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Variation in nitrogen components of sheep milk in sub-Mediterranean area

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Abstract

The aim of this study was to evaluate seasonal variation in urea content and other nitrogen compounds (protein, casein, non-protein nitrogen content) of sheep milk as a tool for monitoring the protein nutritional status over the period of two years. The study was performed on three family farms with 150 to 300 sheep per farm using semi-extensive farming management based on pasture, typical for sub-Mediterranean area. Bulk-tank milk samples were taken during the entire milking period (from March to July or August, depending on the year) and were analysed for protein, casein, non-protein nitrogen (NPN) and urea content. Significant effect of the year was observed on urea and NPN content ($P<0.05$). In addition, seasons had a significant effect on milk protein, urea and casein content ($P<0.01$). Variations in nitrogen components of sheep milk in Dalmatian hinterland have been significant due to the fact that the semi-extensive farming systems could be strongly affected by climate which directly influence on the quantity and quality of pasture.

Key words: sheep milk, nitrogen components, sub-Mediterranean

Introduction

In the area of Dalmatian hinterland (sub-Mediterranean area) sheep are mainly farmed under extensive or semi-extensive management based on artificial, natural or semi-natural pastures using local sheep breeds such as Dalmatian Pramenka. Dalmatian Pramenka is the most numerous indigenous breed in Croatia with more than 200,000 animals and is mainly used for lamb production. However noticeable farmers intend to breed Dalmatian Pramenka for milk and traditional sheep cheese production. Sheep spend most of the year on pasture and are often exposed to a lack of adequate supplementary feeds. Consequently a deficiency of some nutrients and poor energy-protein ratio appear during lactation and seasonal period. This strongly influences the composition of sheep milk and could be overcome by supplementation of dairy sheep, since in Mediterranean regions, concentrates represent a high proportion of energy intake in grazing dairy ewes (Molle et al., 2007). Extensively reared indigenous breeds of dairy animals produce milk of a very different composition from the milk produced by specialized breeds (Bonanno et al., 2013). Fat and protein are the most important milk components that are recovered in the cheese making process and are directly related to the cheese yield (Jaeggi et al., 2005). Proteins in milk consist of approximately 95 % true protein and 5 % non-protein nitrogen (NPN). This NPN fraction is mainly urea, from 20 to 75 % (Journet et al., 1975), and this proportion is related to the protein and energy supply (Geerts et al., 2004). Therefore, milk urea nitrogen concentration is a reliable indicator of protein energy balance in dairy animals (Jelinek et al., 1996; Campanile et al., 1998; Trevaskis and Fulkerson, 1999; Geerts et

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al., 2004; Bendelja et al., 2009; Ghavi Hossein-Zadeh and Ardalan, 2011). Milk urea nitrogen concentration may be used to estimate the crude protein content of the diet fed to ewes or, with lower precision, their crude protein intake, which would be especially important for grazing animals which intake estimation represents a major problem (Cannas et al., 1998). Nutritional factors that might affect milk urea are feed protein intake, ratio of protein to energy in the diet (Trevaskis and Fulkerson, 1999), feeding strategy (diet composition and frequency of feeding; Geerts et al., 2004) and quality of pasture (botanical composition of pasture; Molle et al., 2003; Totty et al., 2013). High levels of urea in milk are interpreted as an indication of excessive protein/nitrogen intake and inefficient utilization of degradable protein as a result of poor energy supply in feed. The reason for the too low urea content in the milk could be due to the lack of crude protein in the feeding (Ghavi Hossein-Zadeh and Ardalan, 2011). Although, the concentration of milk urea is mostly affected by nutritional factors, its concentration can be influenced by some non-nutritional factors like breed, season, parity and stage of lactation, milk yield, time of milking and somatic cell count (Trevaskis and Fulkerson, 1999; Hojman et al., 2004; Jilek et al., 2006; Kuchtik et al., 2008; Bendelja et al., 2009; Ghavi Hossein-Zadeh and Ardalan, 2011). The aim of this study was to evaluate the seasonal variation in urea content and other nitrogen compounds of sheep milk as a tool for monitoring the protein nutritional status of sheep over the period of two years.

Materials and methods

The study was performed on three family farms in the hinterland of middle Dalmatia (area from Knin to Sinj, Croatia), over the period of two years (2007 and 2008). The number of animals per flocks varied between 150 and 300 animals. The lambing was in January and February, depending on the year. After weaning (60 to 90 days of suckling period), sheep were milked from March. The milking period finished in July or August; depending on individual characteristics of animals and farming management. The key part of sheep diet in all farms during the entire year was natural pasture, while the sheep were also supplemented with hay, especially in the winter season. During the milking season sheep were on pasture in the period between morning and evening milking, while overnight they were confined. In addition, sheep were fed up to 150 g of wheat and barley grit per head per day.

