Effects of a dietary supplement of β-carotene given during the dry period on milk production and circulating hormones and metabolites in dairy cows

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SUMMARY

The objective of this study was to investigate whether a supplement of β-carotene given during the dry period is able to 1) improve milk production and milk composition and 2) modify hormone and metabolic status in dairy cows during the dry and postpartum periods. This study was conducted using 40 Holstein, primiparous and multiparous cows. On the day of drying-off, cows were allocated to one of two dietary treatments: control diet (n=20) or control diet plus 1g/cow/d β-carotene (n=20). The β-carotene supplement was given individually to the cows until calving. Blood samples were obtained regularly and the concentrations of β-carotene in blood and circulating metabolites and hormones in plasma were measured. Live weight and body condition score (BCS) were monitored once a month. Daily milk production was recorded starting 5 days after calving. Milk composition was measured every 15 days. The dietary supplement of β-carotene increased blood concentrations of β-carotene during the dry period (*P<0.05*) and although the difference decreased postpartum β-carotene concentrations remained higher compared to the control group until the end of experiment. Live weight, BCS, milk production and composition: milk protein, milk fat, milk urea and somatic cell count, were unaffected by treatment (*P>0.05*). Plasma concentrations of insulin, insulin-like growth factor-1, glucose, non-esterified fatty acids and urea were unaffected by dietary supplementation with β-carotene (*P>0.05*). In conclusion, supplementation with β-carotene during the dry period increased blood concentrations of β-carotene but had no effect on milk production and milk quality nor on hormone and metabolic status.

Keywords: β-carotene, supplement, dairy cow.

RÉSUMÉ

Effets d'une supplémentation en β-carotène pendant le tarissement sur la production laitière ultérieure et les concentrations circulantes d'hormones et de métabolites chez la vache laitière

Les objectifs étaient d'étudier, chez des vaches laitières, l'influence d'une supplémentation en β-carotène pendant le tarissement sur 1) la production laitière et la qualité du lait et 2) le statut hormonal et métabolique pendant le tarissement et après la mise-bas. Quarante vaches Holstein primipares et multipares ont été réparties en deux lots le jour du tarissement. Le groupe contrôle (n=20) a reçu un aliment témoin et le groupe β-carotène (n=20) a reçu le même aliment supplémenté d'1g/vache/j de β-carotène distribué individuellement jusqu'à la mise-bas. Des échantillons de sang ont été prélevés régulièrement afin de mesurer la concentration sanguine en β-carotène et la concentration plasmatique d'hormones et de métabolites. Le poids vif et la note d'état corporel ont été mesurés une fois par mois. La production laitière journalière a été enregistrée à partir de 5 jours après la mise-bas et la composition du lait a été mesurée tous les 15 jours. La supplémentation en β-carotène a augmenté les concentrations sanguines de β-carotène pendant le tarissement (*P<0,05*). Bien que la différence ait diminué après la mise-bas, les concentrations en β-carotène sont restées plus élevées que chez les témoins jusqu'à la fin des mesures. Aucun effet de la supplémentation en β-carotène n'a été observé sur le poids vif, la note d'état corporel, la production de lait ou sa composition (TP, TB, urée et taux de cellules) (*P>0,05*). Les concentrations plasmatiques d'insuline, d'IGF-1, de glucose, d'AGNE et d'urée n'ont pas été affectées par le traitement (*P>0,05*). En conclusion, une supplémentation en β-carotène pendant le tarissement augmente les concentrations sanguines de β-carotène mais n'a pas d'effet sur la production laitière et la composition du lait ni sur le statut hormonal et métabolique.

Mots clés : β-carotène, supplémentation, vache laitière.

Introduction

In the dairy cow, the peripartum period is a stressful time due to the dramatic physiological and metabolic adaptations required during the change from pregnancy to lactation. Energy demand is increased due to the need to meet the requirements for rapid foetal growth and milk production. During this period, cow immunity is suppressed leading to an increase in susceptibility to a number of diseases. It has been reported that neutrophil function before parturition is impaired [1] and this has been linked to mammary gland and uterine infection. The changes in metabolism associated with rapid foetal growth, parturition and initiation of lactation results in increased production of free radicals. When the production of free radicals exceeds the antioxidant defence mechanisms present in the body, the cow is in oxidative stress. Oxidative stress in

