

β -carotene concentration in blood serum of cows from herds with impaired fertility

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β -carotene has many important functions in the body: as a precursor of vitamin A, it works as antioxidant and affects the fertility of cows. This paper presents the results of measurements of the content of β -carotene in the blood serum of cows from different farms in Slovenia. Blood samples of cows ($n = 604$) from 176 farms with impaired fertility were analysed for β -carotene content using Yudkin's photometric method. The descriptive statistics and the proportion of samples that deviated from the reference values were calculated. Influence of month of sampling and farm on β -carotene level was studied as well. The median value of β -carotene in the investigated samples was 5.02 mg/L (minimum 0.63 mg/L, maximum 16.10 mg/L) and was within the reference range. In 32.8% of cow samples, the content of β -carotene was below the reference value (4.0 mg/L). According to the results, we believe that this is not a negligible problem in Slovenian farms, and that it is advisable to control β -carotene status in cows, especially in herds where fertility is impaired and in farms where the composition of the diet indicates the possibility of β -carotene deficiency.

Keywords: dairy cattle, reproduction, β -carotene

1 Introduction

Low reproductive efficiency in cows is complex and often multifactorial in its nature and it is an economic burden for the cattle industry. The positive role of carotenoids, especially β -carotene, in cow fertility has been reported in several studies (Hurley and Doane, 1989, Kawashima et al., 2009, Gouvêa et al., 2018). Carotenoids are a family of more than 600 molecules that are synthesized by higher plants and algae and are involved in photosynthetic processes. Among carotenoids, β -carotene is the main dietary precursor of vitamin A (retinol) in cattle. β -carotene (provitamin A) can be converted into vitamin A in numerous cell types, but this occurs mainly in enterocytes and hepatocytes (Nozière et al., 2006b). Vitamin A is stored mainly in the liver, some also in adipose tissue, from where it is mobilized as necessary.

A deficiency in vitamin A may reduce reproductive efficiency in dairy cows, especially through impaired ovarian function and increased incidence of abortion (Hurley and Doane, 1989). β -carotene acts as an antioxidant in the body and promotes the production of progesterone (de Ondarza et al., 2009). Carotene deficiency mainly negatively affects fertility of cows, causes more frequent occurrence of endometritis, and in pregnant cows, it may lead to the birth of non-vital or stillborn calves (Stöber and Scholz, 2006, Rakes et al., 1985). In cows, the need of vitamins (including β -carotene) is greatest during the transition period, which represents physiological stress for them (Johansson et al., 2014). During late pregnancy, dry matter intake decreases, and the need for nutritive substances including vitamins increases due to the formation of colostrum and intensive growth of the foetus. During transition period and early lactation, a significantly lower concentration of β -carotene in blood of cows was found compared with middle (3-5 months after calving) and end of lactation (7-9 months after

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calving) (Johansson et al., 2014). Slightly higher serum β -carotene concentration was found in older cows compared with cows in the first and second lactation (LeBlanc et al., 2004).

β -carotene and vitamin A are also important for health of cows and their calves. Vitamin A is important for cell differentiation, normal proliferation of lymphocytes (B and T), maintenance of normal epithelial structure (skin, mucous membranes, glands and retina), tissue growth, normal foetal development in the uterus, acceleration of secretion of growth hormone, and in males it is important for semen quality (Stöber and Scholz, 2006). Calves in the first weeks of life depend on the amount of vitamin A in colostrum and milk, as they have only a small reserve of this vitamin at birth. The concentration of β -carotene and vitamin A in colostrum (0.94 mg/L and 2.55 mg/L, respectively) is about ten times higher than in milk (0.12 mg/L and 0.24 mg/L, respectively) (Johansson et al., 2014). In postpartum cows, it improves resistance to infections, especially against mastitis. An increase in blood serum retinol concentration of 100 ng/mL reduced the risk of mastitis by 60% (LeBlanc et al., 2004). Animals with vitamin A deficiency are more prone to skin, respiratory, and gastrointestinal infections (Rucker and Morris, 1997).

