

# Prediction of Vitamin A, Vitamin E, Selenium and Zinc Status of Periparturient Dairy Cows Using Blood Sampling During the Mid Dry Period

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<sup>1</sup>Department of Obstetrics and Gynaecology, <sup>2</sup>Department of Animal Nutrition and Management, and <sup>3</sup>Department of Ruminant Medicine and Veterinary Epidemiology, Swedish University of Agricultural Sciences, Uppsala, <sup>4</sup>Department of Chemistry and <sup>5</sup>Department of Ruminant and Porcine Diseases, National Veterinary Institute (SVA), Uppsala, Sweden.

**Meglia GE, Holtenius K, Petersson L, Öhagen P, Persson Waller K: Prediction of vitamin A, vitamin E, selenium and zinc status of periparturient dairy cows using blood sampling during the mid dry period. Acta vet. scand. 2004, 45, 119-128.** – Vitamins A and E, and the trace elements selenium (Se) and zinc (Zn) are essential for the health and performance of dairy cows. Their concentrations often decrease around calving and extra supplementation is sometimes recommended at that time. However, the need for this varies, for example depending on quantity and quality of feedstuffs in the diet. The aim of this study was to measure the concentrations of serum vitamin A (S-vit A) and vitamin E (S-vit E), plasma Se (P-Se) and serum Zn (S-Zn) in blood samples taken at several time points from one month before to one month after calving, and to evaluate if a blood sample taken during the mid dry period can accurately predict the blood concentration at calving and early lactation. Dairy cows on 3 different feeding regimens during the dry period were included in the study. A significant decrease in the concentrations of S-vit A and S-vit E, and S-Zn, was observed at calving, and P-Se was significantly lower during the dry period and at calving than in early lactation. The blood concentrations of S-vit E and P-Se in the mid dry period significantly predicted the occurrence of values considered marginal or deficient at the time of calving. The data indicate that a mid dry period concentration of  $\geq 5.4$  mg/l of S-vit E and  $\geq 0.09$  mg/l of P-Se will result in a 90% chance that the cow stays above marginal levels at calving given that a feed of the same quality is offered.

***Vitamin A; vitamin E; selenium; zinc; dairy cows; parturition; supplementation.***

## Introduction

Vitamins A and E and the trace elements selenium (Se) and zinc (Zn) are essential for the health and performance of dairy cows (e.g. Reddy & Frey 1990, Herdt & Stowe 1991). These micronutrients are cellular antioxidants, preventing peroxidative damage, either in cell membranes (vitamins) or in the cytoplasm (trace elements), and are essential for a well-functioning immune system (Miller *et al.* 1993, Weiss 2002).

The immune system of dairy cows is suppressed around parturition resulting in an increased susceptibility to infectious diseases (e.g. Mallard *et al.* 1998, Kehrl *et al.* 1998). This may partly be due to a decrease in blood concentrations of vitamins A and E, and Zn observed at this time (Goff & Stabel 1990, Miller *et al.* 1995, Meglia *et al.* 2001). The concentration of Se is more variable, both increases (Weiss *et al.* 1990, Meglia *et al.* 2001) and de-

creases (Miller et al. 1995) have been reported at parturition, relative to the dry period. Micronutrient deficiencies around calving have been associated with diseases like retained foetal membranes, endometritis, and mastitis (e.g. Eger et al. 1985, Erskine et al. 1997, Weiss et al. 1997).

The cow's requirement of micronutrients must be provided in the diet. However, the contents vary substantially between different feedstuffs, and can be negatively influenced by factors like soil type, harvest and storage conditions (e.g. Herdt & Stowe 1991, Puls 1994ab, Underwood & Suttle 2001). The cow's uptake and requirement of micronutrients can also vary due to stage of lactation and health status (e.g. Hogan et al. 1993, Hemingway 1999, Underwood & Suttle 2001). Therefore, extra supplementation of micronutrients is sometimes warranted. Adequate micronutrient intake is particularly important during the late dry period and the early stages of lactation in order to prevent diseases around parturition. However, as the type and quality of the dry cow diet can vary considerably between and within herds, it can be difficult to predict the need for extra supplementation. To avoid unnecessary costs, inadequate supplementation and, at least for Se, the risk for toxicity, it may be useful for the farmer to evaluate the blood concentrations of micronutrients in a sub-sample of cows collected during the mid dry period given that this sample can give information of importance also for the period around parturition. Therefore, the aim of this study was to measure the concentrations of vitamin A, vitamin E, Se and Zn in blood samples taken at several time points from one month before to one month after calving, and to evaluate if a blood sample taken during the mid dry period can accurately predict the blood concentration at calving. Dairy cows on 3 different feeding regimens during the dry period were included in the study.

