BioAnalyt

Rapid Measurement of Vitamin E and ß-Carotene at Cow-Side with iCheck

Vitamin E and ß-carotene are key to cattle fertility and healthy immune system

What is the role of ß-carotene in cattle?

ß-carotene is an important precursor of vitamin A and is closely associated with cattle fertility and health. It is also an antioxidant and can directly enhance immunity. Reproductive disorders resulting from ß-carotene deficiency bear significant costs for cattle breeders. Between a quarter and half of all herd cullings are caused by these disorders. The majority of basic feedstuff for dairy cows is low in ß-carotene. In pastures, ß-carotene content is highly influenced by the season. Thus, blood levels in cattle are low in the winter season and high in the summer when cattle have access to fresh pasture[1].

Maintaining high levels of blood ß-carotene in the immediate pre-calving period significantly improves subsequent fertility rates[2]. Researchers demonstrated that ß-carotene plasma levels before calving determined when cows produce the first dominant follicle after calving. Irrespective of post-calving plasma levels, ovulating cows had significantly higher concentrations of plasma ß-carotene in the last three weeks of the dry period, whilst the anovulatory cows had a lower status (Fig 1).

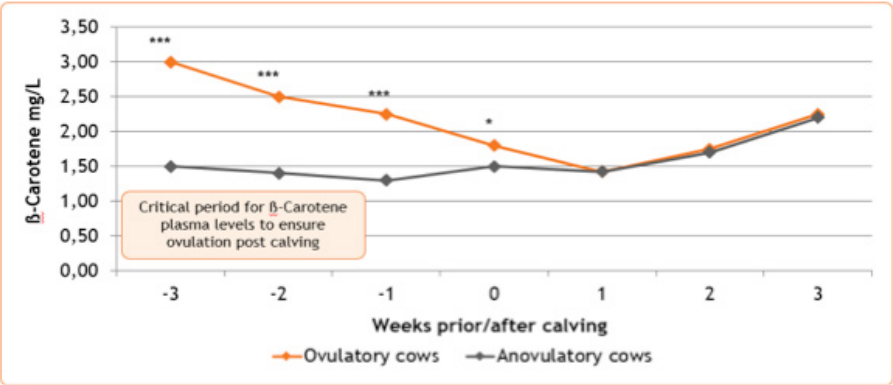


Figure 1: Importance of maintaining high plasma ß-carotene concentrations in the immediate pre-calving period on subsequent ovulation rates[2].

What are the optimal levels of ß-carotene?

When measured in cattle blood, the optimal concentration of ß-carotene is from 3.5 mg/L and up. When blood levels are below 1.5 mg/L, it is defined as deficient and supplementation of 500 mg ß-carotene/cow/day is recommended. For marginal levels defined as 1.5 – 3.5 mg/L, the recommended supplementation is 300 mg ß-carotene per cow per day[1].

What is the role of vitamin E in cattle?

Vitamin E is essential for the cattle's immune system. Similar to ß-carotene, it is an antioxidant. It also plays a role in lactation and improves milk yield when combined with selenium[3]. Vitamin E is abundant in whole cereal grains, particularly the germ; however, it is extremely variable in common feeds. As with ß-carotene, vitamin E blood levels in cattle are low in the winter and high in the summer when cattle have access to fresh pasture[1].

Plasma vitamin E (measured as **α**-tocopherol) levels around calving decline by about 50% and reach levels that would be diagnostic of chronic deficiency (Fig 2). The loss is partly due to sequestration in colostrum. But vitamin E is also consumed at a higher rate because of increased immunologic and metabolic stress before calving. Lower levels also lower resistance to disease.

Cows with low plasma vitamin E levels before calving have a 9 times higher probability of clinical mastitis compared to cows with an acceptable blood level[4]. This was recognized by the National Research Council Committee for Animal Nutrition in the USA (NRC) already in 2001. In its supplementation guidelines, NRC recommends higher dietary vitamin E levels for transition and lactating cows to maintain the minimum plasma vitamin E concentration for optimum immune response.