periparturient cows may be a contributory factor for disease susceptibility. Independent of its role as provitamin A, β-carotene may affect immune function. β-carotene has been found to enhance immune function through its ability to regulate membrane fluidity, gap junction communication and as an antioxidant [2, 3, 4]. *In vitro* studies showed that β-carotene enhanced bovine blood and mammary gland phagocytic cell killing ability and enhanced lymphocyte proliferation induced by a mitogen [5, 6, 7]. β-carotene is a crucial member of a group of molecules which are free radical scavengers. β-carotene and other carotenoids are especially effective at quenching singlet oxygen and can prevent the subsequent formation of secondary reactive oxygen species (ROS) [8]. CHAWLA and KAUR [9] reported a positive correlation between plasma β-carotene concentrations and antioxidant power measured by a ferric reducing antioxidant power assay in cows supplemented with β-carotene during the dry period. They also suggested that there is a need to give cows a β-carotene supplement during the dry period in order to improve their plasma antioxidant status and health after parturition and to improve milk production and quality due to reduced mammary gland infection. Negative energy balance commonly occurs in the early postpartum period and reaches a maximum during the first or second week postpartum [10, 11]. Similarly, plasma concentrations of β-carotene and vitamin E in non-supplemented cows decrease throughout the dry period and reach their lowest levels during the first week postpartum [7, 12]. Thus, the decrease in plasma concentrations of β-carotene could be associated with the occurrence of metabolic and infectious diseases during the peripartum in dairy cows. Additionally, the concentrations of several metabolites and metabolic hormones are modified by the adaptations which occur around calving. However, there have been few reports on the effect of supplemental β-carotene on milk production and quality and on blood metabolites in peripartum cows. Therefore, this study aimed to investigate whether a supplement of β-carotene given during the dry period is able to 1) improve milk production and milk composition and 2) modify hormone and metabolic status in dairy cows during the postpartum period.

Material and Methods

ANIMALS AND MANAGEMENT

Forty high-producing Holstein, primiparous and multiparous, cows were used in the experiment (average annual milk production 10 000kg). Cows were dried off 60 days before presumed calving date. On the day of drying-off cows were paired according to live weight, BCS, age, milk production level of previous lactation, expected calving date, and blood β-carotene concentrations and randomly allocated to one of two dietary treatments: control diet (C group: n=20) or control diet plus 1 g/cow/d β-carotene (BC group: n=20, DSM Nutritional Products Ltd., Paris, France). Treated and control cows were housed together in the same barn on straw bedding where they received a total mixed ration (TMR), formulated to meet average requirements for maintenance and production [13]. Three different diets were formulated (Table 1) and fed to the cows depending on their requirements (1st or 2nd month of the dry-period or lactation). The cows were given fixed quantities (limited compared to appetite) of diet during the dry-period and the lactation diet was given *ad libitum*. The diets contained a vitamin and mineral mix which covered vitamin A requirements. Cows had free access to water and salt licks. Before giving the meal in the morning the cows were restrained with head-lock stanchions and the β-carotene supplement was given individually to the cows by top-dressing on 500 g rapeseed meal. Cows in the control group only received the rapeseed meal. Once the treatments had been eaten the diet was distributed. The basal concentrations of β-carotene in the 1st and 2nd month dry-cow diet, lactating-cow diet and in the rapeseed meal were 2.35, 4.32, 3.72 and 0.63 mg/kg dry matter, respectively.

BLOOD SAMPLING

Blood samples were obtained at -8, -6, -4, -2 weeks before calving, at calving and at 1, 2, 3, 4, 6, 8 and 10 weeks after calving by caudal venipuncture before the morning feed. Blood was collected into 9 mL heparin-coated tube and placed immediately in ice-cooled water. Blood concentrations of β-carotene were measured and the sample was then centrifuged for 10 min at 4ºC and 2 500 rpm. Plasma was collected and frozen (-20ºC) until required for metabolite and hormone assay.

BLOOD CONCENTRATIONS OF β-CAROTENE

On-farm measurement of blood concentrations of β-carotene was performed on the day of sampling. The concentrations of β-carotene in blood were determined as previously described [14] after extraction of β-carotene (iExTM) and measurement with a carotene photometer (iCheck™; BioAnalyt GmbH, Germany).