## Materials and methods

### *Animals and management*

Twenty-three high producing, clinically healthy dairy cows of the Swedish Red and White breed, housed in individual tie stalls at the university research farm were monitored from approximately one month before to one month after calving. The cows were allocated to 3 groups, low, medium and high, depending on the amount of feed supplied during the dry period. The groups contained 8, 8 and 7 animals, respectively, and were matched for lactation number and calving dates. All animals were dried off approximately 10 weeks before the predicted day of parturition and introduced to the experimental diets 8 weeks before parturition was expected. The groups received 6 (low), 9 (medium) and 14.5 (high) kg of dry matter (DM) of a dry period total mixed ration (TMR) mix, respectively. The diets provided on average 75, 110 and 178%, respectively, of the energy requirements for maintenance and pregnancy according to Swedish feeding recommendations (Spörndly 1999). From parturition, all cows received the same amounts of another TMR mix. A detailed description of the study design and composition of the TMR feeds is given in Agenäs et al. (2003). The Uppsala Local Ethics Committee approved the experimental design and all handling of the animals.

### *Blood sampling*

From each cow, jugular blood samples were collected in the morning at 5 time points, i.e. 4-5 weeks and 7-10 days before predicted calving, and 0-3 days, 7-10 days and 4-5 weeks after calving. Before sampling, the skin was cleaned with Milli-Q-Water (Milli-Q, Millipore Corp., Bedford, MA, USA). Blood without additives was taken for vitamin A and vitamin E analysis, while heparinized blood was used for analyses of Se. Blood collected in Zn-free vacutainer tubes without additives (Becton Dick-

Table 1. Least square mean (SE) concentrations of serum vitamin A (S-vit A) and vitamin E (S-vit E), plasma Se (P-Se) and serum zinc (S-Zn) in blood samples taken at 5 time points around calving from each of 20 dairy cows.

Variable	Units	Mean (SD) days relative to calving*				
		-32 (5)	-11 (4)	2 (2)	7 (2)	28 (3)
S-Vit A	mg/l	0.36 (0.01) <sup>a</sup>	0.33 (0.02) <sup>ab</sup>	0.21 (0.01) <sup>c</sup>	0.27 (0.02) <sup>bc</sup>	0.40 (0.02) <sup>a</sup>
S-Vit E	mg/l	3.80 (0.05) <sup>a</sup>	3.10 (0.05) <sup>ab</sup>	2.30 (0.05) <sup>c</sup>	2.96 (0.05) <sup>bc</sup>	5.20 (0.05) <sup>d</sup>
P-Se	mg/l	0.075 (0.003) <sup>ab</sup>	0.072 (0.003) <sup>a</sup>	0.070 (0.003) <sup>a</sup>	0.083 (0.003) <sup>b</sup>	0.081 (0.003) <sup>b</sup>
S-Zn	mg/l	0.84 (0.03) <sup>a</sup>	0.75 (0.03) <sup>ab</sup>	0.63 (0.03) <sup>b</sup>	0.71 (0.03) <sup>ab</sup>	0.77 (0.03) <sup>a</sup>

\* Values with different letters within each row differ significantly ( $P < 0.05$ ).

inson Vacutainers Systems, Meylan, France) was used for analyses of Zn. All tubes were centrifuged at  $1500 \times g$  for 35 min to obtain plasma or serum, which was frozen at  $-20^\circ\text{C}$  until analysis.

#### Analyses of micronutrients

Vitamins A (S-vit A) and E (S-vit E) were extracted from serum samples with hexan, and the separation was done by High Performance Liquid Chromatography (HPLC) on a C18 column (Jones Chromatography, Hengoed, UK). S-vit A and S-vit E were determined by using ultraviolet and fluorescence detection, respectively, according to standard procedures at the Department of Feed, National Veterinary Institute, Uppsala, Sweden.

The determinations of plasma Se (P-Se) and serum Zn (S-Zn) were performed using hydrid generation (HG) inductively coupled plasma atomic emission spectrometry (ICP-AES, Jobin Yvon 238 emission-spectrometer, Jobin Yvon S.A.S., Longjumeau, Cedex, France) and ICP-AES, respectively, with set-up and conditions according to methods accredited by SWEDAC (Swedish Board for Accreditation and Conformity Assessment, Borås, Sweden).

