Schultz and Saville (2003) reported higher MUN concentration in the summer but only for the low producing herds (average 6850 kg milk/lactation/cow). They also reported that effect of season was much less evident in the high producing herds, that could be explained by lower dry meter and protein component intake in the summer. Hojman et al. (2004) reported higher concentration MUN in the spring and the highest in the beginning of the summer in June (18.1 mg/dL), similar results reported Abdouli et al. (2008).



a,bMeans with different superscripts significantly differ (P<0.01)
a,bVrijednosti označene različitim slovima značajno se razlikuju (P<0,01)

Figure 3. Means of milk urea nitrogen concentration (MUN) regarding parity
Grafikon 3. Prosječne vrijednosti uree u kravljem mlijeku s obzirom na redoslijed laktacije



a,b,cMeans with different superscripts significantly differ (P<0.01)
a,b,cVrijednosti označene različitim slovima značajno se razlikuju (P<0,01)

Figure 4. Means of milk urea nitrogen concentration (MUN) by season
Grafikon 4. Prosječne vrijednosti uree u kravljem mlijeku tijekom sezona

Higher MUN concentration in the winter period was reported by Yoon et al. (2004) and Jilek et al. (2005). Total protein and true protein (mostly casein) in milk were lower and NPN especially MUN increased during summer months (Carlsson et al., 1995).

Descriptive information about milk production, protein, and fat percentage and SCC, stratified by MUN category, are summarized in Table 3. Association between MUN concentration and milk protein percentage was negative. The data suggest that milk protein percentage was the highest when MUN values ranged from 15.00 to 25.00 mg/dL. The concentrations of MUN above 30.00 mg/dL had negative effect on milk protein percentage (P<0.01). Similar relation was reported by Johnson and Young (2003), with exception of protein percentage which was the highest (3.40 %) when MUN values ranged from 6.01 to 8.00 mg/dL. The same inverse relation was observed between MUN categories and milk fat percentage. When MUN increased, milk fat decreased, but these changes were very small. Negative

Table 3. Means and standard error for daily milk yield, protein and fat percentage and somatic cell count (SCC) by milk urea nitrogen concentration (MUN) categories (LSM±SE)
Tablica 3. Prosječna proizvodnja mlijeka, udjel bjelančevina i mliječne masti te broj somatskih stanica pri različitom sadržaju uree u mlijeku (LSM±SE)

MUN (mg/dL) Urea (mg/dL)	n	Milk (kg/day) Mlijeko (kg/dan)	Protein (%) Bjelančevine (%)	Milk fat (%) Mliječna mast (%)	SCC (000) BSS (000)
≤15.00	1671	23.26±0.087[a]	3.40±0.008[a]	4.05±0.018[a]	333±14.273[a]
15.01-20.00	7013	24.46±0.083[b]	3.41±0.004[a]	4.06±0.009[a]	287±6.953[a]
20.01-25.00	16687	25.22±0.054[c]	3.41±0.003[a]	4.05±0.006[a]	249±4.500[b]
25.01-30.00	20166	25.86±0.049[d]	3.40±0.002[ab]	4.03±0.005[a]	217±4.072[c]
30.01-35.00	13732	26.27±0.060[e]	3.39±0.003[b]	4.01±0.006[b]	204±4.935[c]
>35.01	8945	26.19±0.077[e]	3.37±0.003[c]	3.96±0.008[c]	195±6.177[c]

[a,b,c,d,e] Means within the same column with different superscripts differ (P<0.01)
[a,b,c,d,e] Vrijednosti u istom stupcu označene različitim slovima značajno se razlikuju (P<0,01)

relation between milk fat and MUN was also reported by other authors (Broderic and Clayton, 1997; Johnson and Young, 2003; Hojman et al., 2004), while positive relation was reported by Abdouli et al. (2008). Carlsson and Bergstrom (1994) mentioned that negative relation between milk fat and MUN may be a result of nutritional variables or a direct negative effect of milk fat on MUN.

In present research (Table 3), SCC and MUN categories were in reverse relation (P<0.01), which is in accordance with negative relation reported by Godden et al. (2001) and Johnson and Young (2003). However, Eicher et. al. (1999) found no significant association between SCC and MUN. Daily milk yield increased with increasing of MUN, however when MUN was higher than 35.00 mg/dL, daily milk yield decreased. Positive association between daily milk yield and MUN has also been reported in previous researches (Oltner et al., 1985; Carrlson et al., 1995; Godden et al., 2001; Johnson and Young, 2003).

Conclusions

Results from this study show significant positive relation between daily milk yield and MUN. Milk fat, protein and SCC were significantly negatively associated with MUN, but differences of their numerical values were very small. Milk urea nitrogen concentration varied depending on season, parity and stage of lactation. These results suggest that previously mentioned non-nutritional factors should be evaluated when the relation between MUN concentration and nutritional management on dairy farms is determined.

Nehranidbeni čimbenici sadržaja uree u mlijeku Holstein krava s velikih farmi u Hrvatskoj

Sažetak

Sadržaj uree u mlijeku pouzdan je pokazatelj uravnoteženosti odnosa bjelančevina i energije u obroku mliječnih krava. Različiti hranidbeni, ali i neki nehranidbeni čimbenici utječu na udjel uree u kravljem mlijeku. Cilj ovog rada bio je utvrditi utjecaj sezone, redoslijeda i stadija laktacije na udjel uree u mlijeku, kao i povezanost između udjela uree, proizvodnje mlijeka, udjela mliječne masti i bjelančevina te broja somatskih stanica u mlijeku. U tu svrhu su provedene mjesečne kontrole mliječnosti na četiri velike farme mliječnih krava Holstein pasmine (ukupno 5061 krava u razdoblju od siječnja 1999. do prosinca 2005. godine). Sezona je značajno utjecala na udjel uree u mlijeku, čija je koncentracija tijekom ljeta i jeseni bila viša nego zimi i u proljeće. Udjel uree bio je najniži u prvotelki (27,34 mg/dL) te se značajno povećavao u krava višeg reda laktacije. Sredinom laktacije (100-200 dana) zabilježen je najveći, a krajem laktacije (>200 dana) najmanji prosječni sadržaj uree u mlijeku. Proizvodnja mlijeka znatno se povećavala do razine od 35,00 mg uree po 1 dL mlijeka, nakon čega je uslijedio pad. Udjel mliječne masti i bjelančevina te broj somatskih stanica u mlijeku bili su u negativnoj korelaciji sa sadržajem uree. Najviši udjel bjelančevina (3,41 %) zabilježen je u mlijeku s rasponom udjela uree od 15 do 25 mg/dL, dok je najviši udjel mliječne masti (4,06 %) zabilježen kad je urea bila u rasponu od 15 do 20 mg/dL. Najveći prosječni broj somatskih stanica ($333x10^3$ mL^{-1}) utvrđen je u mlijeku sa <15 mg/dL uree. Rezultati ovog istraživanja upućuju na zaključak da analizirani nehranidbeni čimbenici značajno utječu na udjel uree u kravljem mlijeku. Na taj način dobiveni rezultati mogu poslužiti prilikom usavršavanja modela za kontrolu uravnoteženosti bjelančevina i energije u obroku mliječnih krava Holstein pasmine.

Ključne riječi: urea u mlijeku, sezona, redoslijed laktacije, stadij laktacije, dnevna proizvodnja mlijeka, Holstein pasmina

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