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INTERSPECIES AND SEASONAL DIFFERENCES OF RETINOL IN DAIRY RUMINANT´S MILK

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ABSTRACT

Milk is an essential source of macronutrients and among lipophilic vitamins is significant source of retinol. The contribution of milk to the reference daily intake for retinol varies from 11% to 16%, worldwide. The most consumed dairy products are fresh, dehydrated and condensed milk in which the amonuts of retinol are not modified to those of in whole milk. Retinol is essential to ensure a good functionality of the immune system and plays a critical role in vision, reproduction, cell differentiation as well as growth and development and is found only in animal tissues. The aim of our study was to evaluate the interspecies differences in the retinol concentration of whole raw bovine, caprine and ovine milk and to observe seasonal variation of retinol in bulk tank milk samples. Samples of raw milk were colleceted on different farms in the Czech Republic between 2013 and 2014. Retinol was measured by ultra high performance liquid chromatography with UV detection (325 nm) in isocratic mode after alkaline saponification with methanolic potassium hydroxide solution and liquid-liquid extraction into non polar organic solvent of whole raw milk. To avoid vitamin losses or degradation during the procedure, antioxidants were added to the sample extraction media. Our results indicate significant interspecies differences between bovine and ovine milk and caprine and ovine milk. Concentration of retinol is very similar in bovine and caprine milk 0.96 ±0.11 mg/L, 0.94 ±0.25 mg/L, respectively. The mean concentration in sheep´s milk is 1.75 ±0.24 mg/L. The seasonal variation of retinol in raw bovine milk was detected as high significant, with the highest concentration during winter. These results contribute to the nutrition evaluation of milk in the Czech Republic and indicate, that the sheep´s milk is the best source of retinol among the milks of ruminants kept in the Czech Republic, however it is not used in its fluid form for human consumption.

Keywords: milk; retinol; season; small ruminant

INTRODUCTION

Milk, especially, appeared to occupy a unique position among the many foods, because it is the sole food for humans and all mammals during the first part of their lives. Therefore, milk contains everything, the young organism needs for growth and development including in particular a sufficient concentration of protein and minerals **(Weber, 1997)**. The valuable nutritional benefits and the higher consumer demand support the production of milk of small ruminants. Consumers are requesting more and more information concerning the nutritional composition of small ruminant´s milk.

The milk fat represents a good dietary source of vitamin A, which is essential for human **(Väänänen et al., 2000; EFSA, 2013)** and is the most quantitatively and qualitatively variable component of milk, depending on the stage of lactation, season, breed, genotype, and feeding **(Raynal-Ljutovac et al., 2008)**. The term "vitamin A" is used for a group of several molecules with the biological activity: all-*trans retinol*, 11-*cis* retinal, retinoic acid and its esters. All-*trans* retinol is considered as the main form of the vitamin and simultaneously sole component of vitamin A that occurs naturally in bovine milk **(Lidén and Erisson 2006)**. Vitamin A is found only in animal tissues and plays a significant role in vision, immunity, cellular differentiation, and embryonic development **(Maijala, 2000)**. In milk and meat, vitamin A occurs mainly as fatty acid retinyl esters, mainly retinyl palmitate, followed retinyl oleate and stearate **(Väänänen, 2000 and EFSA, 2013)**. Retinoic acids are considered as the molecular species responsible for all the functions attributed to vitamin A, with the exception of vision, where only retinal is able to exert an action **(EFSA, 2013)**. Vitamin A or precursor carotenoids with provitamin A activity are poorly soluble in water and relatively unstable during food processing and storage due to its chemical structure, which contains many double bonds susceptible of degradation. Further the stability of vitamin A is also affected by acidity and at pH below 5.0 this compound is more easily destroyed. Trace metals (especially iron and copper) and ultraviolet light accelerate the degradation of vitamin A **(Andrés et al., 2014)**.