In all farms sheep were hand-milked. Samples of bulk-tank milk (n = 53) were taken twice a month during the above mentioned milking periods. Bulk-tank milk samples were obtained every three days before milk transportation to a dairy plant. Milk samples were stored in 200 mL sterile plastic bottles under sterile conditions and were transported (under controlled conditions - up to +4 °C) to the laboratory of Dairy Science Department, Faculty of Agriculture, University of Zagreb. Analysis of chemical composition of sheep milk included determination of protein content by the Kjeldahl method (ISO 8968-2:2003), non-protein nitrogen content (ISO 8968-5:2003), casein content (ISO 17997-1:2004) and urea content (RU 4.2.1-KA-10, 2006).

Data for average monthly precipitation (expressed in mm) in the farm locations were obtained from the Croatian Meteorological and Hydrological Service Institute (Table 1).

Table 1. Data for average monthly precipitation (mm) on area covered by the investigation of years 2007 and 2008

Month	Year	
	2007	2008
March	130.0	113.1
April	14.4	91.6
May	96.6	48.9
June	102.2	199.6
July	30.3	23.1
August	157.2	6.1

Seasonal variation of nitrogen compounds within the year was tested by the analysis of variance using flock as a random effect (PROC GLM, SAS Inst. Inc., 2001). Effects were considered significant if $P<0.05$. The obtained results are presented as the least squares means (LSM) ± standard error (SE).

Results and discussion

Extensive or semi-extensive farming of sheep Pramenka which is typical for Dalmatian hinterland and for other sub-Mediterranean regions is strongly affected by the environmental conditions. Consequently, significant variation in milk composition and quality of milk could be expected (Arranz et al., 2001; Martini et al., 2008). The poor diet management, typical for dairy sheep production systems based on grazing, can substantially limit the ability of sheep producers to control and modify the quality of milk (Pulina et al., 2006). Nutritional imbalance can lead to a severe energy deficit which in turn results in reduced protein and fat content, altered amino acid, fatty acid and mineral profile of milk. Such modifications are responsible for reduced nutritional and technological property of milk (Sevi, 2007). In addition, milk production systems based on grazing pasture are frequently subjected to climatic conditions and meteorological events that influence the quantity and quality of pasture (Lucey, 1996; Pulina et al., 2006). The total amount of precipitation during the milking period was 530,7 and 482,4 mm in year 2007 and 2008, respectively (Table 1).

It is essential to emphasize that during early stages of vegetation growth in April 2007 there was an extremely dry period without almost any precipitation, while in 2008 during the milking period the amount of precipitation was evenly spread out. Pasture was predominant in sheep diet during the milking period, and such diet is characterized by easily-digestible nitrogen components, poor energy and low non-fibrous carbohydrates intake (Kalit, 2008; Mikolayunis et al., 2008). In turn this affects the content of proteins in milk, as well as the content of non-protein nitrogen in milk. Hence, a higher content of NPN was expected in 2008, but a lower NPN concentration ($P<0.01$) was determined (Table 2). Proteins in sheep milk consist of approximately 5 % non-protein nitrogen. NPN is composed of several nitrogenous compounds which in cow milk include urea, amino acids, creatinine, creatin, ammonia, uric acid, and uncountable nitrogen. Sheep milk varies somewhat in the content of these NPN components compared to cow milk, partially due to factors in their diet such as protein and NPN levels (Park et al., 2007). Variations of nitrogen content on the NPN fraction of milk are minor in comparison to the variations on total nitrogen or protein nitrogen in milk (Journet et al., 1975). According to that, in 2008 higher milk urea concentrations ($P<0.05$) were determined (Table 2). Hojman et al. (2004) conducted a research on influences of production and environmental factors on milk urea and proved significant influences of year on the milk urea concentration in cow dairy herds. Also studying influences of environmental factors on milk urea nitrogen in South African Holstein cows Kgole et al. (2012) has shown a significant effect of year on milk urea concentration which they explained with different management practices or nutrition.

A significant effect of year on gross milk composition was proved by Ploumi et al. (1998) for flocks of Chios sheep and Matutinovic et al. (2011) for Dalmatian Pramenka sheep.

As Dalmatian Pramenka spends most of the year on pasture, significant effect ($P<0.01$) of seasonal changes on the content of protein, casein and urea of sheep milk was proven (Fig. 1, 2 and 4).

