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Quantitation of vitamins A and E in raw sheep milk during lactation period

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Abstract. In this article, the influence of breed and lactation stage on vitamin A and E content in raw sheep milk was studied. The milk of the East Friesian, Romanov and Lacaune sheep breeds was included in the study. The samples were taken once a month throughout lactation. The total average content of vitamin A in raw milk of all sheep breeds during lactation was 0.76 ± 0.19 mg kg^{-1} of milk and the total average content of vitamin E was 2.86 ± 0.99 mg kg^{1} of milk. The content of vitamin A and E during lactation varied in respect to the breed type. The highest average content of vitamin A and E was detected in the Romanov sheep:1.01 ± 0.19 mg kg^{-1} of milk for vitamin A and 4.26 ± 1.90 mg kg^{-1} for vitamin E. The lowest average value of vitamin A was found in the milk of the East Friesian sheep (0.56 ± 0.10 mg kg^{-1}). This milk showed also the lowest content of vitamin E 2.11 ± 0.53 mg kg^{-1}. The highest content of vitamins was found in summer and at the end of lactation, which is in September, and the lowest values appeared in early lactation, which is in April.

Key words: Lactation, sheep milk, sheep breed, vitamin A, vitamin E.

INTRODUCTION

The production of sheep milk has been increasing in the Czech Republic in recent years. People are becoming more and more aware of the milk and dairy products of these small ruminants. Due to the increasingly expanding network of farmers' markets, it is much easier to get hold of these products. The East Friesian and Romanov sheep are the most widely bred breeds in the Czech Republic. The Lacaune sheep have gained popularity only recently in the Czech Republic. Sheep milk is much easier to digest than cow milk. Its fat is dispersed in smaller fat beads (Jandal, 1996). In addition, due to the better digestibility of sheep milk proteins, sheep milk is an important constituent of the diet of people suffering from allergies. Milk contains relatively low amounts of vitamin A and E; however, due to its frequent consumption in various forms, it represents an important dietary resource, taking into account the higher average fat content in sheep milk (7%) (Raynal-Ljutovac et al., 2008). Thus, a higher expression of lipophilic vitamins in sheep milk when compared to cow and goat milk is to be expected.

Vitamin A plays a role in the biochemical pathways related to visual perception, affects the growth, differentiation and maturation of gametes, and is important for fetal development, growth, and bone development (Debier et al., 2005). It plays a role in the synthesis of proteins, nucleic acids, and lipoproteins. Vitamin A is also an effective antioxidant. Vitamin deficiency is associated with vision disturbances (night blindness), inhibition of growth and deformities of bone and reproductive organs. High doses of vitamin A result in increased hepatic reserve. In pregnant women, it may have teratogenic effects (Miller et al., 1998). The recommended daily dose for an adult is in the range of 0.8 mg (2,600 IU) – 1 mg (3,300 IU) and for children from 0.4 to 0.6 mg (1,300–2,000 UI) (Capito and Calleja, 2006).

Vitamin E is a very important antioxidant. It has an important function in protecting the body against free oxygen radicals, which can lead to DNA damage. It is also a factor that slows down the ageing of the body and plays a role in the prevention of cardiovascular diseases and cancer (Eitenmiller & Junsoo, 2004). Vitamin E is present in food, being dissolved in fats, and is released and subsequently absorbed during their cleavage in the intestine. The recommended daily dose of vitamin E is from 10 to 15 mg for adults, this value is around from 5 to 8 mg for children (Monsen, 2000). Vitamin E deficiency is often associated with disorders of fat absorption or distribution or cystic fibrosis (Pekmezci, 2011).

The content of vitamins in raw milk is influenced by many factors. These include animal species, breed, stage of lactation, and individual health status. According to Zervas & Tsiplakou (2011), another important aspect is the nutrition of the animal and the specific character of farming.

There are not many studies, which focus on the investigation of vitamin content in raw sheep milk in the Czech Republic. The aim of our study was to determine the content of fat-soluble vitamins A and E in the milk of sheep from private farms in the Czech Republic and to assess the effect of lactation period on the content of these vitamins.

