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Milk Urea Content and $\delta^{13}C$ as Potential Tool for Differentiation of Milk from Organic and Conventional Low- and High-Input Farming Systems

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ABSTRACT

The influence of farming type (conventional or organic) and production system (low-and high-input) on various quality characteristics of milk have been in the focus of studies over the last decade. The aim of this work was to evaluate the impact of different dairy management and production systems on carbon stable isotopes ratio ($\delta^{13}C$) and milk urea content. The samples of raw milk were collected each two weeks at certified organic high-input and low-input farms, conventional high-input and low-input farms in late indoor period and outdoor period. Data analysis showed clear difference between milk from organic high- and low-input farms with non-overlapping range between -22.90 ‰ and -24.70‰ for $\delta^{13}C$ in protein fraction (equal 1.80‰) and between -25.90‰ and -28.20‰ (equal 2.30‰) for $\delta^{13}C$ in fat fraction independently from season factor, as for $\Delta\delta^{13}C$ (protein-fat) values in milk from high-input (1.50-3.00‰) and low-input (3.20-6.30‰) organic farms. Analysis of correlation between $\delta^{13}C$ in protein fraction and milk urea content values showed that during late indoor period the most significant difference was detected between milk from organic low-input and conventional high-input farms (5.85‰ for $\delta^{13}C$ in protein fraction and 4.65 mg/100 g of milk urea content). During outdoor period, the non-overlapping range was established for low-input and high-input organic farms (3.40‰ for $\delta^{13}C$ in protein fraction and 10.77 mg/100 g of milk urea content). Results of $\delta^{13}C$ values in fat and protein milk fractions, as combination of $\delta^{13}C$ in protein fraction and milk urea content could be a potential tool for the distinguish of milk from different farming types, based on different feed composition.

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Introduction

Organic product market has been growing rapidly during the last decade (Willer and Lernoud, 2016). Particularly, in 2013 the global organic dairy market grew up by 22.6% compared to 2007 and amounted $7.672 million. In 2015, in the UK, sales of organic dairy products occupied 7.5% of total sales volume of dairy products (OMSCo, 2015). This can be explained by decreasing of trust to conventional food through contamination incidents, as well as the idea of more health benefits of organic products. Due to higher cost of organic dairy products compared to conventional, the confirmation of organic dairy authenticity is becoming an important aspect of consumer protection.

According to the European certification of the organic dairy management, the hallmarks of organic dairy farming is the account of acceptability of animal breed for chosen region, the selection of feeding in order to maximize coverage of its needs for maintenance, growing and lactation, using only organic feed, which is produced mostly in the same region, providing as much as possible cattle access to land and pasture (Council Regulation (EC) № 834/2007; Commission Regulation (EC) № 889/2008).

Besides, dairy farms could be divided on low-input (LI) and high-input (HI) production systems depending on feeding ration and regime. The LI production system, which is usually more suitable for small farms, involves grazing pastures in summer and hay in winter, minimal using of concentrates and lack of clover and maize silage. In contrast, the HI production system, which is usually more suitable for big farms, involves more extensive feeding by silage, concentrates, a small part of fresh grass during a year.

The influence of farming type (conventional or organic) on various quality characteristics of milk, including its authenticity have been in the focus of studies over the last decade. The results of physical and chemical parameters obtained by various research groups have shown the difference between organic and conventional milk (Kouřimská et al., 2014, Shröder et al., 2011; Kuhnen et al., 2015; Adler and Steinshamn, 2009; Butler et al.,2008), as there absence (Petrov et al., 2016; Olivo et al., 2005; Croissant et al., 2007).

However, it was shown that organic farming type increases the content of ω-3 fatty acids, α-linolenic acid, α-tocopherol and Fe in milk compared to conventional (Średnicka-Tober et al., 2016). It was found that both organic and conventional low-input production systems differed from high-input by a higher content of polyunsaturated fatty acids (PUFA) and antioxidants (Butler et al., 2008). Organic and conventional low-input milk differed from each other only in the second half of grazing period by α-linolenic acid and linoleic acid conjugates (CLA) content (Butler et al., 2008). The content of omega-3 fatty acids could be a marker for differentiation organic and conventional milk, and the marker for intensification level could be CLA and vaccenic acid content (Kushe et al., 2014).