#### Statistical analyses

Analyses of variance with repeated measures

were performed using Statgraphic Plus 3.1 (Rockville, MD, USA) to evaluate the effect of time relative to parturition for each variable. To achieve a normally distributed data set the S-vit E data were square-root transformed. When significant effects of time were observed, the differences between time periods were evaluated with the Scheffe test, and probabilities less than 0.05 were considered significant. The results are presented as least square mean and standard error (SE) of the mean.

Standard logistic regression (*SAS Institute Inc.* 1999) was performed for each micronutrient to evaluate the relationship between the concentration at the mid dry period sampling and the occurrence of a concentration considered marginal or deficient (MD), or adequate (A), at calving and 7-10 days after calving. Threshold levels for MD and A for the different micronutrients were selected based on *Puls* (1994ab). Values below MD were considered marginal or deficient, while values above A were considered adequate. The MD values used were 0.2 mg/l, 3.0 mg/l, 0.06 mg/l and 0.59 mg/l for S-vit A, S-vit E, P-Se and S-Zn, respectively. The corresponding A values used were 0.3 mg/l, 4.0 mg/l, 0.08 mg/l and 0.78 mg/l. If a significant relationship between the samples was established, the mid dry period concentration (x) associated with a probability (p) of 0.10 of a

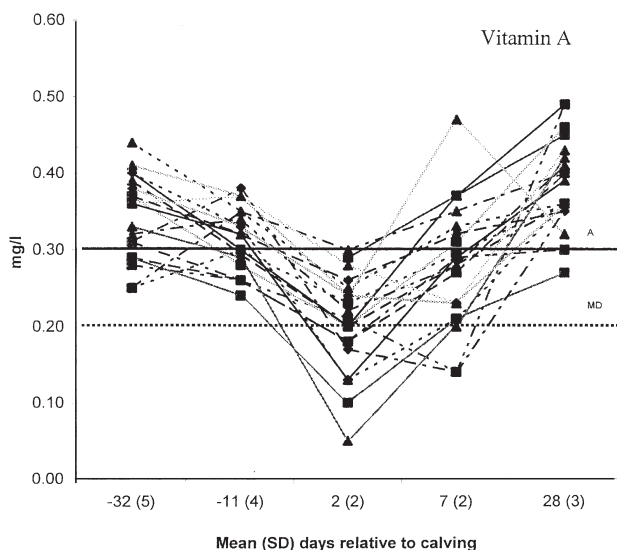


Figure 1. The concentration of serum vitamin A (mg/l) in blood samples taken from 20 dairy cows at 5 different time points relative to calving. The cows were fed low (■), medium (▲) or high (◆) amounts during the dry period. Values considered marginal or deficient (MD), and adequate (A) are indicated in the figure with horizontal lines.

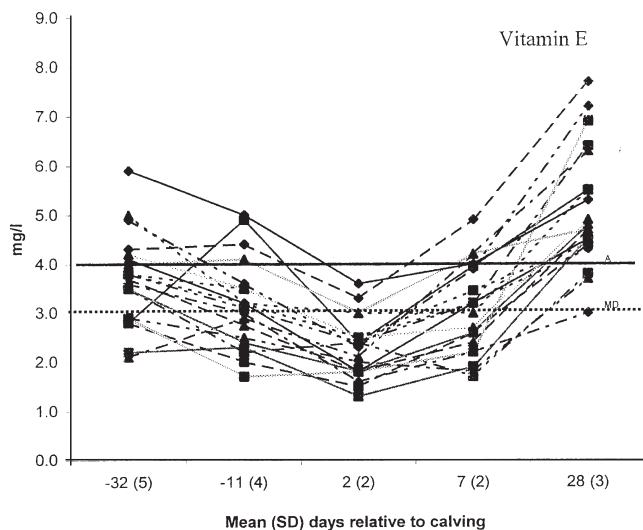


Figure 2. The concentration of serum vitamin E (mg/l) in blood samples taken from 20 dairy cows at 5 different time points relative to calving. The cows were fed low (■), medium (▲) or high (◆) amounts during the dry period. Values considered marginal or deficient (MD), and adequate (A) are indicated in the figure with horizontal lines.