MATERIALS AND METHODS

Experimental material

Pooled milk samples of the East Friesian sheep (a herd of 380 head), the Romanov sheep (a herd of 130 head), and Lacaune sheep (a herd of 85 head) were collected throughout the lactation from April to September on private farms once a month, in cooperation with the Dairy Research Institute. The milk samples were taken first 20 days after parturition.

The ration of all breeds was based on full-day pasture *ad libitum*, hay, and silage. Mineral licks have been used in all cases as a dietary supplement: MILLAPFOS, BIOSAXON for the Romanov sheep, RUMIHERB, NATURMIX for the East Friesian sheep, and SANO for the Lacaune sheep. A mixture of pressed grains was also supplemented.

The chemicals. Determination of vitamins

For the preparation of the analytical samples, we used the following standards and chemicals: DL-α-tocopherol, 98.2% (CALBIOCHEM, Canada), tocopherol set (CALBIOCHEM, Canada), retinol, > 99% (Sigma-Aldrich, Germany), pyrocatechol, > 99.5% (Sigma-Aldrich, Germany), potassium hydroxide, min. 85% (Lachema, Czech Republic), methanol, p.a., content 99.5% (Lachner, Czech Republic), hexane, clean min. 95.0%, Penta, Czech Republic, methanol, super gradient, content min. 99.9% (Lachner, Czech Republic), and treated distilled water (Milipore, France).

Measurement of vitamin E and A content in milk samples

Vitamin E (or the individual tocopherols (T) and tocotrienols (TKT)) and vitamin A were determined by high performance liquid chromatography with fluorescence and spectrophotometric detection, respectively.

Approx. 1 g of homogenized sample was weighed in a plastic tube with a lid. 200 ml of methanol pyrocatechol (0.2 g ml^{-1}) was added, then 5 ml 1M KOH, and the mixture was vortexed for 20 seconds. Subsequently, the sample was saponified for 10 minutes on ultrasound. Then the mixture was vortexed again for 20 seconds. Then, 5 ml of hexane and 1 ml of distilled water were added to the mixture. The mixture was vortexed for 1 minute. Subsequently, 3 ml were taken from the upper hexane layer and evaporated on a rotary evaporator until dry. The residue was dissolved in 0.5 ml of methanol and an aliquot was transferred through a nylon filter into a 1 ml Eppendorf tube, which was placed in the freezer (-20ºC) for 30 minutes. Subsequently, the sample was centrifuged for 2 minutes (by 14.4 rpm) and drained off into a dark vial. For the analytic extension we used the chromatographic system Ultimate 3,000 (Dionex, USA), consisting of a quaternary high-pressure pump, an autosampler, a column thermostat, a fluorescence detector and a diode array detector. We employed an analytical column with a precolumn packed with Develosil 5µm RPAQUEOUS (250 × 4.6 mm); Develosil 5µm C30 UG-100A (10 × 4 mm) (Phenomenex, USA), which allows the separation of all forms of tocopherols and tocotrienols (Fig. 1).