The study of the carbon stable isotopes ratio $^{13}C/^{12}C$ ($\delta^{13}C$) have a potential for authentication of cattle diet and consequently, production system. The $\delta^{13}C$ values in protein fraction obtained from organic and conventional milk (in Germany) have shown a clear year-round difference between the two kinds of milk with no overlap: from −27.03‰ to −23.75‰ in organic milk and from −23.29‰ to −21.16‰ in conventional. It was proposed that value of −23.5‰ could be a derived point for the authentication of organic milk (Molkentin and Giesemann, 2010). The study in Ukraine has shown that the average values $\delta^{13}C$ of protein fraction in organic milk (-23.05‰) were significantly lower than in conventional (-19.15‰) (Petrov et al., 2016).

The variation of $\delta^{13}C$ fat values were from −30.33‰ to −26.71‰ in organic and −26.60‰ to −23.25‰ in conventional milk where feeding was based on corn silage and concentrates (Molkentin and Giesemann, 2010; Molkentin and Giesemann, 2007). It should be mentioned, that $\delta^{13}C$ threshold of -26.5‰, which could be applicable to German organic dairy was not spread for products from other countries (Molkentin, 2013). Previous study in Ukraine has shown that the average values of $\delta^{13}C$ fat in organic milk (-26.00‰) were significantly lower than in conventional (-23.14‰) (Petrov et al., 2016), that could be explained by a different amount of maize in cattle diet.

Investigation of Korean retail organic milk in contrast to European had higher $\delta^{13}C$ values (-22.39‰) than conventional (-23.60‰) because of addition plant of C_4-photosynthesis type in cow's diet, but, possibly, not of corn, due to a high price for this feed in this country (Chung et al., 2014). It should be mentioned that measurement were conducted on whole milk samples.

Thus, geographical and climatic conditions have critical impact on the cattle ration and $\delta^{13}C$ values in milk. However, the aspect of influence of production system type (low- or high-input) on this parameter has not been studied.

Cattle diet and feeding mode are one of the most significant factors influencing the urea content in milk. This parameter is an important indicator of utilization of dietary nitrogen by cow (Biswajit Roy et al., 2011), because unbalanced diet can lead to a lack of limiting amino acids and the oversupply of dietary protein, which is deaminated in the cow's liver. This process leads to an increase of urea plasma and milk urea (NRC, 2001; Spek et al., 2013). Thus, different approaches to cows' feeding on different types of farms could determine the urea content in milk.

The aim of this work was to evaluate the impact of different dairy management and production systems (organic and conventional high-input and low-input) on the carbon stable isotopes ratio ($\delta^{13}C$) and urea content in milk.

Materials and Methods

Sample Collection

The samples of raw milk were collected each two weeks at farms of 4 types (14 samples from each): certified organic high-input farm (ORG-HI) in Zhytomyr region of Ukraine and certified organic low-input farm (ORG-LI) in Chernihiv region of Ukraine, conventional high-input farm (CONV-HI) and conventional low-input farm (CONV-LI) in Kyiv region in March – April (late indoor period) and May – September (outdoor period) of 2016.

Data on cattle diet characteristics, dry matter intake (DMI) were obtained from farm records and collected by questioning farmers.

Analysis of Milk Samples

The milk samples were analysed for isotope ratio $^{13}C/^{12}C$ separately in fat and protein fraction of milk. Milk protein fraction was obtained by centrifugation of milk samples at 8000 rpm during 30 min, followed by washing with petroleum ether. The fat fraction was obtained by extraction with organic solvents according to ISO 14156: 2001.