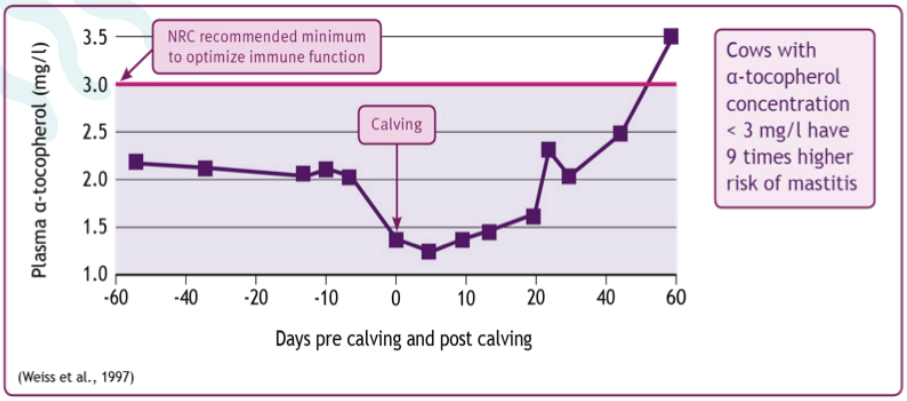


Figure 2. Minimum plasma vitamin E levels recommended by the NRC (2001) to reduce the incidence of mastitis.

What are the optimal levels of vitamin E?

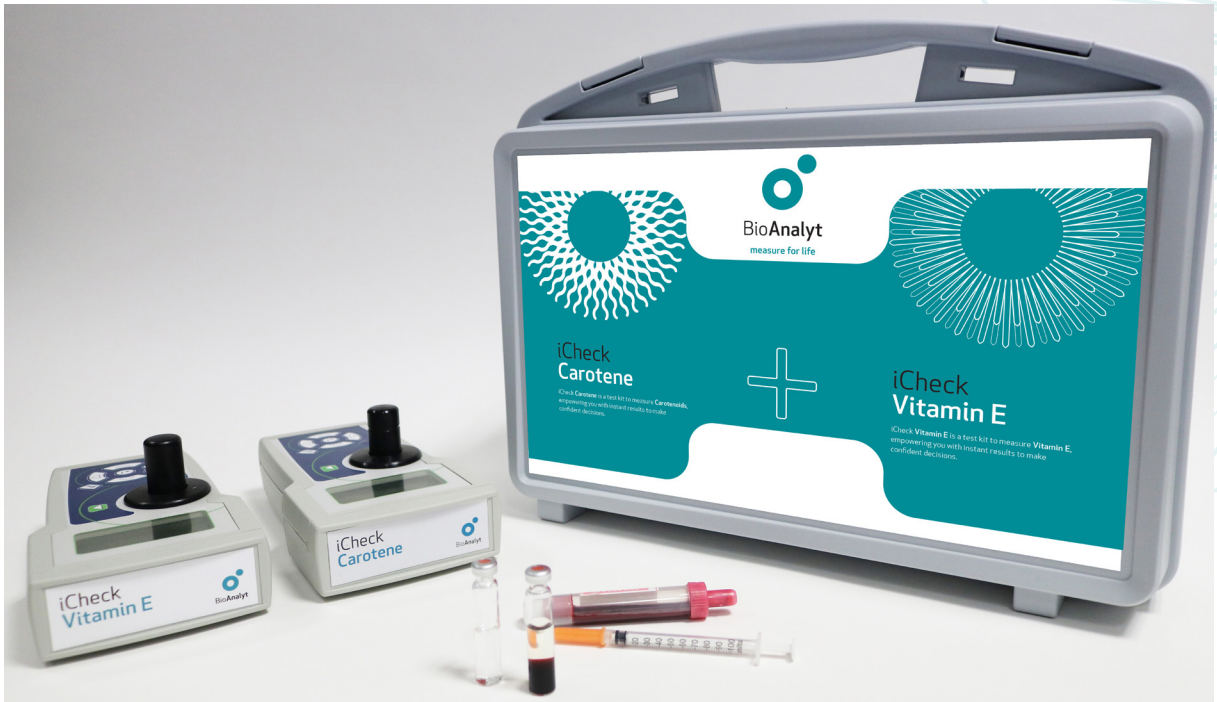
NRC recommends that a minimum of 3.0 mg/L of vitamin E in cattle blood is maintained. When blood levels are below 1.0 mg/L, it is defined as deficient. Optimal vitamin E supplementation for a dairy cow is 1,000 mg of dl-**α**-tocopheryl acetate per day per cow during the dry far-off period (when the cow is not being milked), 2,000 mg per day during the pre-fresh period (close to calving, usually about two weeks to due date) and 500 mg per day during lactation for optimum reproduction and udder health[5].