The carotene stores in the body are sufficient for 2-4 days and should be supplemented every day (Žust et al., 2009). Animals get β -carotene from feed; therefore, β -carotene content of feed significantly affects the supply of cows. The concentration of β -carotene in cow serum reflects the amount consumed by the cow (Torsein et al., 2018). Switch from grass silage to hay diet induced a rapid decrease in β -carotene concentrations in plasma of cows from 3.8 mg/L to 1.7 mg/L from day 1 to 10 (Nozière et al., 2006a). Concentration of carotenoids in the forages vary highly according to development stage and length of conservation (Nozière et al., 2006b). In grass and grass silage, the content of β -carotene is higher than in hay or corn silage, which is also reflected in the content of β -carotene in the milk of cows fed with different voluminous feed (Mogensen et al., 2012). It should be noted that the content of β -carotene might also be quite different in grass and grass silages (Lindquist et al., 2012, Johansson et al., 2014).

In this paper, we will present the results of measurements of β -carotene concentration in the blood serum of dairy cows from different farms in Slovenia, where they encountered fertility problems.

2 Material and methods

The results of β -carotene concentration in blood samples of cows ($n = 604$) sent to the Clinical Laboratory of Veterinary Faculty (Ljubljana, Slovenia) during a five-year period were analysed. The cows were of different breeds and originated from 176 farms. The samples were sent to the laboratory because of fertility problems (low conception rate, ovarian cysts) of the cows. Mostly 2 to 4 samples per farm (range 1 to 25) were sent to the laboratory.

Blood samples were collected in evacuated tubes without anticoagulants intended to obtain blood serum and sent to the laboratory within the same day. Blood samples were centrifuged at $1,300 \times g$ for 10 min, than serum was transferred to sample tubes and centrifuged at $1,400 \times g$ for 10 min to get clean serum without cells. Samples were stored at 6°C until analysis, which was performed within the same or the next day. β -carotene concentration was measured in blood serum samples by the photometric method according to Yudkin (1941).

Using the SPSS statistical program (ver. 22), descriptive statistics and the proportion of samples that deviated from the reference values for cows were calculated. The data were not normally distributed therefore we calculated median, quartile, minimum and maximum values. The influence of month of sampling on serum β -carotene content was assessed with Kruskal-Wallis test for independent samples. Comparisons between months were assessed with Dunnett's T3 multiple comparison post-hoc test. Data were normalized through log-transformation and analysis of variance was applied to test the effect of farm and month. Complete data about breed, age and days in milk were not available and thus could not be accounted for in statistical analysis.

3 Results and discussion

The median of β -carotene concentration in the tested samples was 5.02 mg/L (minimum 0.63 mg/L, maximum 16.10 mg/L) and was within the reference values. Indeed, β -carotene content in blood serum of cows should be above 4.0 mg/L (Jazbec, 1990). In 32.8% of analysed samples, β -carotene content was below 4.0 mg/L.

Our results revealed wide variation of β -carotene concentration between cows and between samples from different farms. The effect of farm was significant ($P < 0.001$). Johansson et al. (2014) found large differences in β -carotene content in the blood plasma of cows at different lactation periods. In cows three weeks before calving (3.86 mg/L), at calving (2.48 mg/L) and 3-4 weeks after calving (2.82 mg/L), the mean β -carotene value was much lower than 3-5 months after calving (10.81 mg/L) and 7-9 months after calving (9.15 mg/L). Kawashima et al. (2009) found that β -carotene concentrations decreased from week 3 prepartum to week 1 postpartum in ovulatory cows and increased after 1 week postpartum in ovulatory and anovulatory group of cows. Some cows in our study were at the beginning of lactation, so the lactation period could have partly influenced the lower values in individual cows, but review of our data revealed that it was more often a herd problem in individual farms, as β -carotene levels were low in several samples from a given farm.

Blood samples were taken and analysed throughout the year and results are presented in Figure 1. The month of sampling was significant ($P < 0.001$) in explaining the variation of β -carotene concentration in the blood of cows. The concentration of β -carotene was highest in November, when it was 5.98 mg/L (minimum 1.50 mg/L, maximum 12.42 mg/L), while the lowest was in February, namely 4.12 mg/L (minimum 1.09 mg/L, maximum 11.11 mg/L). The β -carotene levels in February and April were significantly lower than the levels in October ($P = 0.002$ and $P = 0.001$, respectively) and November ($P = 0.005$ and $P = 0.002$, respectively).