MILK YIELD AND MILK COMPOSITIONS

The individual daily milk yields (milk collected in the evening and the following morning) were measured with milk meters (MM15, DeLaval Inc., Elancourt, France) connected to Alpro (Alpro, DeLaval Inc., France). Daily milk production was recorded starting on the 5th day postpartum. Milk yield per cow per day was average during the first 100 DIM.

Milk composition was measured individually once every two weeks on a composite mixture of milk from successive evening and morning milkings (mixture 50:50). The samples were kept at room temperature with a preservative (Bronopol, Lanxess, Langenfeld, Germany) until analysis. Samples were sent to the laboratory of the Milk Recording Organisation (Syndicat Interdépartemental de l'Elevage, Le Mée, France) to determine milk fat, protein and urea concentrations by infrared spectrophotometry (MilkoScan 6000, Foss Electric, Nanterre, France). Somatic cell counts (SCC) were evaluated by flow cytometric measurement (Fossomatic 5000, Foss Electric, Nanterre, France). In the present experiment there were no clinical cases of mastitis.

	Dry period		Lactation
	1st month	2nd month	
Diet composition	(kg DM/cow/d)	(kg DM/cow/d)	(% DM)
Maize silage	5.2	5.9	38.74
Brewers grains	-	-	6.44
Sugar beet pulp	-	-	9.26
Molasses	-	-	0.84
Orange peel	0.6	-	-
Rapeseed meal	1.4	0.9	18.04
Grass hay	2.6	2.6	14.33
Barley	-	-	6.92
Mineral and vitamin mix*	0.2	0.2	1
Urea	-	-	3.62
Salt licks	ad lib	ad lib	ad lib
$CaCO_3$	0.02	-	0.12
MgCl	-	0.05	-
Sodium bicarbonate	-	-	0.4
Nutritional values	(/cow/day)	(/cow/day)	(/kg DM)
NE_L (Mcal)	14.11	13.50	1.632
PDIN (g)	703	577	106
PDIE (g)	755	673	101
Calcium (g)	52.3	40.0	7.6
Phosphorus (g)	38.8	33.4	4
Starch (%)	-	-	16.8
Crude fiber (%)	-	-	19.3
β-carotene (mg)	42.3	41.8	2.35

**Mineral and vitamin mix contained 240g Ca, 35g P, 40g Na, 50g Mg, 1g Cu, 3.6g Zn, 3.6g Mn, 66mg I, 22mg Co, 20mg Se, 400,000 IU vitamin A, 66,700 IU vitamin D3, and 1200 IU vitamin E per kg as fed (Centralys, Trappes, France).*

NE_L = Net energy for maintenance and lactation

PDIN = true intestinal digestible proteins, when degraded N is the limiting factor

PDIE = true intestinal digestible proteins, when energy is the limiting factor

TABLE I: Composition and nutritional value of the diets given to dairy cows during the dry period and during lactation.

LIVE WEIGHT AND BODY CONDITION SCORE

Live weight and BCS were recorded on the day of drying-off, 1 month after drying-off, just after calving, 1 and 2 months postpartum. Body condition score was evaluated by the same person on a scale of 1-5 with a 0.25 increment where 1 represents extremely thin or emaciated cows and 5 represents extremely fat or obese cows [15].

INSULIN AND INSULIN-LIKE GROWTH FACTOR–1 (IGF-1)

Insulin and IGF-1 were analysed by radioimmunoassays respectively based on porcine insulin (Insulin-CT®, CIS Bio International, Gif-sur-Yvette, France) and human recombinant IGF-1 (IGF-1-RIACT®, CIS Bio International, Gif-sur-Yvette, France) in the samples collected at -8, -4, 0, 2, 4, 6 and 10 weeks in relation to calving. IGF-BPs were removed following the manufacturer's instructions. Intra-assay coefficients of variation were 4.8% at 153.8 pmol/l and 3.8% at 57.45 ng/ml for insulin and IGF-1, respectively.

MEASUREMENT OF BLOOD METABOLITES

Plasma samples were analysed by photometric methods for glucose (Glucose-RTU®, BioMérieux, Lyon, France), NEFA (NEFA C®, Wako Chemicals, Neuss, Germany), urea (Urea-kit S®, BioMérieux, Lyon, France) in the samples collected at -8, -6, -4, -2, 0, 1, 2, 3, 4, 6, 8 and 10 weeks in relation to calving. Inter-assay coefficients of variation were 4.3% at 3.47 mmol/l, 12.65% at 0.31 mmol/l, 8.3% at 2.99 mmol/l, for glucose, NEFA, and urea, respectively.