An innovative and rapid method for vitamin analysis at cow-side

To ensure optimal dietary intake of ß-carotene and Vitamin E, cattle feed is supplemented with both. In order to determine the right levels of supplementation in the feed, the status of ß-carotene and α-tocopherol (vitamin E) in cattle must be measured.

Traditional methods for estimating ß-carotene levels in blood include color charts, giving qualitative results, and spectrophotometry or high-performance liquid chromatography (HPLC), giving quantitative results. Vitamin E can only be measured with HPLC. These methods, however, have significant limitations. Color deviations due to hemolysis or increased bilirubin levels often lead to an overestimation of the ß-carotene concentration, especially in the marginal range critical for diagnosis. Spectrophotometric and HPLC methods provide highly accurate results, but require extensive sample preparation steps and expensive equipment in a laboratory setting.

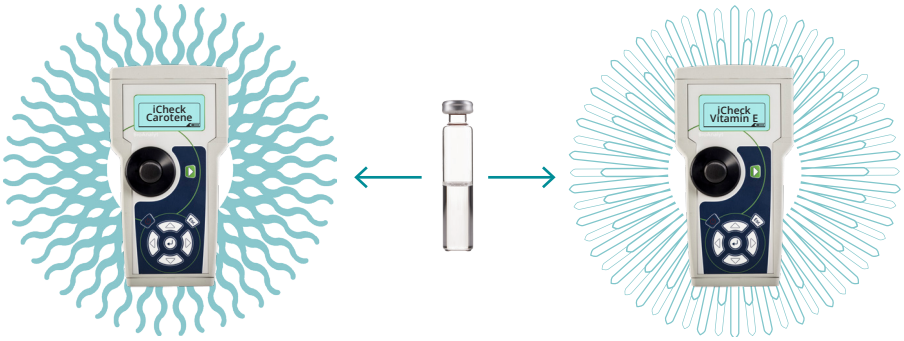
To fill this gap, BioAnalyt GmbH, in cooperation with DSM Nutritional Products, developed iCheck Carotene and iCheck Vitamin E. These are portable devices for rapid assessment of ß-Carotene and vitamin E in whole blood and plasma. iCheck delivers lab-level accuracy at a fraction of the cost of lab-bound methods and can be used directly on the farm.



How it works

Vitamin levels in blood measured in under 10 minutes

iCheck consists of 2 parts: a ready-to-use reagent vial and a portable measuring device.

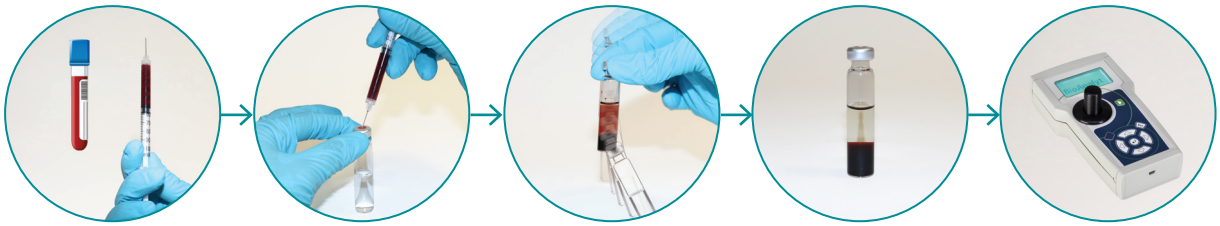


iCheck Carotene is a portable photometer. The device measures total carotenoids and displays the result in mg/L.

iCheck Reagent Vial contains a precise amount of solvents optimized for the extraction of Vitamin E & ß-carotene. Same vial may be measured with iCheck Carotene & iCheck Vitamin E.

iCheck Vitamin E is a portable fluorometer. The device measures vitamin E and displays the result in mg/L.

HOW DOES IT WORK?



