

## Seasonal Variation in Bovine Luteal Concentrations of $\beta$ -Carotene

Şevket ARIKAN

Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale - TURKEY

Richard G. RODWAY

Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2, 9JT-UK

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**Abstract :**  $\beta$ -Carotene is present in extremely high concentrations in bovine corpus luteum. The aim of this study was to examine the extent of the seasonal variation in concentrations of luteal  $\beta$ -carotene in cows.

A total of 102 corpora lutea collected throughout all 12 months of the year were analysed by high performance liquid chromatography (HPLC). The mean  $\beta$ -carotene concentration of the luteal tissues was  $58.7 \pm 4.5 \mu\text{g/g}$ . Bovine corpora lutea collected in the warmer months (May-Oct.) contained significantly ( $P < 0.01$ ) higher  $\beta$ -carotene concentrations than those corpora lutea collected in the cold months (Nov-Apr). However, the  $\beta$ -carotene concentrations of luteal tissues were highly variable among the animals used in the study. The  $\beta$ -carotene content of individual bovine corpora lutea ranged from  $5.8 \mu\text{g}$  to  $216 \mu\text{g}$  per gram of tissue.

The results of the present study show the fact that bovine corpora lutea collected in summer contained more  $\beta$ -carotene than those collected in winter, indicating the existence of a feed-dependent factor on  $\beta$ -carotene accumulation in bovine luteal tissue.

**Key Words:** Carotene, corpus luteum, bovine.

### İnek Luteal Dokusunun İçerdiği $\beta$ -Karoten Miktarında Gözlenen Mevsime Bağlı Değişimler

**Özet :** İnek korpus luteumunun çok yüksek konsantrasyonda  $\beta$ -karoten içermesi dikkate alınarak, bu çalışmada ineklerin luteal dokusundaki  $\beta$ -karoten konsantrasyonlarında, mevsime bağlı olarak meydana gelen varyasyonların incelenmesi amaçlanmıştır.

Yılın 12 ayı boyunca toplanan toplam 102 adet korpus luteum yüksek performanslı sıvı kromatografisi (HPLC) kullanılarak analiz edildi. Analizi yapılan bu dokulardaki  $\beta$ -karoten konsantrasyonu ortalaması  $58.7 \pm 4.5 \mu\text{g/g}$  olarak bulundu. Sıcak geçen aylarda (Mayıs-Ekim) toplanan korpus luteumlar, soğuk aylarda (Kasım-Nisan) toplanan korpus luteumlara oranla daha fazla  $\beta$ -karoten içermekteydi ( $P < 0.01$ ). Bununla beraber, bu çalışmada kullanılan luteal dokuların  $\beta$ -karoten konsantrasyonlarının hayvanlar arasında oldukça değişkenlik gösterdiği gözlemlendi. İneklerin korpus luteumlarının  $\beta$ -karoten içeriği bireysel olarak  $5.8 \mu\text{g/gr}$  ile  $216 \mu\text{g/gr}$  arasında değişmekteydi.

Sonuç olarak yaz aylarında toplanan korpus luteumların kış aylarında toplananlardan daha fazla  $\beta$ -karoten içermesinden hareketle, bu çalışmadan ineklerin luteal dokusunda  $\beta$ -karotenin birikmesinde mevsime bağlı beslenme faktörlerinin rolü olduğu sonucuna varılmaktadır.

**Anahtar Sözcükler:** Karoten, korpus luteum, inek

### Introduction

$\beta$ -Carotene is present in extremely high concentrations in the bovine corpus luteum (CL) (1), giving the CL its characteristic bright yellow colour. As well as acting as a precursor for vitamin A, there is increasing evidence that  $\beta$ -carotene may be necessary for optimal steroid production (2), possibly acting as an anti-oxidant (3, 4).

Carotenoids are present in tissues of green plants. The most common carotenoid in all green plants is  $\beta$ -carotene. Animals cannot synthesise  $\beta$ -carotene *de novo*, and they depend on feed for their supply. Therefore, concentrations of  $\beta$ -carotene in bovine milk fat and serum vary depending on the season and nutritional background of the animals (5, 6, 7).

It is evident from the literature that breed also has a marked effect on the  $\beta$ -carotene content of the blood of cows (8, 9, 10). Of the more common breeds, Guernseys appear to have the highest level of  $\beta$ -carotene in their blood with Jerseys having the next highest concentration. Holsteins appear to have somewhat less  $\beta$ -carotene in comparison to the Guernsey and Jersey breeds (8, 9).