Analysis of stable isotope ratios of carbon was performed with isotope-ratio mass spectrometer МИ-1201СГ (NPO "Electron", Ukraine), according to Gerstenberg and Herrman, 1983. Isotope ratio is given in ‰ on a δ scale and was calculated as follows:

$$\delta = \frac{R1-R2}{R2} \times 1000‰$$

Where are C – Carbon, R1 – the ratio $^{13}C/^{12}C$ in the sample, R2 – the ratio $^{13}C/^{12}C$ in the internal standard PEF-1.

The isotope ratios ($\delta^{13}C$) were measured by the international standard PEF-1 and converted to the international standard VPDB.

The content of milk urea was measured according to "Manual of methods of analysis of foods" (with modifications) (Milk and milk products, 2015) on spectrophotometer "Unico S2100" (USA).

Briefly, 5 ml aliquots of working standard solutions of urea (5 mg/ml) was used in 25 ml test tubes and added 5 ml DMAB solution (1.6 g of p-Dimethyl amino benzaldehyde was dissolved in 100 ml ethyl ethanol and added 10 ml concentrate HCl) to each for the generation of standard curve. We prepared reagent blank by mixing 2.5 ml of 7.0 pH buffer (3.403 g anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) and 4.355 g anhydrous dipotassiummonohydrogen orthophosphate (K_2HPO_4) were dissolved separately in 100 ml of distilled water and mixed in 1 liter of distilled water) and 2.5 ml of trichloroacetic acid (TCA) (24%) and then added 5 ml of DMAB solution. Tubes were shaken thoroughly and let stand for 10 minutes.

Milk sample (10 ml) was mixed with 10 ml of TCA (24%) to precipitate the proteins, centrifuged samples at 8 000 rpm during 30 min and after that filtered using filter paper. Then 5 ml of filtrate was treated with 5 ml of DMAB reagent to develop the colour. The optical density of the yellow colour was measured at 420 nm. The amount of milk urea content was calculated from standard curve.

Statistical Analysis

The data were characterized using the mean and the standard deviation (SD). The impact of the "farming type" and "production system" was evaluated using one-way analysis of variance (ANOVA) in MS Excel 2010.

Results and Discussion

Diet analysis showed that feed composition in late indoor period differed outdoor period for all the farms (Table 1). Cattle diet on organic farms mostly consisted of fresh grass and forage (such as cereals and legumes), and the percentage of corn silage was less than on conventional farms. Plants, which were used for feed, were distinguished by their type of photosynthesis. Legumes and many cereals, which form the basis of forage and pasture grass in the cattle diet belong to plants of the C_3-photosynthesis type, and corn, which was base for silage or fresh fed, as well as sorghum, millet, plantain, which were on the pasture belong to plants of C_4- photosynthesis type. Accordingly, these plants were differed in isotopic carbon profile: C_3-type plants have lower values $\delta^{13}C$ – from -30‰ to -23‰, and C_4-type plant –higher, from -14‰ to -12‰ (Camin et al., 2016; Molkentin and Giesemann, 2007). Differences in the $\delta^{13}C$ values of feed are also affected on isotopic carbon profile of milk fat and protein. The values of $\delta^{13}C$ positively correlated with an increasing of corn percentage in the diet and decreased with an increasing the percent of hay or fresh herbs in the ration (Camin et al., 2008; Bontempo et al., 2012; Kaffarnik et al., 2014).

In addition, it should be noted that the analysis accuracy was influenced by the purity of the extracted fat and protein fractions (Molkentin and Giesemann, 2007).

Also, the influence of mass fraction of protein and fat in milk on the values and accuracy of $\delta^{13}C$ in fat and protein fractions remains unexplored. While there is an assumption that the percent of plant with C_4-photosynthesis type in the cattle diet have a more pronounced effect on the fat fraction (Molkentin and Giesemann, 2010), however, the effect on protein fraction could also be essential (Petrov et al., 2016).

The results of stable isotope ratio $^{13}C/^{12}C$ study in the fat fraction ($\delta^{13}C_{fat}$) and protein fraction ($\delta^{13}C_{protein}$) of milk are presented in Table 2.