STATISTICAL ANALYSIS

Statistical analysis was performed using SAS (Version 9.1; SAS Institute, Cary, NC, USA). The parameters measured in this study were repeated measures over time on individual cows. The MIXED procedure was used to determine the difference between treatment groups including a random female effect and contrast statement was used in the model [16]. The somatic cell counts (SCC) were log transformed before analysis

since the results did not have a normal distribution. Results are presented as LSmeans ± standard error of the mean (SEM). Significant differences are reported at *P<0.05*.

Results

BLOOD β-CAROTENE CONCENTRATIONS

There was no significant difference in blood concentrations of β-carotene between the two treatment groups before the start of the dietary supplement (Figure 1). There was a significant effect of treatment before (BC > C, *P<0.001*) and after calving (BC > C, *P<0.001*) on blood concentrations of β-carotene.

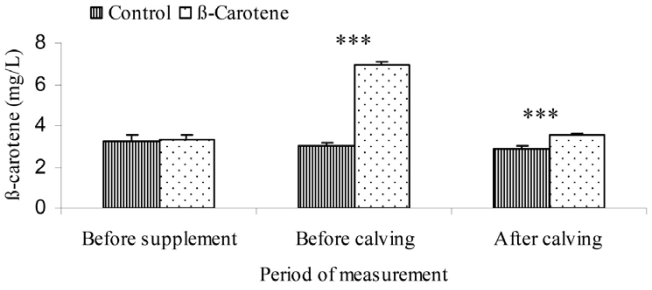

FIGURE 1: Blood β-carotene concentrations in Holstein cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until the day of calving. LSmean ± SEM. Significant difference between dietary treatments, *** *P<0.001*

LIVE WEIGHT AND BODY CONDITION SCORE

Neither live weight (*P=0.95*) nor BCS (*P=0.92*) were modified by dietary treatment (Figure 2). However, both parameters increased to a peak 1 month prior to calving and then decreased during the rest of the study (*P<0.001*).

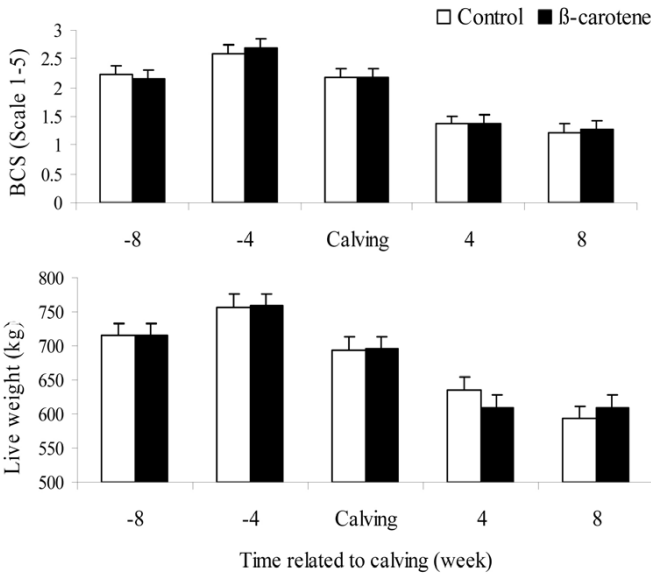

FIGURE 2: Evolution in body condition score (BCS) and live weight in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting -8 wks before calving until the day of calving. LSmean ± SEM.

MILK PRODUCTION

Average milk yield per day during the first 100 DIM was not affected by treatment (BC= 40.0 ± 6.1 kg/d, C=39.0 ± 7.2 kg/d; *P=0.24*). Milk production increased with time after calving to reach a plateau (*P<0.0001*) but there was no interaction between treatment and time (*P=0.83*).

INSULIN AND INSULIN-LIKE GROWTH FACTOR–1

Neither insulin (*P=0.78*) nor IGF-1 (*P=0.87*) (Figure 3) differed between treatments. Plasma concentrations of insulin increased slightly during the first month of the dry period and then drastically decreased at calving. The lowest blood concentrations of insulin were observed at calving in the BC group and at 2 weeks postpartum in the control group. Similarly, plasma concentrations of IGF-1 increased slightly during the first month after drying-off and then decreased and reached a minimum at 2 weeks postpartum. Thereafter IGF-1 increased gradually throughout the rest of the study.