The vitamin A deficiency is a major public health nutrition problem **(Klemm et al., 2010)**. Deficiencies of vitamin A caused by malnutrition include chronic illnesses, liver disease, and fat malabsorption conditions such as celiac disease and cystic fibrosis **(Maijala, 2000)**. Vitamin A deficiency can be severe and results in nyctalopia (night blindness), xerophthalmia (conjunctival dryness with keratinization of the epithelium), keratomalacia (ulceration and necrosis of the cornea), poor resistance to infection and abnormal epithelial morphology **(Weber et al., 1997)**. In the year 2005 suffered by xerophtalamia 5.2 million pre school children and 9.8 million pregnant women **(WHO, 2009)**. The recommended dietary allowances depend on life stage group. In accordance with food and nutrition board are the smallest doses for children and the highest for women in lactation. Daily reference intake according to Regulation (EU) No 1169/2011 on the provision of food information to consumers is for retinol 0.8 mg. The contribution of milk to the reference daily intake for retinol varies from 11% to 16%, worldwide. However retinol is removed with the fat during the separation process thus making it a requirement to replenish the lost vitamin in the form of a vitamin premix in skim and partially skimmed milks. In some countries (i.e. Canada) vitamin A is added into skim and partiallly skimmed milks is mandatory in level 0.7/100 ml **(CFIA, 2013)**. Fresh, dehydrated and condensed milk represent approximately 46% of world dairy products and the amounts of vitamin A are not modified compared to those in whole milk **(Sauvant et al., 2003)**.

A number of spectroscopic and separation methods are used for the determination of vitamin A in food. At presence, the technique of choice is high performace liquid chromatography (HPLC). The normal-phase and reversed-phase HPLC conditions coupled to UV and fluorescence detection has been widely used, as well as the mass spectrometry detection system. Recently ultra-high performance liquid chromatography (UHPLC) has emerged as a liquid chromatographic technique offering much rapid and efficient separation **(López-Cervantes J. et al., 2006)**.

In our study we investigated how is the retinol concentration influenced by the season and observed the differences between the bovine, caprine and ovine milk. These results are needed for nutritional evaluation of milk from different ruminant´s species.

MATERIAL AND METHODOLOGY

Samples of farm milk in the number of 155 were collected and analysed during 2013-2014. The breed representation on the dairy farms was Holstein Friesian cattle and their cross-breeds, Czech Fleckvieh cattle and their cross- breeds, Jersey and Montbeliarde. From the goats were included white short-haired goats and from sheep different breeds – Lacaune, Cigaja and East-Frisien sheep. The feeding management on the farm was balanced during whole year.

Whole raw milk samples were kept frozen prior to the analysis. The samples were homogenized before processing and analysed in duplicate.

Chemicals used for extraction and HPLC analyses were methanol, acetonitril and n-hexane, all HPLC gradient grade (Merck, Germany), sodium-1-heptane sulfonate (Sigma-Aldrich, Germany), potassium hydroxide, hydroquinone, ascorbic acid, sodium sulphate anhydrous, hydrochloric acid all in p.a. purity (Penta, Czech Republic). Vitamin A–acetate concentrate 50 % (w/w) in peanut oil was purchased from Fluka (Buchs, Switzerland). The stock standard solutions of vitamin A for analyses were prepared to the concentration of approximately 500 mg/L of retinol and in methanol. Working standard solutions at concentrations 0.1 – 20 mg/L were prepared by dilution of the stock solution in methanol.

The sample preparation procedure consisted of direct saponification with methanolic potassium hydroxide and liquid-liquid extraction in n-hexane. Antioxidants hydroquinone and ascorbic acid were added to the sample. The separation of retinol was provided by the analytical reverse phase UPLC system Acquity with dual wavelength absorbance detector (Waters, USA). The chromatographic column Acquity BEH C8 (Waters, USA) was used. The analysis was performed in isocratic mode and the mobile phase consisted of methanol and water (93:7) at a flow rate of 0.4 mL/min, injection volume was 4 μL, run time 2.7 minutes. The UV detection was performed at the wavelength of 325 nm.