Whole blood is collected*, and exactly 0.5 mL of blood sample is taken up into the provided syringe.

Sample is directly injected into the iCheck Reagent Vial.

Reagent Vial with blood sample is vigorously shaken for a minimum of 10 seconds.

The vial must now stand still for a minimum of 5 minutes for vitamin extraction and phase separation.

The vial is inserted into either iCheck Vitamin E or iCheck Carotene** is determined.

* Anti-coagulants, such as heparin or EDTA used during blood collection do not interfere with the measurement.
** When 0.5 mL sample is used and the reading is made with iCheck Carotene, the displayed result must be multiplied with 0.8. For details contact support@bioanalyt.com.

iCheck Vitamin E and iCheck Carotene Technical Data

TECHNICAL DATA		
Sample	iCheck Carotene	iCheck Vitamin E
Analyte:	Total carotenoids	Alpha tocopherol
Sample:	Food: Premix, roots (i.e. cassava), beverages, eggs, salmon; Biological fluids: colostrum, cattle whole blood & serum	Cattle serum and whole blood
Sample preparation:	For solid or highly concentrated samples: dilution in distilled or bottled water	Not necessary
Sample volume per analysis:	0.4 mL (400 µL)	0.5 mL (500 µL)
Device		
Analytical method:	Photometric determination of total carotenoid concentration using absorption at 450 and 525nm.	Autofluorescence of alpha tocopherol
Units displayed:	mg/L	mg/L
Linear range:	0.15 - 15.00 mg/L	1.0–25.0 mg/L
Calibration:	Factory set (standards included for control)	Required if Vitamin E Standard values deviate from the linear range
Time per analysis:	< 10 min	
Environment:	20 –30°C, no direct sunlight	
User training:	1 day training	
Use:	Laboratory and field	
Data output:	Sample #, Batch #, Result, Date, Time (in transferred data)	
Connectivity and data:	Results are stored in the device and transferred to a PC via USB	
Power source:	NiMH rechargeable batteries included; AA 1.2 or 1.5V	
Warranty:	2 years	
Device weight:	0.45 kg	
Device dimensions:	11 x 4 x 20 cm (W x H x L)	
Test Kit		
Content:	100 reagent vials; 100 syringes - 1.0 mL; 100 needles	
Chemical composition:	n-Hexan and alcohols	
Volume per reagent vial:	2.0 mL	
Shelf life:	12 months at 20 –30°C, no direct sunlight, upright	
Dimension of test kit:	26 x 14.5 x 16.5 cm	
Disposal instructions:	Hazardous waste	

Lab-level accuracy in minutes and at 10% of lab costs

Comparison of iCheck Carotene and iCheck Vitamin E to HPLC reference method.

The iChecks have been validated against standard method of HPLC. During the validation, both whole blood and serum were measured on iChecks, while serum was only used for HPLC measurements. All whole blood results have been adjusted by a factor of 32% based on the average hematocrit level in cattle.

There was a positive Pearson correlation (R2 = 0.95) between the HPLC method and iCheck Vitamin E for the measurement of vitamin E in the blood of dairy cattle and calves (Fig 3 A). This strong correlation between the two methods was reflected in the Bland-Altman plot for vitamin E measurement using the two methods, as shown in Figure 3 B. The Bland-Altman plot showed that the differences in vitamin E values were not outside the 95% acceptability limits, suggesting no significant systematic error between the two methods (unpublished data).

For the measurement of ß-carotene, a strong positive Pearson correlation (R2 = 0.97) also exists between HPLC and iCheck Carotene for the measurement of ß-carotene in the blood of dairy cows and their calves (Fig 3C). This was in agreement with an earlier study that obtained a correlation of 0.98 between the two methods[6].