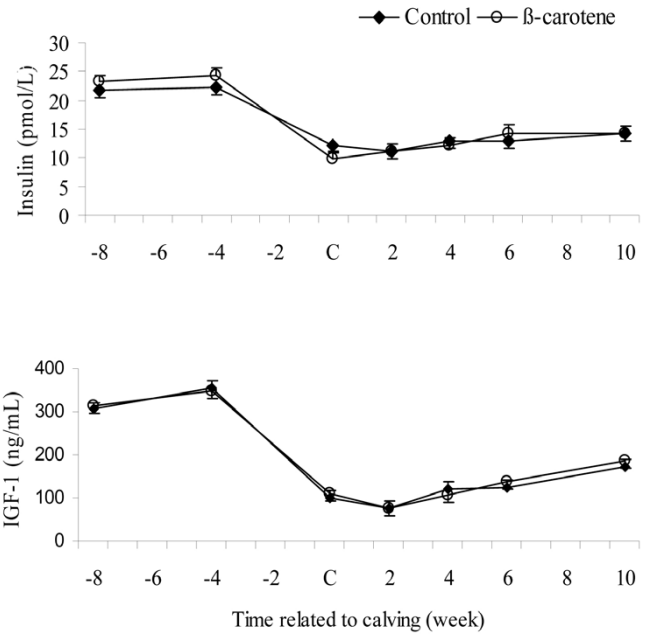

FIGURE 3: Plasma concentrations of a) insulin and b) insulin-like growth factor-1 (IGF-1) in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting -8 wks before calving until the day of calving. LSmean ± SEM. C=calving.

BLOOD METABOLITES

The dietary supplement of β-carotene did not affect blood concentrations of glucose (*P=0.20*), NEFA (*P=0.11*), and urea (*P=0.59*) (Figure 4). Plasma concentration of these metabolites were affected by time postpartum (*P<0.0001*) but there was no interaction between time and treatment (*P>0.05*).

MILK COMPOSITION

Milk composition (protein (*P=0.71*), fat (*P=0.85*) and urea (*P=0.61*); Figure 5) and SCC (log transformed, (*P=0.26*) Figure 6) were not affected by treatment. Milk protein and fat were influenced by time postpartum (*P<0.0001*) while urea

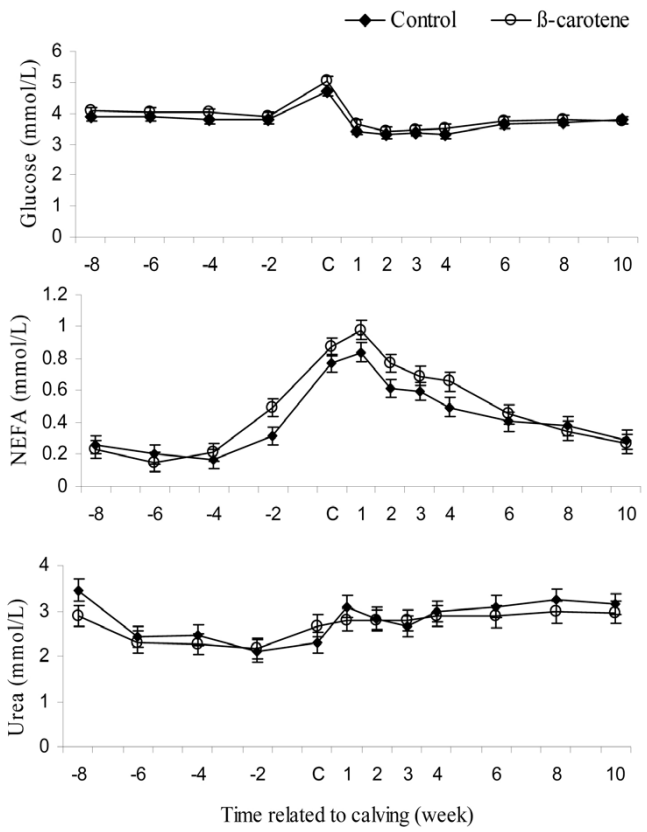

FIGURE 4: Plasma concentrations of glucose, non-esterified fatty acids (NEFA), and urea in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting -8 wks before calving until the day of calving. LSmean ± SEM. C=calving.