Table 2. Effect of the year on sheep milk composition in sub-Mediterranean area (LSM±S.E.)

Item	Year		Significance P-value
	2007	2008	
Protein content (g/100 g)	6.09±0.06	6.10±0.05	ns
Casein (g/100 g)	4.65±0.07	4.76±0.06	ns
Urea (mg/100 mL)	32.23±1.82	37.55±1.66	*
Non protein nitrogen (g/100 g)	0.27±0.02	0.20±0.02	**

**P<0.01; *P<0.05; ns - non significant

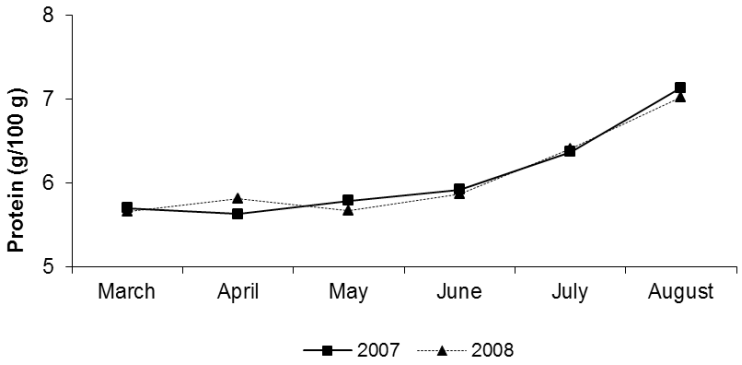

Figure 1. Seasonal variation of protein content in sheep milk in sub-Mediterranean area, n=53, P<0.01

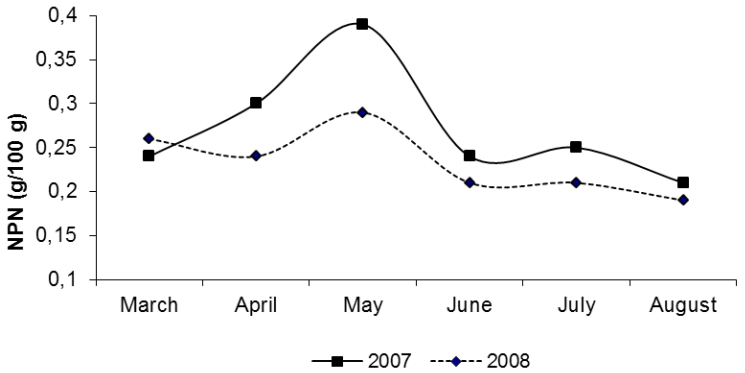

Figure 2. Seasonal variation of casein content in sheep milk in sub-Mediterranean area, n=53, P<0.01

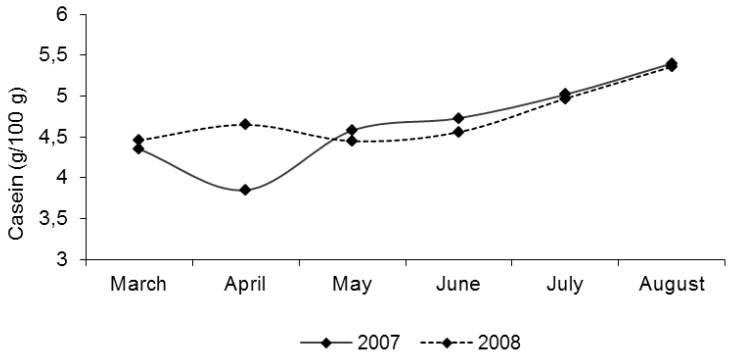

Figure 3. Seasonal variation of non-protein nitrogen (NPN) content in sheep milk in sub-Mediterranean area, n=53

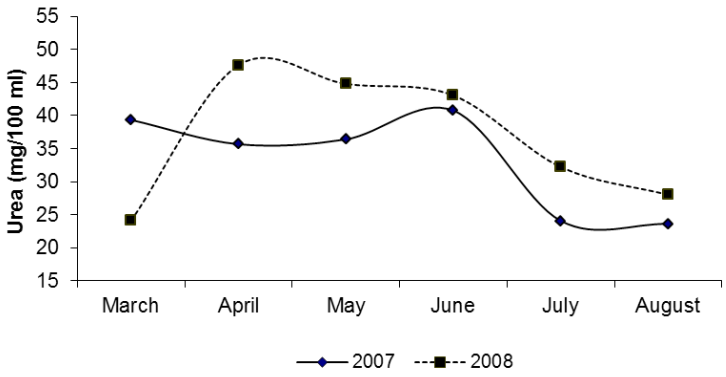

Figure 4. Seasonal variation of urea concentration in sheep milk in sub-Mediterranean area, n=53, P<0.01

