Concentration of vitamin E in bovine plasma and erythrocytes

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Abstract:

The aim of this study was to determine and compare the plasma and erythrocyte (RBCs) levels of vitamin E in cows of 3 different categories (2–3 weeks *antepartum*, 1–3 weeks and 2.5–3 months of lactation) and 1-month-old calves on dairy nutrition. We were interested in the degree of correlation of vitamin E in plasma and RBCs within the individual categories. Blood on EDTA was collected from 61 cows and 12 calves. As a part of the haematological examination, we determined haematocrit (HCT) immediately after the collection. We determined vitamin E from plasma and RBCs by a standard HPLC method. We compared the results of vitamin E in plasma and RBCs and correlated them. The concentration of vitamin E in the plasma and RBCs was 6.98 and 3.45 μmol/l, respectively, in cows 2–3 weeks *antepartum*; 1–3 weeks of lactation it was 4.98 and 3.34; 2.5–3 months of lactation 11.76 and 2.80 μmol/l; and in the case of calves 12.07 and 6.29 μmol/l. Weak correlations were observed between vitamin E in plasma and the RBC concentrations in the *antepartum* category $R^2 = 0.2076$; 1–3 weeks of lactation $R^2 = 0.0369$; 2.5–3 months of lactation $R^2 = 0.2403$ and calves on dairy nutrition $R^2 = 0.4628$. Vitamin E concentrations in RBCs were shown to be more stable than in plasma, where the concentrations varied. It is possible that vitamin E in RBCs could tell us more about the longer-term reserves of vitamin E in the organism. The highest concentration of vitamin E in plasma and RBCs, as well as a stronger correlation was found in calves.

Correlations, calves, dairy cows, haematocrit

The most common material for determination of vitamin E for diagnostic or scientific purposes is plasma or serum. Blood collection in dairy cows and calves for plasma or serum is relatively easy and it is generally considered a good method for determination of vitamin E concentration in organism. However, certain human medicine studies suggest that vitamin E concentrations in other blood components (mainly erythrocytes, RBCs) may be even a better indicator of the body's total vitamin E reserves (Lehmann 1981; Lehmann et al. 1988; Cuerq et al. 2016).

A number of studies have been carried out on the importance of vitamin E for the health of both dairy cows and calves (Weiss et al. 1997; Johansson et al. 2014; Mikulková et al. 2020; Kadek et al. 2021; Strickland et al. 2021).

Vitamin E is known to act primarily as an antioxidant and it is localized in hydrophobic structures (plasma lipoproteins and biological membranes). In the case of oxidative stress, lipid peroxyl free radicals are produced from unsaturated fatty acids to an increased extent. Phospholipids, which are also found in membranes, are particularly sensitive to oxidative damage. The orientation of vitamin E in the biological membrane is crucial for its function as an antioxidant — the hydrophobic part of its molecule is immersed in the membrane and the hydrophilic chromate ring is on the surface. In this way, it can interact with other antioxidants such as vitamin C, and thus contribute to its auto-regeneration.

In plasma, vitamin E is transported in plasmatic lipoproteins. Unlike other fat-soluble vitamins, vitamin E has no specific plasma carrier (Kayden and Traber 1993; Traber 1994).

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It is incorporated by vitamin E binding proteins into low density lipoproteins and then dispersed in cells and cell membrane phospholipids. Alpha-tocopherol binding protein (alpha-TBP), a liver cytosolic protein, acts as a regulator of its concentration in the bloodstream and preferably incorporates alpha tocopherol into low density lipoproteins. The plasma membrane alpha-tocopherol binding protein (TBPpm) was characterized in human RBCs and liver, and it has also been suggested that it may regulate tocopherol concentrations in these cells (Bellizzi et al. 1997a, b; Dutta-Roy 1999).

The aim of our study was to determine and compare the concentration of vitamin E in plasma and RBCs in cows of 3 different categories (2–3 weeks before farrowing, 1–3 weeks of lactation, 2.5–3 months of lactation) and in 1-month-old calves on dairy nutrition. We also observed a correlation between vitamin E in plasma and RBCs.

Materials and Methods

Animals

A group of 61 adult Holstein cows and 12 calves aged approximately 1 month on dairy nutrition were selected from a Holstein cattle farm as a part of metabolic testing. Only clinically healthy animals without having overcome metabolic or infectious diseases in the past with a body condition score (BCS) of 3–3.5 were selected. These 61 cows were divided into three categories: 22 *antepartum* cows (2–3 weeks before farrowing), 20 freshening cows (1–3 weeks of lactation), and 19 cows 2.5–3 months of lactation. Blood samples (2.5 ml of blood) were collected into sampling tubes containing the ethylenediaminetetraacetic acid (EDTA) anticoagulant. Sampling was performed once from all animals.