Table 2. Results (p-values) of logistic regression analyses evaluating the relationship between the concentrations of serum vitamin A (S-vit A) and vitamin E (S-vit E), plasma selenium (P-Se) and serum zinc (S-Zn) in the mid dry period and the occurrence of concentrations considered marginal or deficient (MD), and adequate (A) levels after calving, in 20 dairy cows

Mean (SD) days after calving	Threshold level <sup>a</sup>	S-Vit A	Vit E	P-Se	Zn
2 (2)	MD	0.29	0.05	0.02	0.34
	A	n.a. <sup>b</sup>	n.a. <sup>b</sup>	0.05	0.75
7 (2)	MD	0.19	0.07	0.54	0.78
	A	0.08	0.09	0.14	0.79

<sup>a</sup> The MD and A levels for each micronutrient are given in the results section.

<sup>b</sup> n.a. = not analyzed; all cows had values below adequate levels.

level below MD and A, respectively, was calculated using the formula:

$$p(x) = e^{\alpha+\beta x} / (1 + e^{\alpha+\beta x}),$$

where  $\alpha$  = the intercept and  $\beta$  = the slope from the logistic regression.

In addition, the relationship between the concentration and the degree of change from mid dry period to calving was evaluated for each micronutrient using scatter plots.

## Results

Three cows, one in each feeding group, were omitted from all analyses due to missing blood samples. In addition, P-Se data from another cow in the high feeding group were missing due to technical problems. The mean (SD) days at the 5 blood samplings were 32 (5) days and 11 (4) days before calving, and 2 (2) days, 7 (2) days and 28 (3) days after calving.

### Vitamins A and E

Time had a significant ( $p < 0.001$ ) effect on the concentrations of S-vit A and S-vit E, and the lowest concentrations were found at, and just after, calving (Table 1). At calving, 50% (10/20) of the cows had vitamin A values below MD, while the corresponding proportion for vitamin E was 90% (Figs. 1 and 2). None of the

cows had S-vit A or S-vit E values above A at this time point. One week after calving, 15% (3/20) and 50% (10/20) of the cows had S-vit A and S-vit E concentration below MD, respectively, while 35% (7/20) and 20% (4/20) of the cows had values above A.

The concentration of S-vit A one month before calving did not significantly predict levels below MD at, or one week after, calving, while it tended to predict the occurrence of levels above A at one week after calving (Table 2). In contrast, the mid dry period concentration of S-vit E predicted the occurrence of levels below MD at calving. A similar tendency was observed for both MD and A at one week after calving. Based on the results, a S-vit E concentration above 5.40 and 4.42 mg/l one month before calving will, in 90% of the cows, result in concentrations above MD at calving and one week after calving, respectively. The magnitude of the S-vit A and S-vit E concentration at the mid dry sampling did not influence the degree of change from mid dry period to calving.

### P-Se and S-Zn

Both P-Se ( $p = 0.013$ ) and S-Zn ( $p < 0.001$ ) changed significantly over time (Table 1). The P-Se concentration was higher after calving than at and just before calving, while S-Zn was

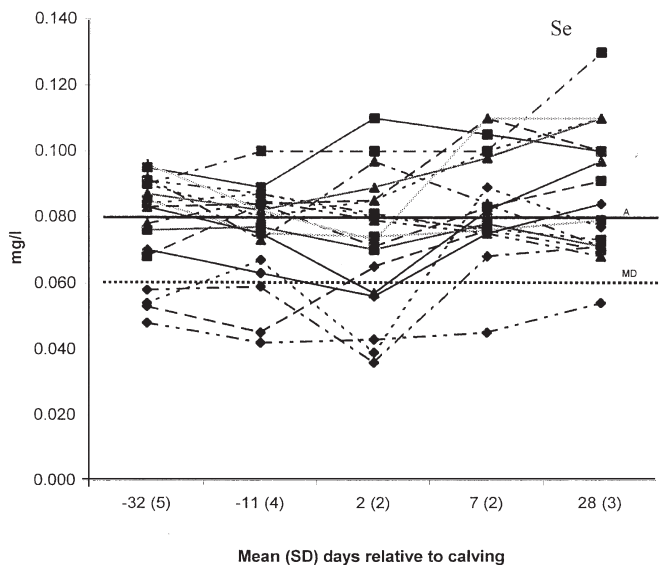


Figure 3. The concentration of plasma selenium (mg/l) in blood samples taken from 20 dairy cows at 5 different time points relative to calving. The cows were fed low (■), medium (▲) or high (◆) amounts during the dry period. Values considered marginal or deficient (MD), and adequate (A) are indicated in the figure with horizontal lines.