For statistical analysis was used Microsoft Office Excel 2010 and Unistat 6.0. The statistical significance was set at p =0.01 and p =0.05.

RESULTS AND DISCUSSION

The characteristic of small ruminant´s milk fat is its globule size. The caprine milk has higher proportion of small globules compared to cow milk. This property should support the hypothesis, that the small ruminant´s milk has higher content of retinol than bovine milk **(Raynal Ljutovac et al, 2008)**. The Table 1 shows the mean retinol concentration in milk of different ruminant´s species hold in the Czech Republic. The determined results from our study are higher in comparison with the available literature **(Debier, 2005; Raynal-Ljutovac et al., 2008; Park and Haenlein 2013; Hulshof 2006)**. This difference should depend on the method of extraction and the type of breed held in different countries. Analysis of retinol is paved with difficulties due to the labile nature of the compound, low concentration, and the need for saponification in order to remove fat and to hydrolyse retinyl esters **(Hulshof et al., 2006)**. There are various endogenous and exogenous factors, which can influence the concentration of biological active compounds, among other seasons of the year, stage of lactation, environmental factors, feed fortification and the diet during the stage of lactation and the difference in retinol precursor concentration in the animal diet and/or a difference in bioavailability of the precursors in the animal body **(Raynal-Ljutovac et al., 2008; Fedele et.al., 2004)**.

Table 1 Concentration of retinol in milk of different ruminant´s species

	Type of milk		
	Cow	Goat	Ewe
Retinol concentration (mg/L)	0.96 ±0.11*	0.94 ±0.25	1.75 ±0.24
Retinol in 1 g of milk fat (μg)	26.25	24.12	29.56
Retinol (mg/L) **	0.3 – 0.52	0.4 – 0.62	0.44 – 0.83

*mean±SD

** Data from Park and Haenlein (2013); Raynal-Ljutovac (2008); Hulshof (2006)

The type of breed is significant endogenous factor, which influences the milk fat content and subsequently the retinol concentration. The mean retinol concentrations in caprine and bovine milk are very similar ($p >0.05$). In bovine milk were the values in the range from 0.28 mg/L to 1.61 mg/L with median 0.66 mg/L and in caprine milk from 0.46 mg/L to 1.75 mg/L with median 0.85 mg/L. The comparison of the results, however, shows greater variability retinol concentration in caprine milk (0.94 ±0,25 mg/L versus 0.96 ±0,11 mg/L). The same results, significant higher concentration of retinol in ewe milk compared to goat´s milk were found by **Kondyli et al. (2012)**.

The significant difference was observed in comparison with ovine milk ($p <0.01$). The concentration in ewe milk is 0.72 – 1.96 mg/L with median 1.39 mg/L. According to **Hulshof et al., (2006)** raw milk contains on average 0.40 mg retinol per 1000g of milk. It should be hypothesised, that goat´s and ewe milk contains higher concentration of retinol in contrast to that of cow´s, because both goat and sheep milk are lacking β-carotene, which is entirely converted into retinol **(Raynal-Ljutovac et al., 2008)**. This fact is not practically observed, because the concentration of retinol expressed for 1 gram of fat is not significant between ruminant´s species. This reason should be in retention of retinol in the liquid and semi/liquid dairy products, which is above 80% **(Hulshof et al., 2006)** and small proportion of the retinol is associated with whey proteins and/or concentrated in the milk fat globule membrane **(Revilla et al., 2014)**, which is not cleaved into the fat extract. Very important factor is the farming system. Two major systems of small ruminant farming are pasture and indoor systems. Between these two farming systems is a wide scope of mixed systems such as summer pasture/winter indoors or alternatively indoors/outdoors subject to climatic differences **(Morand-Ferhr et al., 2007)**. According to the study **Fedele et al., (2004),** is found the higher concentration (often significantly) in goat ´s milk from goats reared on pasture system than on indoors farming system. The concentration on pasture was observed 650.5 ±133.9 μg/100 DM and in indoor system, the concentratrion was 498.6 ±49.9 μg/100 DM. The green herbage increased the total retinol content by 20% (**Fedele et al., 2004)**.

