Supplementation of prepartum dairy cows with β -carotene

R. C. Oliveira,* B. M. Guerreiro,† N. N. Morais Junior,* R. L. Araujo,* R. A. N. Pereira,‡ and M. N. Pereira*¹

*Departamento de Zootecnia, Universidade Federal de Lavras, 37200-000, Lavras, Brazil

†Departamento de Reprodução Animal, Universidade de São Paulo, 05508-270, São Paulo, Brazil

‡Empresa de Pesquisa Agropecuária de Minas Gerais, 37200-000, Lavras, Brazil

ABSTRACT

The prepartum supplementation of dairy cows with β -carotene was evaluated. Cows were blocked by parity and expected calving date and assigned to a treatment: β -carotene (1.2 g/cow per d) or control (no supplementation). The same total mixed ration batch was offered to all cows, and β -carotene was top dressed to individual cows once per day. The data set contained 283 Holsteins that received a treatment for >14 d (29.1 ± 6.9 d). Frequency distributions were analyzed with the GENMOD procedure of SAS using logistic regression for binomial data. Continuous variables were analyzed with the MIXED procedure of SAS. Within parity, nonparametric estimates of the survivor function for reproductive variables were computed using the product-limit method of the Kaplan-Meier method with the LIFETEST procedure of SAS. Plasma β -carotene concentration before supplementation was similar between supplemented and nonsupplemented cows ($2.99 \mu\text{g/mL}$) and peaked at $3.26 \pm 0.175 \mu\text{g/mL}$ on d -15 ± 2.4 precalving for supplemented cows ($2.62 \pm 0.168 \mu\text{g/mL}$ for control). Colostrum density, milk yield, and milk composition were similar between treatments. β -Carotene tended to increase milk protein content from 2.90 to 2.96% and to decrease the proportion of primiparous cows with a milk fat to protein ratio >1.5 from 22.6 to 6.4%. The proportion of primiparous and multiparous cows with difficult calving, metritis, progesterone >1 ng/mL at 21 d and at 42 d in lactation, % conception at first service, and % pregnancy at 90 and 150 d in lactation were similar between treatments. A trend for decreased incidence of somatic cell count >200,000 cells/mL was present in multiparous cows supplemented with β -carotene (38.9% vs. 28.1%). Beta-carotene was associated with a reduction in the proportion of multiparous cows with retained placenta 12 h postpartum from 29.9 to 21.7%; time of placenta release

was 392 min (340 to 440) for β -carotene and 490 min (395 to 540) for control (median and 95% confidence interval). For primiparous cows, placenta release was not affected by β -carotene (incidence was 15.4%). The intervals from calving to first estrus, to first service, and to conception were not affected by β -carotene supplementation in either parity. However, independent of treatment, cows with improved reproductive efficiency had increased postpartum β -carotene concentration in plasma. The prepartum supplementation of β -carotene increased plasma concentration around calving. No response in milk yield or reproductive performance was detected. β -Carotene supplementation was associated with a lower incidence of retained placenta in multiparous cows.

Key words: β -carotene, retained placenta, transition period

INTRODUCTION

Carotenoids, such as β -carotene, can be converted to vitamin A in cells of the intestinal mucosa, liver, uterus, and ovaries (Chew et al., 1982a; Borel et al., 2005; Morales et al., 2006). Carotenoids also have antioxidant function, protecting cells and cell components against reactive oxygen species (Chew and Park, 2004). The antioxidant defense system of periparturient dairy cows may be overloaded with reactive oxygen species, predisposing animals to immune-system-related diseases, such as retained placenta, metritis, and mastitis (Bernabucci et al., 2002; LeBlanc et al., 2004; Castillo et al., 2005). β -Carotene supplementation increased the proliferative response of lymphocytes to mitogens and enhanced the phagocytic ability of polymorphonuclear neutrophils (Tjoelker et al., 1988; Michal et al., 1994). β -Carotene deficiency can affect reproduction, lactation performance, and health negatively, justifying its supplementation to dairy cows around calving.

In confinement dairy production systems, total farm reliance on preserved forages is not uncommon. The carotenoid content of forages is reduced during storage as silage or hay (Chauveau-Duriot et al., 2005), and the content in grains is low (Holden et al., 1999).

Received October 29, 2014.

Accepted June 7, 2015.