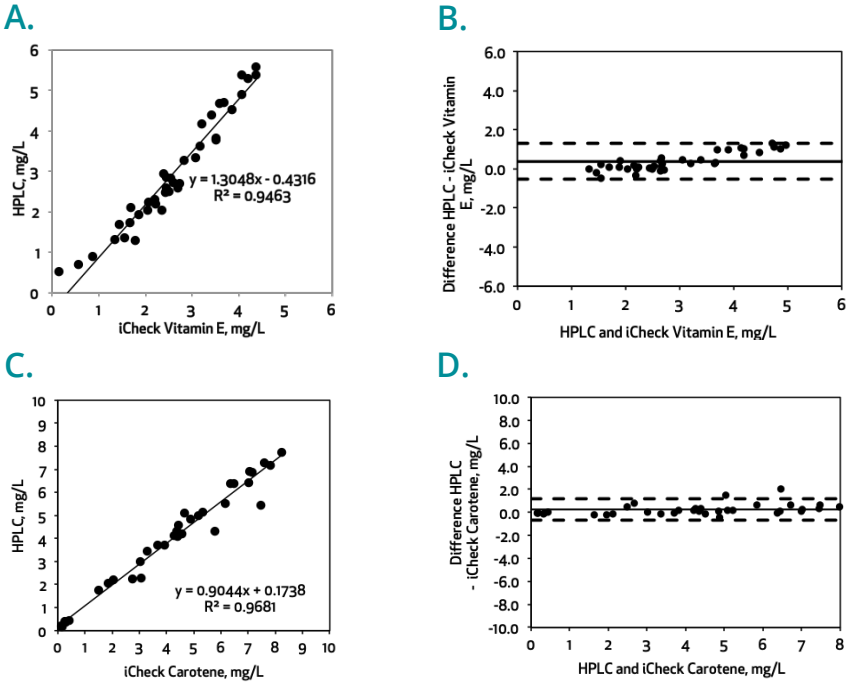


Figure 3: Pearson correlation and Bland-Altman plots showing the relationship between values in blood plasma obtained by HPLC and iCheck Vitamin E in dairy cow and calves (1A and 1B). Pearson correlation and Bland-Altman plots showing the relationship between ß-carotene values in blood plasma obtained by HPLC and iCheck Carotene in dairy cow and calves (1C and 1D).

Benefit

Optimized fertility, milk production and disease resistance

With iCheck devices vitamin E and ß-carotene blood levels can be rapidly measured on the farm, allowing for direct optimization of the nutritional interventions together with relevant stakeholders such as farmers, feed consultants and veterinarians.

- **Speed:** results in less than 10 minutes vs. hours with traditional lab methods, or days if the samples must be shipped to external lab.
- **Economy:** cost is only 10% of conventional lab methods taking into account equipment and personnel costs
- **Easy implementation:** only 1-day training required
- **Scalability:** no set up calibration required to operate*

If you would like to know more about the validation study, make a purchase of the iCheck, or get hands-on training regarding the use of the devices please contact us on:

Email: contact@bioanalyt.com
WhatsApp: +49 1573 1111234

*iCheck Vitamin E only requires a manual calibration if Standard falls outside the linear range by more than 10%

References

1. Production and Reproduction response of Dairy Cows to Supplemental Beta Carotene.
   De Ondarza M. B., et al. Penn State Dairy Cattle Nutrition Workshop, 2009.
2. Relationship between plasma ß-carotene concentrations during the peripartum period and ovulation in the first follicular wave postpartum in dairy cows. Kawashima C., et al. Animal Reproduction Science. Vol. 111 (1), 2009.
3. The Technical and Financial Effects of Parenteral Supplementation with Selenium and Vitamin E during Late Pregnancy and the Early Lactation Period on the Productivity of Dairy Cattle. Bayril T., et al. Asian Australas. J. Animal Science, Vol. 28 (8), 2015.
4. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. Weiss W. P., et al. J. of Dairy Science, Vol. 80, 1997.
5. How Much Supplemental Vitamins do Cows Really Need? Weiss B. Department of Animal Sciences, The Ohio State University. Proceedings from the Tri-State Dairy Nutrition Conference, April 16 - 18, 2018.
6. Determination of ß-carotene in whole blood of cattle: Comparison of a new cow-side assay with HPLC. Raila J., et al. Vet. Clinical Pathology, Vol. 1 (3), 2011.

Detailed information available here:

- https://www.dsm.com/anh/products-and-services/products/vitamins/vitamin-e.html
- https://www.dsm.com/anh/products-and-services/products/vitamins/vitamin-a.html

iChecks are manufactured in Germany, used in over 80 countries and validated against standard laboratory methods. Learn more at www.bioanalyt.com/products