The seasonal variation in cow´s farm milk samples (Figure 1) was observed at significance $p <0.01$. However, the statistical difference was found between winter and the other time of year. The mean concentration in winter was 1.01 mg/L and the lowest was observed in autumn 0.77 mg/L. **Hulshof et al. (2006)** investigated the seasonal

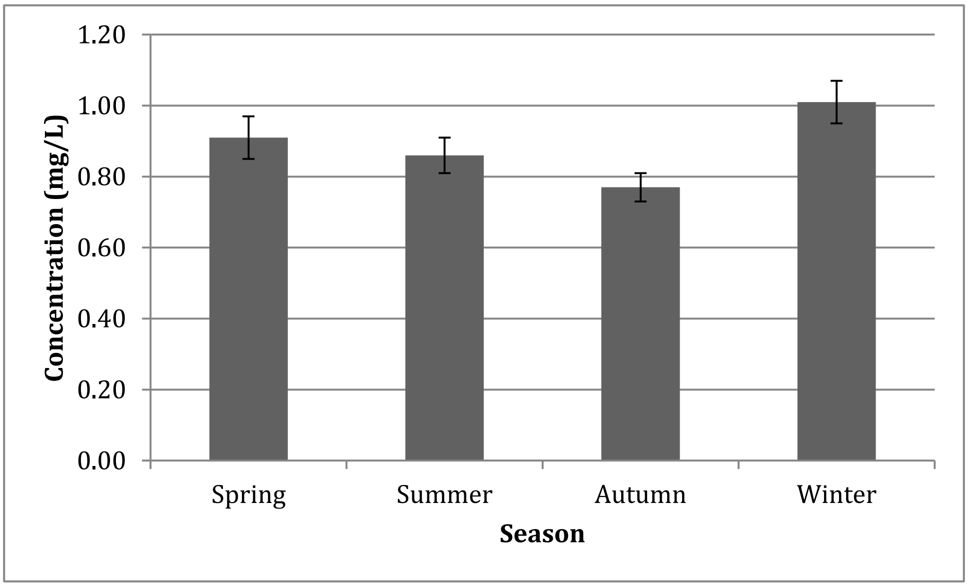

Figure 1. The seasonal variation of retinol in cow´s farm milk (mean ±SD).

variation in retinol content in Dutch milk. They observed opposite results than in our study. Winter milk contained 20% less retinol compared to summer milk. The mean concentration per g fat was 9.2 µg and was dependent of the fat content. Seasonal differences in animal feeding practices may be the main cause for these differences in nutrient content. During winter and spring cows stay in stables and are mainly fed in silage. The highest levels are normally found during spring and summer, when the cows are fed on fresh vitamin rich pasture **(Sauvant et al., 2002)**. This feeding management is not so used in the Czech Republic. The breeders are trying to compile the diet balanced during the year, but the dairy cows are often in the stable whole year and on the pasture are approximately half of the farms from the May to September.

CONCLUSION

Based on our results we can conclud that milk is good source of vitamin A and the highest concentration was observed in the ewe milk, respectively sheep´s cheeses, where its content is increasing during the processing. From the perspective of the consumer our research shows an important finding that the concentration of vitamin A in farm cow's milk, which the consumer buys in milk vending machine, is high throughout the year and against expectations is rising during winter. This finding also applies to milk purchased for processing dairies. From the nutrition point of view the whole bovine milk contains approximately 192 µg of vitamin A per glass (200 mL), which provides about 24% of the daily vitamin A requirement.

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