The comparing of $\delta^{13}C$ values in milk from organic and conventional farms with different production systems found variations depending on production system, late indoor and outdoor period, fat and protein fractions.

The range of fluctuation in $\delta^{13}C_{protein}$ values were less than difference in $\delta^{13}C_{fat}$ at all farms, which were under investigation. Also, seasonal difference of $\delta^{13}C_{protein}$ absolute values were detected in milk from all the farms. It was estimated that the values of $\delta^{13}C_{protein}$ in milk from ORG-HI farm were not below than -22.90‰, and values in milk from ORG-LI farm – were not higher than -24.70‰ (not overlapping range amounted 1.80‰) (Table 2). In outdoor period the ORG-HI farm diet has changed and corn silage occupied a 2-fold greater percentage in DMI, compared to late indoor period, which reflected on increasing of $\delta^{13}C_{protein}$ values by 0.77‰. The use of surplus silage during outdoor period is explained by climatic conditions in some regions of Ukraine – hot dry summer (more than +30°C) leads to a lack of fresh grass.

During outdoor period the feeding on the ORG-LI farm consisted only of fresh grass and it reflected on the $\delta^{13}C_{protein}$ value, which decreased by 1.77‰, compared to late indoor period.

Table 1 Feed composition at farms of different types

Feed, %DMI	ORG-HI	CONV-HI	ORG-LI	CONV-LI
Late indoor period				
Forage	71.26	41.91	100	46.14
Corn silage	9.09	52.76	0	17.1
Concentrates	19.65	5.33	0	31.96
Other feed	0	0	0	4.8
Outdoor period				
Pasture, fresh-cut grass	34.95	69.40	100	51.64
Forage	45.81	0	0	0
Corn silage	18.85	20.38	0	0
Concentrates	0.39	10.22	0	48.36

Table 2 Stable isotope ratio $^{13}C/^{12}C$ in milk

Parameter,‰		ORG-HI		CONV-HI		ORG-LI		CONV-LI	
		LIP	OP	LIP	OP	LIP	OP	LIP	OP
$\delta^{13}C_{protein}$	Mean	-22.40[a]	-21.63[c]	-18.56[a]	-24.60[c]	-24.93[a]	-26.70[c]	-22.04[a]	-24.10[c]
	Min	-22.90	-22.50	-18.85	-24.15	-25.40	-27.30	-22.70	-25.60
	Max	-21.60	-20.80	-18.00	-25.05	-24.70	-25.90	-21.40	-23.00
	SD	0.92	1.20	0.53	0.64	0.35	0.99	0.92	1.84
$\delta^{13}C_{fat}$	Mean	-24.73[b]	-23.73[c]	-21.51[b]	-28.30[c]	-29.33[b]	-31.94[c]	-25.32[b]	-28.07[c]
	Min	-25.90	-24.70	-22.60	-27.55	-30.10	-32.9	-26.20	-30.70
	Max	-24.00	-22.40	-20.42	-29.05	-28.20	-30.5	-24.50	-25.20
	SD	1.34	1.62	1.54	1.06	0.42	1.70	1.20	1.80
$\Delta\delta^{13}C$ ($\delta^{13}C_{protein}$- $\delta^{13}C_{fat}$)	Mean	2.33[b]	2.10[b]	2.95[b]	3.40[b]	4.40[b]	5.24[b]	3.28[b]	3.97[b]
	Min	1.60	1.50	2.36	3.10	3.90	3.20	2.50	2.20
	Max	3.00	3.00	3.58	3.70	5.00	6.30	4.00	5.10
	SD	0.70	0.53	0.86	0.30	0.56	1.25	1.06	2.05

LIP: Late indoor period, OP: Outdoor period, SD – standard deviation, [a]significance P=0.05, [b] significance P=0.001, [c] significance P=0.0001

Variation of this parameter in milk from CONV-HI farm were much higher, than on other farms. During late indoor period values of $\delta^{13}C_{protein}$ were not below than -18.85‰ and during outdoor period were not higher than -24.15‰ ($\delta^{13}C_{protein}$ difference was 5.30‰), which could be explained by decreasing of corn silage percent in ration (from 52.76%DMI to 20.38%DMI) and changing in other diet components.