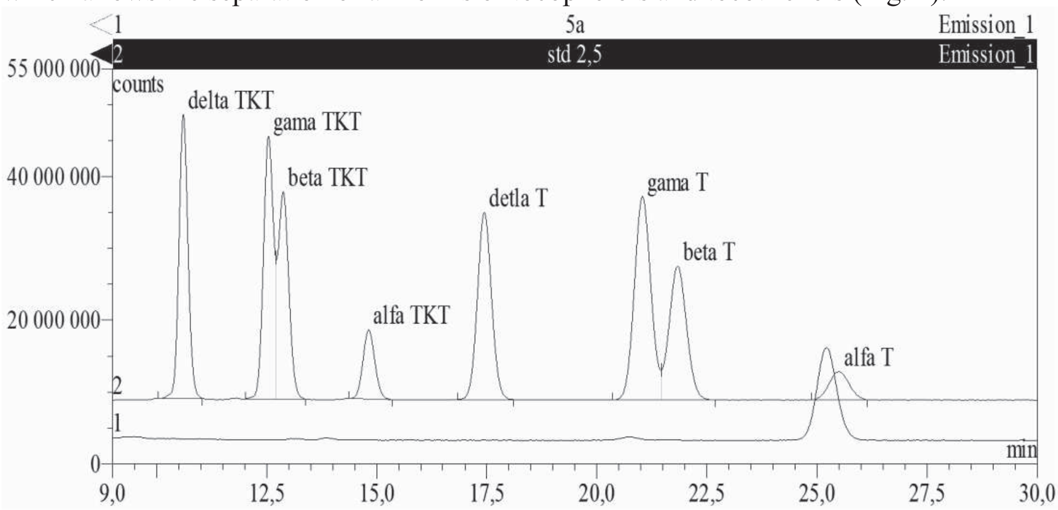

Figure 1. Chromatogram of vitamin E (compared to the standard): 2 – standard, 1 – sample.

A mixture of MeOH: deionized water (97 : 3, v/v) was used as the mobile phase; flow rate of 1 ml per minute. Column temperature was 30°C and 10 ml sample injection was applied. For the detection of tocol wavelengths we selected: excitation 292 nm and emission at 330 nm. Vitamin A was determined by spectrophotometric detection at $\lambda = 325$ nm (Fig. 2).

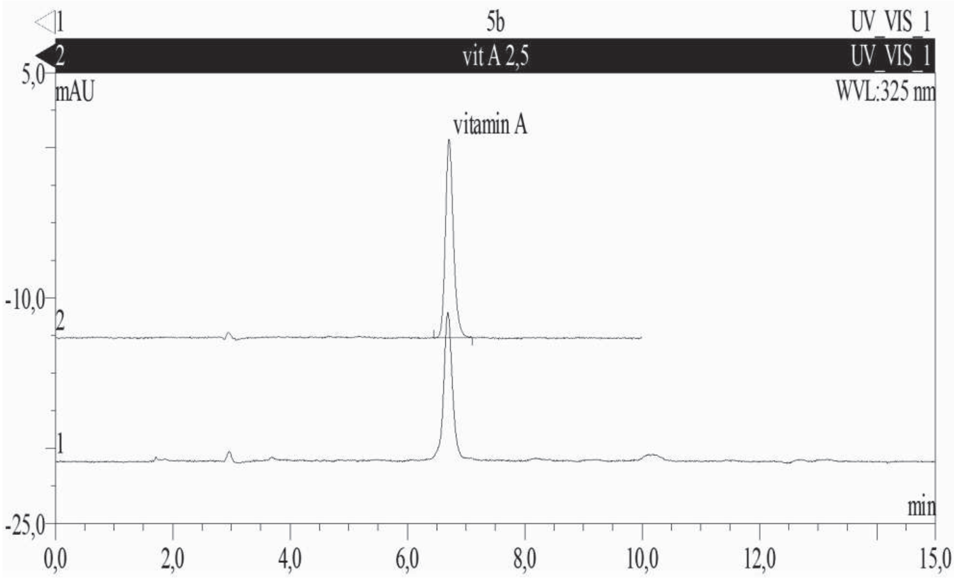

Figure 2. Chromatogram of vitamin A (compared to the standard): 2 – standard, 1 – sample.

The content of analytes in the samples was evaluated by external calibration. Calibration curve of all tocols and vitamin A was linear in the range from 0.05 to 10 µg ml^{-1}. The detection limit for each tocol, expressed as a ratio of three times the value of the signal-to-noise ratio, was as follows: δ-tocotrienols and δ-tocopherol 0.01 µg ml^{-1}, β-tocotrienol, γ-tocotrienol, β-tocopherol and γ-tocopherol 0.025 µ ml^{-1}, α-tocotrienol and α-tocopherol 0.05 µ ml^{-1}, vitamin A 0.025 µ ml^{-1}. The results were processed with Chromeleon and MS Excel. The results were expressed as m kg^{-1} of milk. Statistical analysis was done in *Statistica* Version 9. The measured values were processed by the analysis of variance method (*ANOVA*), using *post-hoc Tukey's* tests for more detailed evaluation.