The amount of  $\beta$ -carotene in grasses varies with plant species and stage of growth. The level of  $\beta$ -carotene in pasture grass, corn, alfalfa, etc. is highest in the young plant but decreases as the plant becomes older (11). Growth in grass varies with the season and in the UK green plants grow only during a limited period of the year. It is, therefore, necessary to preserve green plants in some form for winter feeding. This can be achieved either by drying (naturally in the field) or by chemical preservation in the form of silage. However, the level of carotenoids in feedstuffs such as hay, straw, fodder etc. suffers serious depletion during the preservation process and storage (12, 13, 14). The concentration of carotene is highest in fresh plants and immediately after harvesting it begins to fall (15, 16). The reduction occurring after harvesting is rapid immediately after cutting, and the rate gradually becomes slower with time (14, 17). The carotene content of freshly cut plant tissues may fall by more than half within 24 h (13). The aim of this study was to determine the extent of seasonal variation in concentrations of luteal  $\beta$ -carotene of cows.

## Materials and Methods

This experiment was designed to investigate the seasonal variation of  $\beta$ -carotene accumulation in bovine corpora lutea. Therefore, every month, heifer ovaries were collected immediately after slaughter from a local abattoir in Leeds, UK and transported to the laboratory within 30-40 min on ice. The ovaries were then stored immediately at  $-40\text{ }^{\circ}\text{C}$  until they were analysed for  $\beta$ -carotene content. Corpora lutea judged to be in the early-to-mid luteal phase were used.

A total of 102 corpora lutea collected throughout all 12 months of the year were analysed by HPLC. Tissue extraction was performed by a slight modification of the method of Schmitz et al. (18). Briefly, 500 mg luteal tissue was added to a 15 ml glass tube wrapped in aluminium foil to exclude light. Samples were homogenised for 1.5 min in 2 ml 1% aqueous sodium

ascorbate and 2 ml absolute ethanol. Subsequently, 1 ml of a saturated aqueous KOH solution was added to the samples, which were then vortexed and saponified at  $60^{\circ}\text{C}$  for 30 min. The tube was then cooled in a beaker of ice and extracted three times with 3 ml of hexane. Finally, the combined extracts were evaporated under nitrogen, and the residue was reconstituted with ethanol. Both organic solvents used in the extraction, ethanol and hexane, contained 0.01% BHT as an antioxidant.

All solvents used were of HPLC grade. The HPLC system used for  $\beta$ -carotene analysis consisted of a Spectra-physic SP8700 solvent delivery system and an SP8750 manual injector (Spectra-physic, San Jose, CA, USA). Samples (50  $\mu\text{l}$ ) were injected onto a 10  $\mu\text{m}$ , spherical, C-18 reverse-phase column (30cm x 3.9 mm; Resolve, Millipore) and eluted with a mobile phase consisting of 50:45:5 (vol/vol/vol) methanol:acetonitrile:tetrahydrofuran (19), with a flow rate of 2 ml/min.  $\beta$ -Carotene was detected at a wavelength of 452 with a Spectraflow 757 model variable wavelength detector (Kratos Analytical Instruments, USA). Peak areas were compared to those of authentic standards for quantification.

Analysis of variance (ANOVA) was used to assess the seasonal differences in  $\beta$ -carotene concentration. Significance was defined as  $P < 0.05$ . Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS). All results are reported as means  $\pm$  SEM.

## Results

The mean  $\beta$ -carotene concentration of luteal tissues collected from 102 heifers throughout the 12 months of the year was  $58.7 \pm 4.5\text{ }\mu\text{g/g}$  ( $\pm$  = SEM). Concentrations of  $\beta$ -carotene were lowest in those luteal tissues collected in January ( $34.5 \pm 8.2\text{ }\mu\text{g/g}$ ) and highest in those collected in September ( $83.4 \pm 25.5\text{ }\mu\text{g/g}$ ) (Figure 1). There were significant differences ( $P < 0.01$ ) between January and September in terms of  $\beta$ -carotene concentrations in the corpus luteum. Bovine corpora lutea collected in the warmer months (May-Oct.), when pasture or green forage provides ample amounts of  $\beta$ -carotene, contained significantly ( $P < 0.01$ ) more  $\beta$ -carotene than those corpora lutea collected in the cold months (Nov-Apr), when the  $\beta$ -carotene supply is usually low (Figure 2). The mean concentrations of  $\beta$ -carotene were  $73.8 \pm 8.2$  and  $44.8 \pm 3.4\text{ }\mu\text{g/g}$  in the warmer and cold months