Values of $\delta^{13}C_{protein}$ in milk from CONV-LI fluctuated from -25.60‰ to -21.40‰ during both late indoor and outdoor periods. The average $\delta^{13}C_{protein}$ difference between two periods drew up 2.06‰.

It has been showed that absolute values of $\delta^{13}C_{fat}$ were lower than $\delta^{13}C_{protein}$ values in all farms types (Table 2).

It has been showed that $\delta^{13}C_{fat}$ values in milk from ORG-HI farm were not below -25.90‰ and milk from ORG-LI farm were not higher than -28.20‰ in both periods (not overlapping range amounted 2.30‰). The difference between late indoor and outdoor periods for $\delta^{13}C_{fat}$ values were +1.00‰ and -2.67‰ for milk from ORG-HI and ORG-LI farms respectively.

In milk from CONV-HI farm the highest values of the $\delta^{13}C_{fat}$ were observed. During late indoor period it was not below than -20.42‰ because of differences in diet, high percent of corn silage. During outdoor period, milk $\delta^{13}C_{fat}$ values from CONV-HI farm showed values from -29.05‰ to -27.55‰ which overlap with values of milk from CONV-LI from -30.70‰ to -25.20‰. The difference between late indoor and outdoor periods for $\delta^{13}C_{fat}$ values were -6.79‰ and -2.75‰ for milk from CONV-HI and CONV-LI farms respectively.

Correlative analysis detected positive correlation between $\delta^{13}C_{protein}$ and $\delta^{13}C_{fat}$ values in milk from all the farms: ORG-HI (r = 0.95), CONV-HI (r = 0.85), ORG-LI (r = 0.88) and CONV-LI (r = 0.92).

The values of $\delta^{13}C$ in protein and fat fractions of milk from farms with HI-production system indicated the high percentage of C_4- photosynthesis type plants in cow diet. At the same time, the $\delta^{13}C$ values of both fractions from low-input farms could be explained by small percentage of such plants or their absence in ration. Thus, these results complied with data about the positive correlation between percent of corn silage and the values of $\delta^{13}C$ (Camin et al., 2008).

Values of $\Delta\delta^{13}C$ also depended on the farming types. It was found that $\Delta\delta^{13}C$ values in milk from low-input farms were higher in comparison with the high-input (Table 2). The range of values in milk from ORG-LI farm was equal to 3.20 - 6.30‰ and in milk from CONV-LI farm – 2.20-5.10‰. At the same time, the $\Delta\delta^{13}C$ values in milk from ORG-HI and CONV-HI farms were lower – 1.50-3.00‰ and 2.36-3.70‰ respectively (Table 2).

Difference in $\Delta\delta^{13}C$ values range between high- and low-input farms could be explained by different percentage of degradable diet protein by cows, which was derived from the plant with C_4-photosynthesis type.

Corn (plant with C_4-photosynthesis type) is characterized by higher percentage of rumen undegradable protein (RUP) (35.3% in corn silage and 47.3% corn grains) compared to fresh grass (25.5% RUP) and hay (30.5% RUP), which belong to plants with C_3-photosynthesis type (NRC, 2001). Rumen degradable protein (RDP) of feed breaks up to ammonia due enzyme fermentation by rumen microorganisms, which could change $^{13}C_{protein}$ values. After that, this ammonia may be used for synthesis of microbial protein by rumen microflora, which will be assimilating by cow's body. At the same time, rumen undegradable protein (RUP) enters to small intestine, where it will be digested by ferments to individual amino acids, that does not impact $^{13}C_{protein}$ values. Thus, possibly, at high-input farm with a high percentage of corn in the cattle diet, percent of common assimilated protein with a higher $\delta^{13}C$ values will be much higher. Consequently, it effects on isotopic profile of milk protein fraction.