Immediately after the collection, haematocrit (HCT) was determined on a veterinary haematology analyzer BC - 2800 (Mindray, Nanshan, Shenzhen, China).

Determination of vitamin E in plasma

The concentration of vitamin E (α- tocopherol) in the plasma of cows was determined by the High-Performance Liquid Chromatography (HPLC) method on the Ultimate 3000 device (Dionex, Sunnyvale, USA) according to Sowell et al. (1994) with minor modifications. ClinCal® Calibrator lyophilized serum (RECIPE, Munich, Germany) was used as a calibrator for the determination of vitamins A and E. Briefly, the samples were deproteinized with ethanol, which was followed by extraction into hexane, then evaporation of the organic layer and dissolution in the mobile phase, methanol.

Preparation of samples for determination of vitamin E in RBCs

The sample preparation for the determination of vitamin E in RBCs as well as the analysis itself was performed according to Bieri et al. (1979) with minor modifications. One ml of blood from a 2.5 ml EDTA collection tube was pipetted into another plastic tube. The RBCs were washed 3 × with saline. After the last wash, the saline was pipetted off and 1 ml 0.5% solution of pyrogallol in water was added to the remaining RBCs sediment. After the addition of pyrogallol, all samples were mixed properly and stored in a freezer (−20 °C) until the analysis.

Determination of vitamin E in RBCs

After unfreezing the samples, 200 µl of the RBCs suspension were pipetted off, 600 µl of cold methanol were added with slow shaking to form a fine suspension without clustering. Subsequently, 1.2 ml of hexane was added, and the mixture was shaken (1 h on the vortex), the organic layer was dried and dissolved in the mobile phase, methanol.

After dilution, the concentration of vitamin E in the RBCs was calculated according to the formula:

$$C_{\text{vit. E in RBCs}} = C_{\text{(measured concentration of vit. E in RBCs)}}\ \text{HCT} + 100\ /\text{HCT}$$

Statistical evaluation

The results were tested for homogeneity of variance (Hartley-Cochran-Bartlett test) and normality of distribution (Shapiro-Wilk test). The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Fisher's LSD *post hoc* test. All results were expressed as the mean value (x) ± standard deviation (SD). A $P \leq 0.05$ value was considered significant. Cross-correlation was performed for some parameters.

Results

The average concentrations of vitamin E in the plasma and RBCs of cows and calves are shown in Fig. 1. The highest concentration of vitamin E in plasma was in the category of cows 2.5–3 months of lactation (11.76 ± 2.78 µmol/l) and calves (12.07 ± 3.64 µmol/l). Calves had the highest concentration of vitamin E in RBCs (6.29 ± 2.01 µmol/l).

No significant differences in vitamin E concentration in the RBCs of cows were observed between the individual categories ($P > 0.05$). However, significant differences were observed between vitamin E plasma concentrations of cows in the 2–3 week *antepartum* and 1–3 weeks lactation categories ($P < 0.01$) and between other cow categories ($P < 0.001$).

The correlation between the vitamin E concentration in plasma and in RBCs within the individual groups of cows is expressed in Figs 2–4 and for calves in Fig. 5. The determination coefficient within the category 2–3 *antepartum* was $R^2 = 0.2076$; 1–3 weeks of lactation $R^2 = 0.0369$; 2.5–3 months of lactation $R^2 = 0.2403$ and in calves on dairy nutrition $R^2 = 0.4628$.