Similar results were observed by Sevi et al. (2004) who studied the effects of lambing season on Comisana ewes whose diet is also based on pasture as well as in our previous study (Matutinovic et al., 2011). Due to the extremely dry period in April 2007, a significant drop (P<0.01) in urea concentration was observed, while the highest milk urea concentration was observed in June 2007 (Fig. 4). In comparison to 2007, the highest recorded concentration of milk urea in 2008 was observed in early spring, in April, (Fig. 4) while the lowest figures were recorded in March. Faye et al. (2010) conducted a research on the variability of urea concentration in camel milk. They proved that the highest value in milk urea was occurring in spring and the lowest in autumn. It is known that increased urea content in milk can change the technological milk properties and characteristics of mature cheese (Martin et al., 1997). Trevaskis and Fulkerson (1999) investigated the relationship between various animal and management factors on milk urea in dairy cows grazing pasture and also detected a significant effect of seasonal changes on the concentration of milk urea. Similar results on sheep were observed by Molle et al. (2003) who studied complementary grazing sources for Sarda dairy sheep. The effect of season was not significant on NPN (Fig. 3) which is in good correspondence with findings of Journet et al. (1975), who stated that the season usually has a minor effect on the NPN content in milk. The highest content of NPN during milking period was observed (0.29 and 0.39 in May vs. 0.19 and 0.21 in August; Fig. 3) which corresponds to results obtained by Abilleira et al. (2010), who conducted a research on flocks of Latxa ewes. It should also be noted that similarly to the urea content drop observed in spring 2007, there was also a significant drop in caseine content during the dry period (Fig. 2). In contrast to that, the content of protein and casein increased as a consequence of lactation progress (July and August; Fig. 1 and 2). Such findings correspond to results obtained by Fuertes et al. (1998); Jaramillo et al. (2008) and Matutinovic et al. (2011). Jaeggi et al. (2005) focused on the effects of season on quality of sheep milk within the North American great lakes region. They proved a decrease of total protein and casein content as a consequence of decreasing solids in milk produced during hot summer temperatures and poorer quality pastures. The results obtained in this study do not correspond with those of Jaeggi et al. (2005).

Conclusions

The semi-extensive farming systems are strongly affected by climate and environmental conditions, especially precipitation which is directly related to the quantity and quality of pasture. According to that, a significant effect of year and seasonal variation on nitrogen components of sheep milk in sub-Mediterranean area was detected. The mentioned farming conditions determine the variation in digestibility of protein and carbohydrates from pasture forage. According to milk composition and nitrogen components, a requirement to add feed supplements into the animal diet should be taken into consideration. Also an appropriate management practices and control of diet to reduce the effects of year and seasonal variations should be implemented.

Varijabilnost dušičnih sastojaka ovčjeg mlijeka sub-mediteranskog područja

Sažetak

Cilj ovog rada bio je utvrditi sezonske varijacije sadržaja uree i drugih dušičnih sastojaka (bjelančevina, kazeina i neproteinskog dušika) ovčjeg mlijeka kao alata za praćenje proteinskog hranidbenog statusa ovaca. Istraživanje je provedeno u razdoblju od dvije godine na tri obiteljska poljoprivredna gospodarstva s polu-ekstenzivnim uzgojem ovaca (od 150 do 300 ovaca po stadu). Uzgoj je baziran na ispaši na prirodnim pašnjacima, što je karakteristično za sub-mediteransko područje. Prikupljeni su skupni uzorci ovčjeg mlijeka tijekom mužnje (od ožujka do srpnja ili kolovoza, ovisno o godini). Uzorci su analizirani na sadržaj proteina, kazeina, neproteinskog dušika (NPN) i uree. Utvrđen je značajan ($P<0,05$) utjecaj godine na koncentraciju uree i NPN-a. Povrh toga, sezona je značajno ($P<0,01$) utjecala na sadržaj proteina, uree i kazeina u ovčjem mlijeku. Varijacije dušičnih sastojaka ovčjeg mlijeka u Dalmatinskoj Zagori posljedica su polu-ekstenzivnog načina uzgoja ovaca, osjetnog utjecaja klimatskih čimbenika koji izravno utječu na količinu i kvalitetu paše.

Ključne riječi: ovčje mlijeko, dušični sastojci, sub-mediteran

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