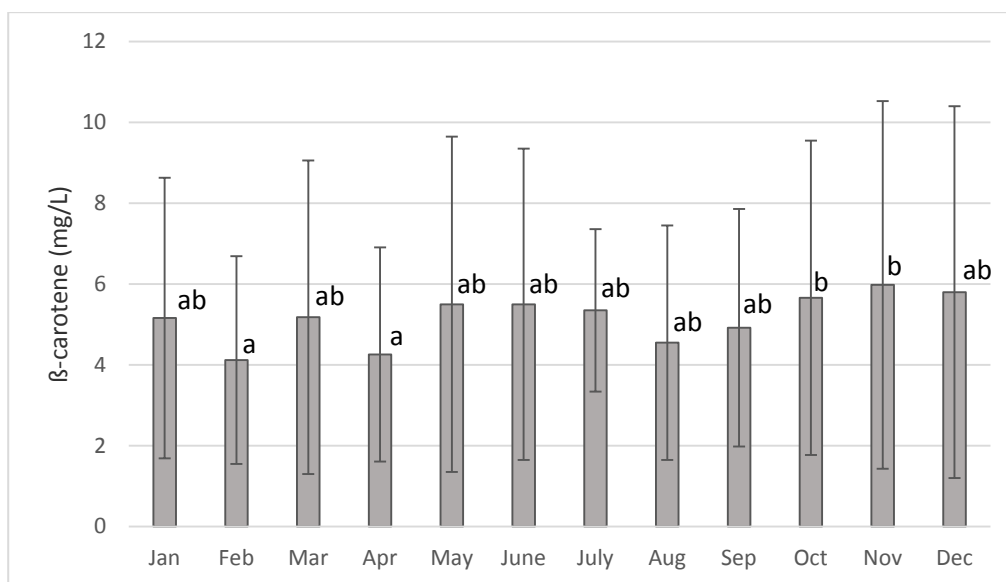


Figure 1 β -carotene concentration (median, 1st and 3rd quartile) throughout the year. Different letters indicate significant differences between months of sampling

The concentration of β -carotene measured in cow serum reflects the total ration consumed by the cow. Having a total ration with a minimum of 20% maize silage (dry matter) was associated to significantly lower concentration of β -carotene in cow serum (Torsein et al., 2018). The concentration of β -carotene in feed was lower in herds using total mixed ration compared to herds not using total mixed ration (Torsein et al., 2018). Development stage and length of conservation affect concentration of carotenoids in the forages (Nozière et al., 2006b). In our study slightly lower median β -carotene content in the winter and early spring months may be affected by diet, as animals are fed conserved feed (grass silage, hay) in which β -carotene content is lower than in fresh grass.

β -carotene is important for cow fertility. Cows with higher blood β -carotene concentration in the postpartum period have shown higher fertility than cows with lower β -carotene (Oliveira et al., 2015). In addition, de Ondarza et al. (2009) found higher fertility in cows fed extra β -carotene after calving. Lower blood β -carotene concentration during the pre-partum period was associated with anovulation during the first follicular postpartum wave. The change of plasma β -carotene concentrations during the peripartum period differed between ovulatory and anovulatory cows in the first follicular wave postpartum (Kawashima et al., 2009). β -carotene and vitamins A, D₃ and E supplementation of grazing beef cows increased the conception rate at the first fixed-time artificial insemination and can be a tool to optimize the reproductive performance (Gouvêa et al., 2018). β -carotene and vitamin A

supplementation had positive effect on reproductive functions and pregnancy rate in dairy cows with chronic fertility impairment and deficit of β -carotene and vitamin A (Trojačanec et al., 2012). De Bie et al. (2016) investigated the association between negative energy balance and β -carotene content in serum and follicular fluid and found a significant increase of serum β -carotene and retinol concentrations regardless of energy status if β -carotene was added to cows feed. The addition of β -carotene also had a positive effect on the size of ovarian follicles, which in turn means better fertility in cows. The addition of 1.2 g β -carotene per cow/day was associated with a lower incidence of retained placenta in multiparous cows and shorter time required for release of placenta (Oliveira et al., 2015). Serum β -carotene levels increased gradually postpartum in cows both with and without retained placenta, but their levels were significantly lower in cows with retained placenta compared to the control group. The calving to first oestrus and calving to first service intervals were considerably longer in the group with retained placenta (Akar and Gazioglu, 2006).

According to the results of the studies β -carotene deficiency may have affected fertility in some of the investigated farms.

4 Conclusions

In one third of the cows, blood β -carotene content was below the reference value. Low β -carotene content may be associated with the physiological period and/or diet of cows. Based on the results, we believe that this is not a negligible problem in Slovenian farms, and it is advisable to control β -carotene status in cows, especially in herds with fertility impairment and in farms where the composition of the diet indicates the risk of β -carotene deficiency.

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