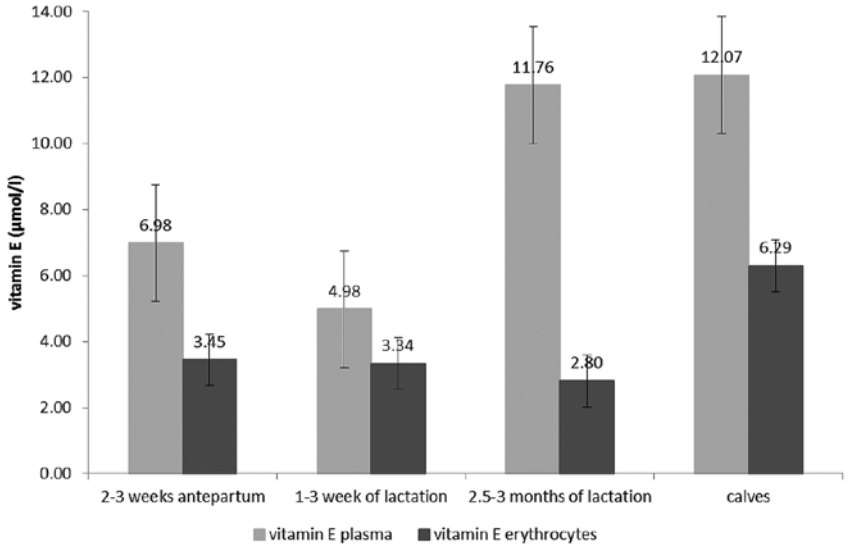

Fig. 1. Concentrations of vitamin E in plasma and erythrocytes of the individual categories of cows and in calves (mean ± standard deviation)

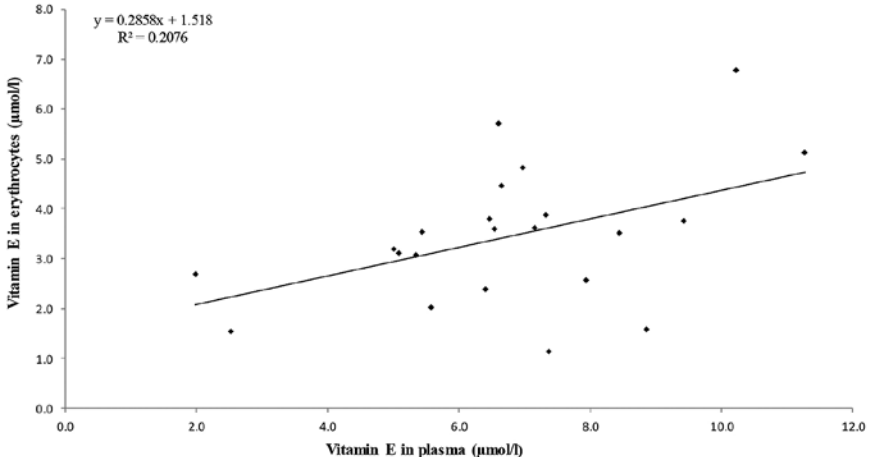

Fig. 2. Relation between vitamin E in plasma and erythrocytes in cows at 2–3 weeks *antepartum*

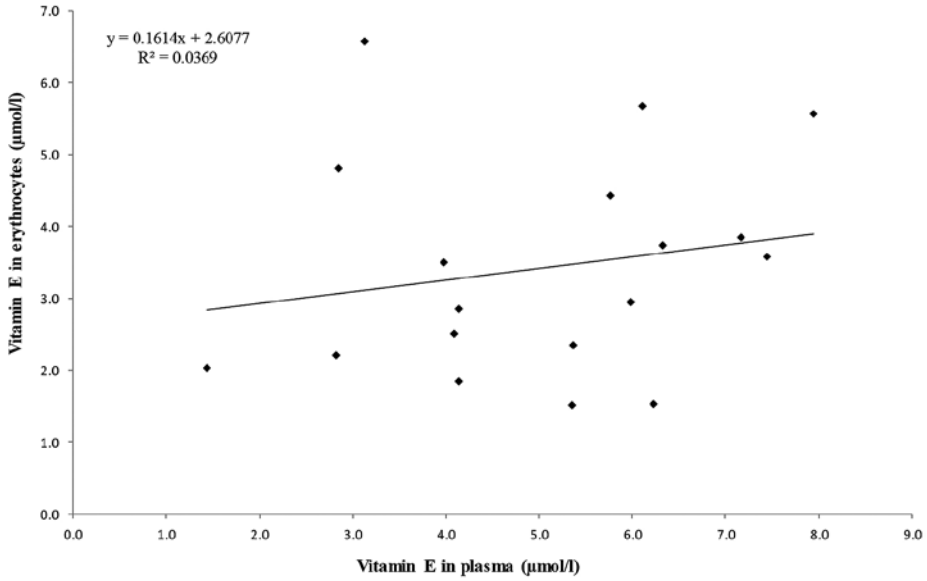

Fig. 3. Relation between vitamin E in plasma and erythrocytes in cows at 1–3 weeks of lactation