The fact that the $\Delta\delta^{13}C_{(protein-fat)}$ values did not overlap in milk from ORG-HI and ORG-LI farms independently from season factor, could be used as potential indicator of milk from organic low-input production system.

It was found that milk urea content (MUC) differed significantly (P=0.05) in milk samples, depending on farming type and production system (Table 3).

In late indoor period, values of MUC in high-input farms were lower than in low-input. In outdoor period, absolute MUC values increased on all the farms, except of ORG-HI farm, compared to late indoor period. Also, values at ORG-LI farm were the highest in this period.

According to obtained results cattle feeding by total mixed ration, as on high-input farms, leads to decreasing of MUC values and feeding mostly by forage or grass, as on low-input farms, leads to increasing of this parameter.

Table 3 Milk urea content in different farming types

Type of farm	Milk urea content, mg/100 g	
	LIP	OP
ORG-HI	20.60±1.01[a]	20.28±1.48[a]
CONV-HI	18.66±1.65[a]	26.01±3.03[a]
ORG-LI	26.24±1.06[a]	37.69±4.83[a]
CONV-LI	25.10±0.54[a]	28.35±3.28[a]

LIP: Late indoor period, OP: Outdoor period, Data are represented as Means ± SD. [a]significance P=0.05

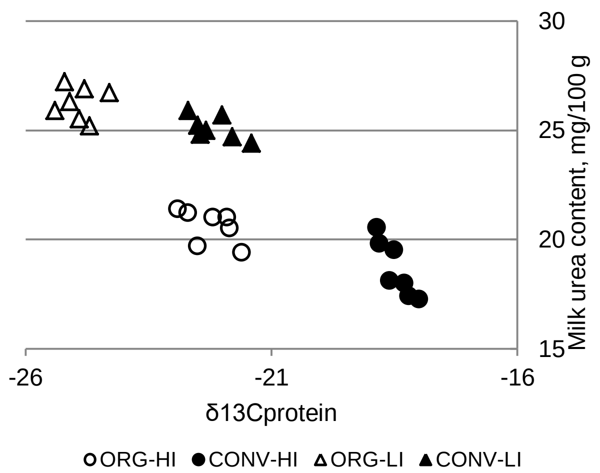

Fig.1 Correlation between $^{\delta 13}C_{protein}$ and milk urea content at late indoor period

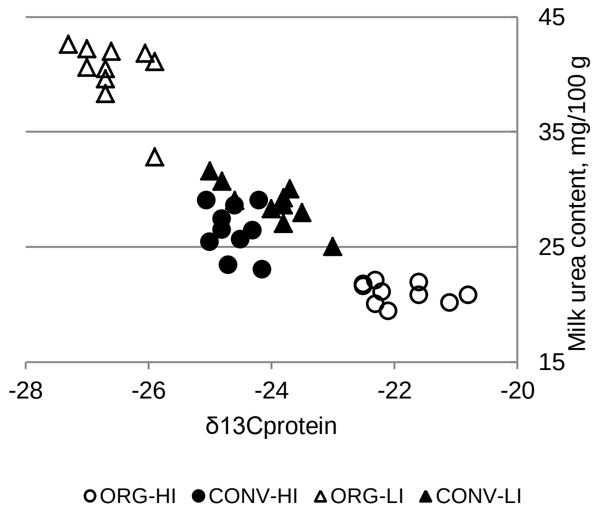

Fig.2 Correlation between $^{\delta 13}C_{protein}$ and milk urea content at outdoor period

It could be explained by the fact that feeding only by grass and forage compared to total mixed ration using, leads to protein- and carbohydrate-rich feed divided intake to a rumen. This causes rapid degradation of proteins in rumen and consequently increases the concentration of urea nitrogen in plasma and milk urea content which agrees with relevant studies (Ikuta et al., 2005; Geerts et al., 2004). In its turn, lack of energy for rumen microflora maintain caused by the absence of concentrates also leads to rumen nitrogen imbalance.