RESULTS AND DISCUSSION

The content of vitamin A and E was detected in raw milk of 3 sheep breeds – the East Friesian, the Romanov and the Lacaune. The average values of vitamin A and E in the milk samples of individual breeds are presented in Table 1. The total average content of vitamin A in raw milk of all sheep breeds during lactation was 0.7–0.1 m kg^{-1} of milk and the total average content of vitamin E was 2.8–0.9 m kg^{-1}. The correlation between the content of vitamin A and vitamin E was found ($R^2 = 0.62$, $P < 0.05$).

The results show significant differences in the content of vitamins A and E in relation to the breed of sheep. The highest ($P < 0.05$) average content of both, vitamins A and E, was found in the milk of the Romanov sheep (1.01 ± 0.19 mg kg^{-1} and 4.26 mg $kg^{-1} \pm 1.90$, respectively) when compared with two other breeds. The average content of vitamin E in the milk of the Lacaune sheep vs. milk of the East Friesian breed was not statistically different while the difference between these two breeds was found for vitamin A in milk (Table 1.) Raynal-Ljutovac et al (2008) found the content of vitamin E in sheep milk 1.1 mg kg^{-1}. The content of vitamin A is according to this author 0.8 mg kg^{-1}. Park et al (2007) gives the value of vitamin A content 0.44 mg kg^{-1} (146 UI). Thus, values of vitamin A content determined in this study correspond to values published in Czech and foreign studies, while the content of vitamin E was higher than most of the published values related to sheep milk.

Table 1. The average content and standard deviation of vitamins A and E in sheep milk

	East Friesian	Lacaune	Romanov
Vitamin A	0.56 ± 0.10^{a}	0.70 ± 0.11^{b}	1.01 ± 0.21^{c}
Vitamin E	2.11 ± 0.53^{a}	2.21 ± 0.84^{a}	4.26 ± 1.91^{b}

The values in the same line marked with different letters (a–d) differ significantly ($P \leq 0.05$)

The amount of vitamins in sheep milk varies during lactation. The lowest measured value of vitamin E (0.15 mg kg^{-1}) among all the breeds was found in the milk of the Romanov sheep in April. The highest value was found in September (6.70 mg kg^{-1}) in the milk of the Romanov sheep, too. The content of vitamin E in the milk of the Lacaune sheep and the East Friesian sheep had a similar time-course to the milk of the Romanov sheep but the values were lower through all the lactation stages. The lowest content of vitamin E among all the breeds was found at the beginning of the lactation period, which is in April. The average value was 0.59 ± 0.39 mg kg^{-1}. The highest average value of vitamin E among all the breeds was recorded at the end of lactation, which is in September ($4.4 \pm$ mg kg^{-1}). In other months, the content of vitamin E was not statistically different from each other. It appears that the content of vitamin E in raw sheep milk rises from April to September, and thus increases during lactation (Fig. 3).

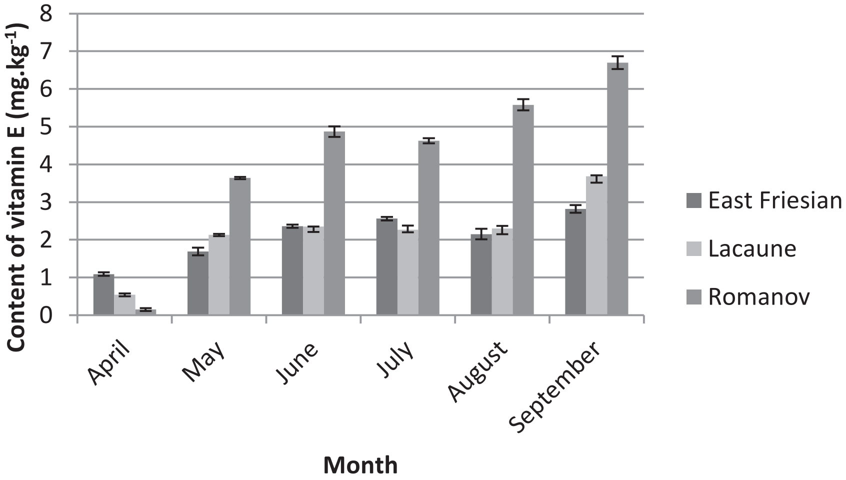

Figure 3. Content of vitamin E during lactation.