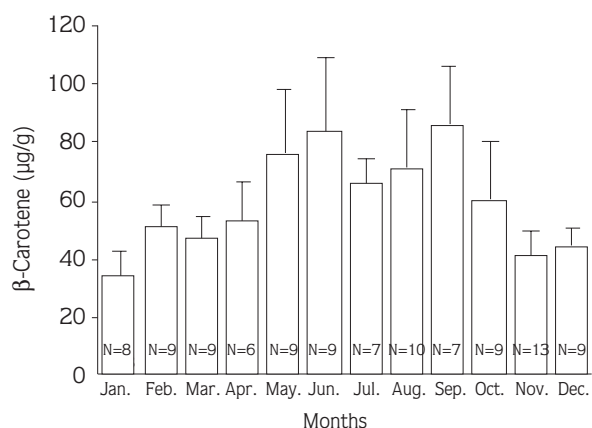


Figure 1. The monthly variations in the concentrations of  $\beta$ -carotene in bovine luteal tissue. ( $\pm$  = SEM). N = Number of animals.

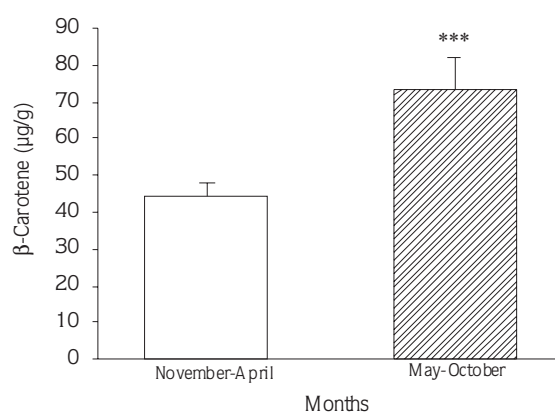


Figure 2. The seasonal variations in the concentrations of  $\beta$ -carotene in bovine luteal tissue. ( $P < 0.01$ ). ( $\pm$  = SEM).

respectively. However,  $\beta$ -carotene concentrations were highly variable among the animals used in the study. The  $\beta$ -carotene content of individual bovine corpora lutea ranged from 5.8  $\mu\text{g}$  to 216  $\mu\text{g}$  per gram of wet tissue.

## Discussion

The evidence from the present study shows that bovine corpora lutea collected in summer contained more  $\beta$ -carotene than those collected in winter, suggesting that seasonal changes in diet may affect  $\beta$ -carotene accumulation in luteal tissues. This is in agreement with previous studies in which the relationship between the season and  $\beta$ -carotene concentrations in bovine plasma has been demonstrated (6, 7). These studies showed that the plasma collected in winter contained less  $\beta$ -carotene than that collected in summer.

$\beta$ -Carotene concentrations of luteal tissues were highly variable among the heifers used in the present study. Little is known about the possible effects of stages of the oestrous cycle on luteal  $\beta$ -carotene content, but as all tissue used was from approximately the same stage of the cycle, this should not have affected the results obtained. A positive correlation between the plasma concentration of  $\beta$ -carotene and corpus luteum size has been reported by previous researchers (21, 22). A similar correlation between the luteal concentration of  $\beta$ -

carotene and the size of the corpus luteum might exist. If it is valid, variation among the corpora lutea might be attributed partly to the corpus luteum size.

The nutritional backgrounds of the animals may also have been different. The variation in  $\beta$ -carotene concentration observed may also reflect breed-dependent differences, which have been demonstrated by measuring the concentration of  $\beta$ -carotene in serum collected from different breeds of animals given the same diet (8, 9, 10, 20).

The ovaries were collected from an abattoir, therefore, we had no knowledge of the feeding background or breed of the heifers used in this study as a source of luteal tissue. The results presented in this study seem to reflect the availability of  $\beta$ -carotene in the feedstuff. Animals cannot synthesise  $\beta$ -carotene *de novo*, and they depend on feed for their supply. Therefore, concentrations of  $\beta$ -carotene in bovine corpora lutea seem to vary depending on the season and nutritional background of the animals. The rapid loss in the  $\beta$ -carotene concentration occurs during the storage of feedstuff in winter. It has been reported that the concentration of  $\beta$ -carotene in feedstuff decreases from 300 ppm to 10 ppm by the end of winter (23). All of these indicate the existence of a feed-dependent factor in  $\beta$ -carotene accumulation in bovine luteal tissue.

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