Also, seasonal changes of MUC values could be explained by difference in cattle feeding. The increasing of the percent of fresh grass during outdoor period at ORG-LI, CONV-HI and CONV-LI farms leads to higher values of MUC. However, the minor changes of this parameter in milk from ORG-HI farm depend on using the mostly stable ration during both periods.

After analysing the data, it has been suggested the existence of relationship between values in $\delta^{13}C_{protein}$ and milk urea content, that can be explained by the percentage of plants with C_4-photosynthesis type in the cattle diet. It has been shown negative correlation between $\delta^{13}C_{protein}$ and milk urea content in milk from all farms: ORG-HI (r=-0.91), CONV-HI (r=-0.85) (Fig.4), ORG-LI (r=-0.78) and CONV-LI (r=-0.89) (Fig.1.2). Possibly, this correlation could also be explained by the fact, that carbonyl group of urea enriched by heavy isotope ^{13}C, while a protein fraction enriched by a lighter isotope ^{12}C.

The most significant difference in $\delta^{13}C_{protein}$ and milk urea content during late indoor period was recorded for milk from ORG-LI and CONV-HI farms. The non-overlapping range between them was from -18.85‰ to -24.70‰ for $\delta^{13}C_{protein}$ values and between 20.55 mg/100 g and 25.20 mg/100 g of MUC (Fig.1).

At the same time, milk from CONV-LI and ORG-HI had a similar range of isotopes, while the average level of urea was lower by 21.49% on the ORG-HI farm than on CONV-LI farm. Possibly, variation of MUC depends on ratio of concentrates to forage (Table 1).

During outdoor period the most significant difference in $\delta^{13}C_{protein}$ and milk urea content was recorded for milk from ORG-LI and ORG-HI farms. The non-overlapping range between them was from -22.50‰ to -25.90‰ for $\delta^{13}C_{protein}$ values and between 22.03 mg/100 g and 32.80 mg/100 g of MUC (Fig.1).

The values in milk from both conventional farms overlapped, which could be explained by similar percent of forage and fresh grass (Table 1).

Thus, such approach could be a potential tool for distinguishing milk from farms with different production systems. Analysis of these parameters could be important for authentication of milk from farms certified by more strict organic standards. For example, standard BioSuisse (Switzerland) provides for mandatory pasture grazing and minimum use of concentrates.

Conclusions

It has been shown clear difference between milk from organic high- and low-input farms with non-overlapping range equal 1.80‰ in $\delta^{13}C_{protein}$ and 2.30‰ in $\delta^{13}C_{fat}$. Also, non-overlapping range $\Delta\delta^{13}C$ in milk from ORG-HI (1.50-3.00‰) and ORG-LI (3.20-6.30‰) farms was detected independently from season factor.

It was found that under the decreasing percent of corn silage (from 52.76% to 20.38% DMI) the values of $\delta^{13}C_{protein}$ and $\delta^{13}C_{fat}$ could decrease down to 5.30‰ and 6.79‰ respectively (as on CONV-HI farm).

Analysis of correlation between $\delta^{13}C_{protein}$ and milk urea content values showed that during late indoor period the most significant difference was detected between milk from ORG-LI and CONV-HI farms (5.85‰ for $\delta^{13}C_{protein}$ and 4.65 mg/100 g of MUC). During outdoor period, the non-overlapping range was established for ORG-LI and ORG-HI farms (3.40‰ for $\delta^{13}C_{protein}$ and 10.77 mg/100 g of MUC). These results could be explained by different production types and, consequently, different percent of corn silage and forage in feed. Thus, non-use of corn silage and concentrates reflects on $\delta^{13}C_{protein}$ and MUC values, which could be used as a potential indicator of milk from organic low-input production system.

Further development in this direction is necessary for searching the authentication criteria, but geographical and climatic conditions of region and the production system type must be taken into account.

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