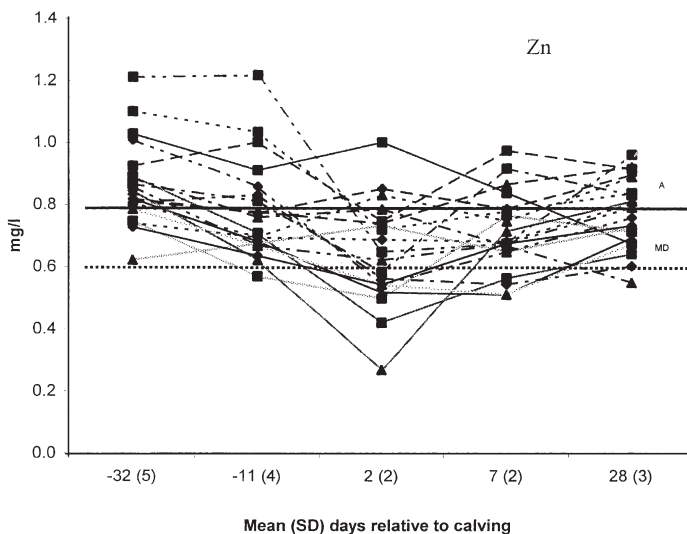


Figure 4. The concentration of serum zinc (mg/l) in blood samples taken from 20 dairy cows at 5 different time points relative to calving. The cows were fed low (■), medium (▲) or high (◆) amounts during the dry period. Values considered marginal or deficient (MD), and adequate (A) are indicated in the figure with horizontal lines.

lower at calving than one month before and after calving. Twenty-six % (5/19) and 45% (9/20) of the cows were below the MD level at calving for P-Se and S-Zn, respectively (Figs. 3 and 4). The corresponding proportions at one week after calving were 5% (1/19) and 20% (4/20), respectively. P-Se concentrations above A were found in 47% (9/19) and 53% (10/19) of the cows at calving and one week after calving, respectively. The corresponding proportions for S-Zn were 15% (3/20) and 25% (5/20), respectively.

The mid dry period sample predicted the occurrence of P-Se levels below MD and above A at calving, but no such relationship was observed for S-Zn (Table 2). A P-Se concentration above 0.09 mg/l one month before calving will, in 90% of the cows, result in a concentration above MD at calving. The magnitude of the concentrations of P-Se and S-Zn at the mid dry sampling did not influence the degree of change from mid dry to calving.

### Discussion

In the present study, the concentrations of S-vit A and S-vit E, P-Se and S-Zn were all at their lowest at calving. The most marked changes were observed for S-vit A and S-vit E, and a large proportion of the cows had S-vit A and S-vit E concentrations at calving considered marginal or deficient according to *Puls* (1994b). In addition, many cows had marginal or less than adequate concentrations one week before and one week after calving. These changes conform with earlier studies (*Michal et al.* 1994, *Weiss et al.* 1997, *Meglia et al.* 2001) and are mainly considered to be due to colostrum formation and to a decrease in dry matter intake around parturition (*Goff & Stabel* 1990, *Weiss et al.* 1990). However, the decline in S-vit E at calving may also be explained by a decreased serum lipoprotein concentration at this time (*Herd & Stowe* 1991).

A relationship between the blood concentration in the mid dry period and the occurrence of MD and A levels at calving was found for S-vit E, but not for S-vit A, and a similar tendency was also observed one week after calving. The results show that a mid dry period concentration of S-vit E at or above 5.4 mg/l will, with 90% probability, result in a concentration at calving above marginal levels if the same diet is given. However, it may be argued that a transient drop in the vitamin concentration at calving may be of less biological significance than a vitamin concentration remaining low also at one week after calving. The results indicate that a mid dry period concentration of S-vit E of 4.42 mg/l would be sufficient to give values above marginal levels at one week after calving. The reasons why a relationship between mid dry period and calving levels was found for S-vit E, but not for S-vit A, are most likely found in differences in uptake and metabolism. Vitamin A is stored for long periods in the liver and is not as sensitive to day-to-day variation as vitamin E (*Scherf et al.* 1996). As vitamin E is only stored in the liver for a few days, daily supplementation through the diet is necessary. However, vitamin E can be stored for longer periods in fat tissue (*Bjorneboe et al.* 1990).