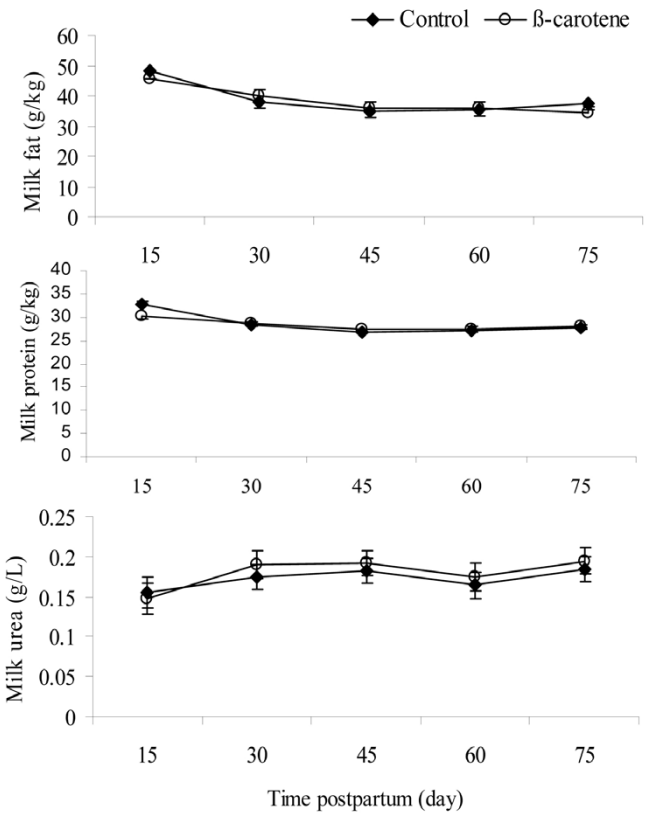

FIGURE 5: Milk composition (milk fat, protein and urea) in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting -8 wks before calving until the day of calving. The samples were taken every 15 days post partum for 10 weeks. LSmean ± SEM.

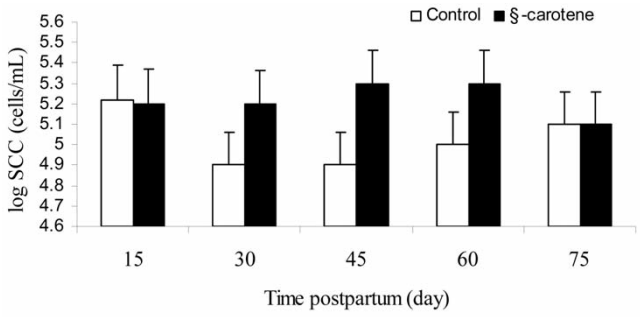

FIGURE 6: Somatic cell count (SCC, log transformed) in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until the day of calving. The samples were taken every 15 days post partum for 10 weeks. LSmean ± SEM.

and SCC were unaffected. There was no interaction between treatment and time on milk composition (*P>0.05*).

Discussion

A dietary supplement of β-carotene given to dairy cows during the dry period increased blood concentrations of β-carotene. This is in agreement with several studies [17, 18, 19, 20].

In the present experiment a β-carotene supplement had no effect on milk production in agreement with other studies [17, 19, 21]. In contrast, a study conducted with heat stressed cows showed that cumulative milk yield increased by 6 to 11% in β-carotene supplemented compared to non-supplemented cows [22]. The heat stressed cows were supplemented with β-carotene starting at approximately 15 to 20 days postpartum and for variable periods, the longest being 170 days. Moreover, OLDHAM *et al*. [23] found that a supplement of β-carotene during the dry period and early lactation could increase milk yield in non-heat stressed dairy cows. They concluded that the effect of β-carotene on milk production warranted additional research. Differences in β-carotene concentrations in the control diet, the initial blood concentration of β-carotene, the level of supplementation, the timing of supplementation, the duration of supplementation may have contributed to the lack of consistency in responses to β-carotene supplementation previously reported.