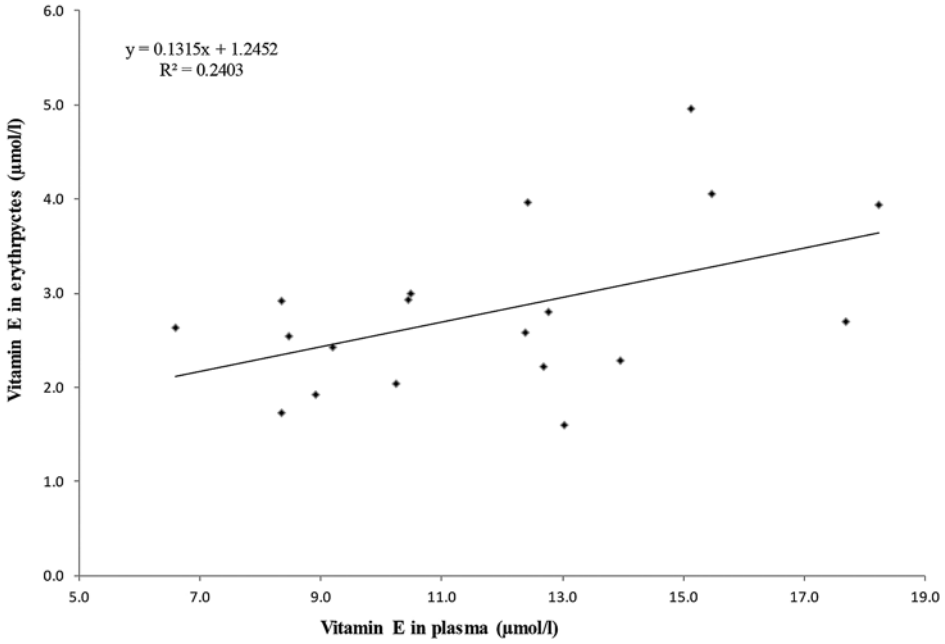

Fig. 4. Relation between vitamin E in plasma and erythrocytes in cows at 2.5–3 months of lactation

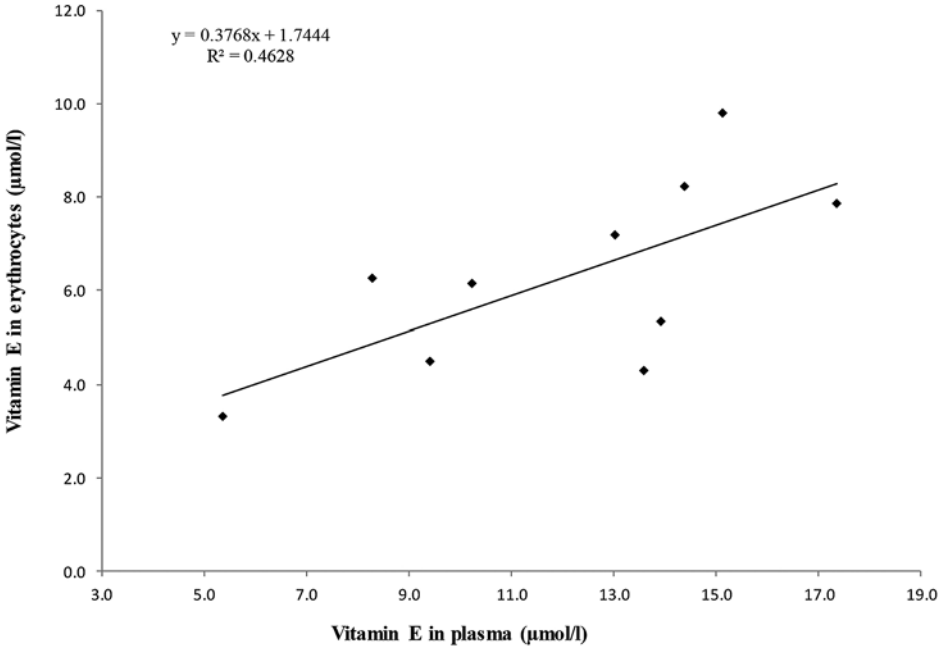

Fig. 5. Relation between vitamin E in plasma and erythrocytes in calves on dairy nutrition

The average ratio of vitamin E in plasma to vitamin E in RBCs was 0.49 in the *antepartum* cows, 0.67 in cows at 1–3 weeks of lactation, 0.24 in cows at 2.5–3 months of lactation, and 0.52 in calves.

Discussion

There was a significant difference between plasma vitamin E concentrations between cow categories. The average plasma concentration of vitamin E in cows in the *antepartum* category and 1–3 weeks of lactation was 6.98 and 4.98 µmol/l, in cows in the period of 2.5–3 months of lactation these values were higher in plasma, at 11.76 µmol/l. Similar values of vitamin E in plasma in unsupplemented cows of these categories were observed in the studies by Weiss et al. (1992) and Píšťková et al. (2019).