The values of vitamin A during lactation are recorded in Fig. 4. The lowest value of vitamin A (mg kg^{-1}) between all the monitored breeds was found in the milk of the East Friesian sheep in August. The highest content 1.25 mg kg^{-1} was recorded in September in the milk of the Romanov sheep. The values of vitamin A had a very similar time-course to that of vitamin E.

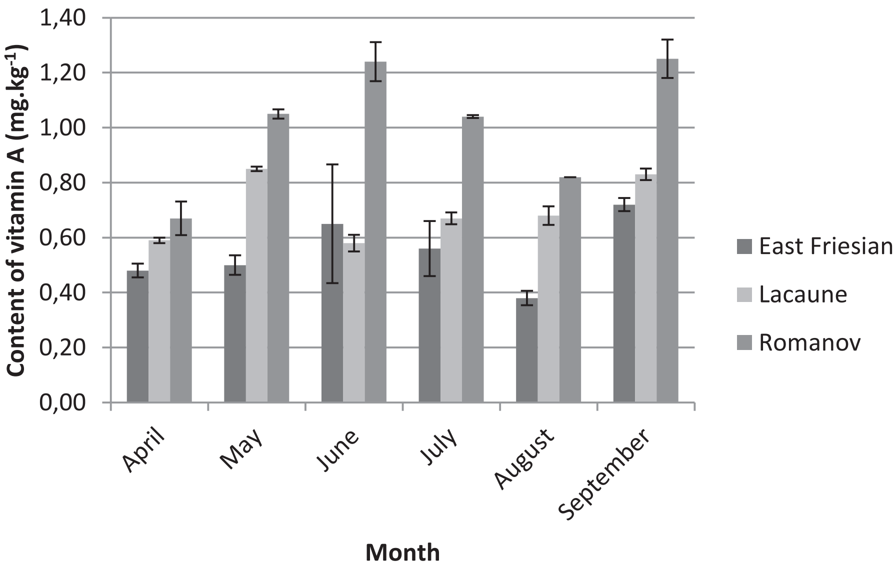

Figure 4. Content of vitamin A during lactation.

The content of vitamin A in milk increases from the beginning of the lactation period till June, then decreases slightly and takes the highest values in September. Statistically highest average content of vitamin A 0.93 mg kg^{-1} was determined in September, the lowest average content 0.58 ± 0.08 mg kg^{-1} in April. The values measured in May, June and July were not statistically different.

It can be presumed that fluctuations in the above mentioned vitamin levels during lactation are associated with warm weather in the summer months and also with changes in the fat content of sheep milk during lactation.

The content of both vitamins in sheep milk experimentally determined in this study was higher than the content of both vitamins in cow milk given in literature. The difference is more striking for vitamin E than for vitamin A (Table 2).

Table 2. Comparison of sheep and cow milk (average content)

	Sheep milk	Cow milk
Vitamin A	0.76 ± 0.19 mg kg^{-1}	0.30–1.00 mg kg^{-1}*
Vitamin E	2.86 ± 0.99 mg kg^{-1}	0.20–1.20 mg kg^{-1}*

*Fox & McSweeney (1998)

CONCLUSION

There were found significant differences in the content of vitamin A and E between three sheep breeds. The highest content of both, vitamins A and E, was found in the milk of the Romanov sheep. The milk content of both vitamins differed in respect to the lactation period as well. The highest content was found in September and the lowest in April. A strong correlation was found between the average content of the two examined vitamins over the entire monitored period. Sheep milk contains a higher amount of both vitamins compared to cow milk.

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