The changes in P-Se around calving were not as dramatic as for S-vit A and S-vit E, and varied between cows. However, lower values were found just before and at calving than one month after calving. The change from dry period diet to lactation diet, and thus a larger intake of Se, is the most likely explanation for the difference. Varying results can be found in the literature in relation to changes in Se around calving (*Weiss et al.* 1990, *Miller et al.* 1995). In a previous study (*Meglia et al.* 2001), we detected a significant increase in the P-Se content at calving compared to one month before and after calving. However, the variation between time periods was small and the P-Se concentration was

comparatively low in that study. The results show that a mid dry period concentration of P-Se of  $\geq 0.09$  mg/l indicates that the concentration at calving will be above MD in 90% of the cows, given the same diet. As excess Se in the diet may have negative effects, for example by increasing the incidence of clinical mastitis (Jukola et al. 1996), overfeeding of Se should be avoided.

In line with other studies (Goff & Stabel 1990, Meglia et al. 2001), the concentration of S-Zn was lowest at calving, but there was no relationship between the mid dry period value and the concentration at calving, or one week after calving. Almost all cows had values above MD, but a minority had values considered adequate at and after calving. The drop in S-Zn is mainly due to colostrum formation and increased stress (Goff & Stabel 1990). Stress induces synthesis of metallothionein, which is associated with Zn metabolism, resulting in redistribution of Zn from blood to other tissues (Spears et al. 1991, Xin et al. 1993).

The cows were fed different amounts of feed, including micronutrients, during the dry period. As the micronutrient contents of the different feed components were not analysed, it is not possible to calculate the exact amounts given to the different groups. However, there was a marked within-group variation in blood concentrations of the micronutrients measured. This illustrates that factors other than the content in the diet are important for the uptake of micronutrients. After calving, all cows received the same diet, and had a similar dry matter intake during the first month after calving (Agenäs et al. 2003). Despite this, animals with relatively low blood levels of micronutrients before and at calving returned only slowly to adequate levels.

## Conclusions

Increasing the amount of micronutrient supplementation around parturition can reduce the risk of sub-clinical deficiencies and minimize the negative effect on health and productivity. However, a beneficial effect will only occur if the content in the diet is sub-optimal. The results indicate that analyses of S-vit E and P-Se, but not of S-vit A and S-Zn, in mid dry period blood samples can be used as a tool to evaluate the need for extra supplementation of vitamin E and Se during the periparturient period. If the mid dry period blood concentration of S-vit E and SeP is  $\geq 5.4$  mg/l and  $\geq 0.09$  mg/l, respectively, the cow has a 90% chance of staying above marginal levels at calving given that a feed of the same quality is offered.

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### Svensk sammanfattning

*Bedömning av vitamin A-, vitamin E-, selen- och zinkstatus hos kor runt kalvning med hjälp av blodprov tagna i mitten av sinperioden.*

Vitamin A och E, och spårelementen selen (Se) och zink (Zn) är viktiga för mjölkornas hälsa och produktion. Koncentrationen av dessa ämnen minskar runt kalvning och extra tillskott rekommenderas ibland under denna tid. Behovet av detta varierar dock till exempel beroende på fodrets kvantitet och kvalitet. Syftet med denna studie var att mäta koncentrationen av vitamin A (S-vit A), vitamin E (S-vit E), Se (P-Se) och Zn (S-Zn) i blodprov tagna vid flera tidpunkter från en månad före till en månad efter kalvning, och att utvärdera om ett blodprov taget mitt i sinperioden kan förutsäga blodkoncentrationen vid kalvning och tidig laktation. Mjölkkor som utfodrades med 3 olika mängder av samma foderstat under sinperioden ingick i studien. En markant minskning i koncentrationen av S-vit A och S-vit E, och till en mindre del i koncentrationen av P-Se och S-Zn, sågs

runt kalvning. Blodkoncentrationen av S-vit E och P-Se mitt i sintiden kunde signifikant förutsäga förekomst av värden vid kalvning som indikerar marginell nivå eller brist. Data tyder på att om blodprovet

mitt i sintiden innehåller  $\geq 5.4$  mg/l S-vit E och  $\geq 0.09$  mg/l P-Se har kon 90% chans att nå högre nivåer vid kalvning än vad som anses marginellt om ett foder av samma kvalitet ges.

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