The vitamin E concentrations in RBCs appeared to be relatively stable; on the contrary, the plasma concentrations differed also in terms of category and were more variable. This is probably due to physiological processes such as colostrum synthesis and the transfer of vitamins from plasma to colostrum/milk.

In our study, the weakest correlation was observed between the plasma vitamin E and RBC concentrations in the case of the 1–3 weeks of lactation group ($R^2 = 0.0369$). Even in some cases, the concentration of vitamin E in RBCs in this category was higher than in the plasma alone. On the contrary, in the group of 2.5–3 months of lactation the plasma concentrations of vitamin E were higher and a weaker correlation was observed ($R^2 = 0.2403$) similarly to the *antepartum* category.

In the study by Weiss et al. (1992), the correlation between the concentration of alpha-tocopherol in plasma and RBCs was similar, but at the same time slightly higher than

in our case ($R^2 = 0.35$). In this work, an experimental group of cows was supplemented with vitamin E. It was shown that the ratio of alpha-tocopherol RBCs to plasma was not affected by the vitamin E supplementation, but it was variable in terms of the stage of pregnancy and lactation. In dry-staying cows, the ratio was around 0.4, but as the time of birth approached, the plasma concentrations of vitamin E decreased and the ratio increased to 0.6. We observed very similar conditions in the same categories of cows in our work (0.49 in the case of cows 2–3 weeks *antepartum* and 0.67 at 1–3 weeks of lactation). Weiss et al. (1992) explains that there was a decrease in blood lipids after delivery, which increased the concentration of vitamin E in RBCs. This suggests a decrease in the plasma transport capacity (lipids) after parturition. In the work of Horwitt et al. (1972) a high correlation was observed between the plasma lipoproteins and plasma vitamin E concentrations (Horwitt et al. 1972).

In our study, a higher correlation was observed between the concentration of vitamin E in plasma and RBCs in calves than in cows ($R^2 = 0.4628$). As in our case, Roquet et al. (1992) observed a similar, but a little weaker correlation between the plasma vitamin E levels and RBCs in calves ($R^2 = 0.37$). The plasma concentrations of vitamin E in calves in this work showed higher changes — fluctuations in response to vitamin E supplementation than the RBCs concentrations. This also indicates a higher stability of vitamin E concentrations in RBCs.

It seems that the concentration of vitamin E in RBCs is likely to reflect the longer-term level of vitamin E in the body; in contrast, its concentration in plasma is subject to immediate fluctuations due to environmental influences, nutrition, or stages of lactation. On the other hand, in the work of Fry et al. (1993), vitamin E concentrations in the liver and RBCs of deficient lambs decreased more rapidly than in plasma and muscle tissue. Probably, in the case of severe deficiencies, the body primarily draws vitamin E from its reserves to increase its plasma concentrations. Although the period around farrowing is challenging for cows, and also in our case the concentrations of vitamins before delivery and 1–3 weeks of lactation were low, with proper nutrition, they usually return to the normal level quickly.

It seems that the standard determination of vitamin E in RBCs in the normal operation of the laboratory could reveal a lot about the longer-term reserve of vitamin E in the body. However, the determination of vitamin E in RBCs in a normal routine operation is difficult due to the preparation (washing and lysis of the RBCs suspension).

In Ghaffari et al. (2019), a new test (iCheck) was described for measuring levels of vitamins E, A and beta-carotene directly in the field and from the whole blood (corrected by HCT) and it was compared to the standard plasma HPLC method. This test turned out to show a very good agreement and accuracy compared to the HPLC method. It is worth considering whether the whole blood might be a more suitable material for determining vitamin E and evaluating its reserves in the body; on the other hand, it has to be pointed out that it would involve mixing two compartments, intra- and extracellular.

In conclusion, vitamin E levels in RBCs appear to be more stable and there were no significant differences in concentrations of vitamin E in RBCs between categories. Conversely, in the case of plasma the vitamin E concentrations were more variable within the cow categories. A weak correlation was observed between the plasma and RBCs vitamin E concentrations. In our case, it was the weakest in the 2nd category (freshening cows at 1–3 weeks of lactation) and the strongest in 1-month-old calves. Vitamin E in RBCs has been shown to be more stable and less subject to changes that accompany a cow at different stages of life. It is possible that the concentration of vitamin E determined in RBCs could reveal longer-term reserves of vitamin E in the body. On the other hand, its determination in RBCs is more demanding and time-consuming for a standard laboratory operation.

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