In this study, the evolution in plasma glucose was in agreement with a previous report [24]. What is commonly seen is that glucose concentrations remain stable or increase slightly during the prepartum period, rise at calving, and then decrease immediately after calving [25, 26]. The increase in plasma glucose at calving reflects an increase in gluconeogenesis which is in response to calving stress. The decrease immediately after calving may be associated with the modest increase in

dry matter intake concomitant with the very high uptake of circulating glucose by the mammary gland for lactose synthesis [27].

The changes in plasma; NEFA, IGF-1 and insulin, together with the changes in live weight and BCS, reflect the nutritional status of the cows. These variables confirm the very large energy demands of cows during the last month of pregnancy. The energy supply from feed intake could not meet the requirements for maintenance and rapid foetal growth. Therefore, body energy reserves, mainly in the form of body fat, are mobilized to provide the energy and to cover requirements. This mechanism results in an increase in NEFA, and a decrease in insulin and IGF-1 and the loss of body condition.

In our study, there was no effect of a dietary supplement of β-carotene on milk protein content which is consistent with previous work [21]. Recently, de ONDARZA *et al*. [28] also reported that a supplement of β-carotene given after calving had no effect on milk protein and SCC but did increase milk fat compared to non-supplemented cows. HINO *et al*. [29] found, in an *in vitro* study, that β-carotene plus α-tocopherol increased the growth of cellulolytic bacteria cultured in fat-supplemented media and increased cellulose digestion. The increase in fibre digestion in the rumen may explain why milk fat increased after β-carotene supplementation. Another possibility is that β-carotene supplementation is associated with altered rumen bio-hydrogenation as has been observed for another antioxidant, vitamin E [30]. A supplement of vitamin E when given with a linseed supplement (rich in C18:3n-3) altered rumen bio-hydrogenation and resulted in an increase in the production of vaccenic acid (*trans*-11 C18:1) and a decrease in the production of *trans*-10 C18:1 in the rumen [31]. Since *trans*-10 C18:1 has been associated with milk fat depression [32] this may explain why a dietary antioxidant supplement can in some situations increase milk fat level. In the present study, although the blood concentrations of β-carotene post partum were different between treatments, the BC cows were no longer receiving the dietary β-carotene supplement therefore it is unlikely that ruminal microbe growth and cellulolytic bacteria function would have been affected. In the studies where the supplement of β-carotene increased milk production, cows also received β-carotene during the post partum period [22, 23, 28]. In the present study we wished to investigate whether there was a carry-over effect of β-carotene supplementation during the dry-period into the lactation period.

β-carotene functions as an antioxidant and may enhance immunity. This role is of interest in the fight against infection. Several studies have been conducted to determine the effect of β-carotene on cow immunity or udder health [9, 23, 34, 35]. Some studies showed positive effects on udder health while others did not. Somatic cell count in cow milk has been used as an indicator of udder health and milk quality and can be related to the response of cellular immunity to pathogens. RAKES *et al*. [21], for example, showed that a daily supplement of 300 mg β-carotene lowered SCC in milk. Others reported that the incidence of clinical mastitis was lower in β-carotene supplemented compared to non-supplemented cows [19]. We, however, found no effect of a supplement of β-carotene on SCC. Although in the present experiment β-carotene remained higher in BC cows compared to C cows postpartum, even though the supplementation was stopped at calving, the difference was probably not large enough to affect SCC. Other studies have shown that β-carotene was ineffective in lowering somatic cell counts and protecting against mastitis [17, 23, 34].

The present study showed that a dietary supplement of β-carotene in a purified form given to dairy cows can escape complete degradation in the rumen and increase circulating concentrations in dairy cows. Although in the literature β-carotene has been shown to have specific effects on metabolism and production in dairy cows, the parameters that we measured in the present experiment were unable to shed light on the physiological functions that require β-carotene. Although the β-carotene supplement was stopped the day of calving, blood concentrations remained higher in the treated cows compared to the controls during the post partum period. There was no effect of β-carotene on milk production and metabolism after the supplement was stopped.

Acknowledgement

The authors thank DSM Nutritional Products for partial financial support. Winai Kaewlamun was supported by the Royal Golden Jubilee PhD Program of the Thailand Research Fund and PHC Franco-Thai project year 2007-2008. Marcel Okouyi was supported by the Gabonese government. Dominique Tristant and the farm workers at the experimental farm of AgroParisTech, Thiverval-Grignon and the technical assistance of Christine Ficheux are also acknowledged.

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