¹Corresponding author: mpereira@dzo.ufla.br

Positive responses in dairy cow fertility and lactation performance have been observed when β -carotene was supplemented after calving (Aréchiga et al., 1998; De Ondarza et al., 2009), although absent (Bindas et al., 1984; Akordor et al., 1986) or negative (Folman et al., 1987) effects on reproduction have been reported. Kaewlamun et al. (2011) and Wang et al. (2013) have reported benefits in uterine health of cows supplemented with β -carotene before calving. Kawashima et al. (2009) also showed in a retrospective study that ovulatory cows during the first follicular wave postpartum had higher plasma β -carotene concentration before calving than anovulatory cows. These studies suggest that β -carotene supplementation before calving may improve fertility in early lactation.

In small confinement systems adopting multiple diets, it is not uncommon for fresh cows to go directly to the most nutrient-dense diet of the farm without having access to a postcalving-specific diet. The permanence time of cows in postcalving groups is also variable, depending on herd management practices and degree of overcrowding. This scenario makes the adoption of nutrition strategies specifically focused on the transition period, usually defined as the period between 3 wk before and 3 wk after parturition (Drackley, 1999), difficult. Thus, the aim of this study was to evaluate the effect of β -carotene supplementation only during the prepartum transition period on lactation performance, reproduction, and health of early lactation dairy cows.

MATERIALS AND METHODS

Experimental procedures were approved by the Federal University of Lavras Bioethic Committee in Utilization of Animals (protocol no. 056/13). The study was conducted in a commercial dairy herd in southeast Brazil. The farm housed primiparous and multiparous cows in separate free-stall pens during the prefresh period and during lactation. The prefresh cow barn with headlocks was split into 2 halves with more stalls than cows. One load of TMR was offered twice daily to all 4 groups of cows. Within parity, cows were blocked based on expected calving date and were randomly assigned to a treatment at d -25 to -31 of the predicted calving date (goal was a 28-d prepartum transition period). Cows were moved from the far off dry cow diet to the prefresh cow diet once per week. Treatments were 1.2 g/d of β -carotene (12 g/d of Rovimix, containing 10% β -carotene, DSM Nutritional Products, São Paulo, Brazil) or control. Beta-carotene was mixed with a cup of ground corn and top dressed to individual treatment cows on the feed offered in the morning, and control cows received nothing. The intake of β -carotene was observed visually. Cows were moved to individual ma-

ternity pens when signs of calving were observed, and treatment allocation was maintained until the day of parturition.

For the 283 cows (66 primiparous and 217 multiparous) that received the treatments for 14 d or more and that had available data for at least one variable, the duration of the precalving transition period was 29.1 ± 6.9 d. The number of cows for each variable is described in Table 1. The period from the beginning of treatment allocations until the day the last cow completed 150 DIM lasted from February 7 to September 27, 2012. Parturitions occurred from February 21 to April 30.

The composition of the diets were defined by the manager without the interference of the research team. The diet for the prepartum transition period contained (% of DM) 55.9% corn silage, 17.2% green chop Tifton grass (*Cynodon* spp.), 8.9% soybean meal, 3.9% finely ground corn, 6.4% citrus pulp, 4.8% whole cottonseed, and 2.9% minerals, vitamins, and additives (18.7% live yeast, 12.4% urea, 5.0% Mycofix, 0.5% Sel-Plex, and 63.4% of a product containing 5% Ca, 1% P, 1% Mg, 1% Na, 1.15% Cl, 0.8% S, 39.3 mg/kg Co, 660 mg/kg Cu, 775 mg/kg Fe, 19.5 mg/kg I, 1,630 mg/kg Mn, 8.98 mg/kg Se, 2,368 mg/kg Zn, 260 kIU/kg of vitamin A, 35 kIU/kg of vitamin D, 2,600 IU/kg of vitamin E, and 100 mg/kg biotin). The lactating cow diet contained (% of DM) 34.8% corn silage, 8.4% green chop Tifton grass, 13.8% soybean meal, 6.1% whole heated soybeans, 18.6% finely ground corn, 11.9% citrus pulp, 2.3% whole cottonseed, and 4.1% minerals, vitamins, and additives (18.1% Megalac-E, 15.8% limestone, 6.8% sodium bicarbonate, 3.4% live yeast, 2.7% NaCl, 1.1% Mycofix, 0.2% Sel-Plex, and 51.9% of a product containing 21% Ca, 15% P, 3% Mg, 3% S, 100 mg/kg Co, 3,000 mg/kg Cu, 180 mg/kg I, 5,000 mg/kg Mn, 80 mg/kg Se, 12,000 mg/kg Zn, 1,000 kIU/kg of vitamin A, 250 kIU/kg of vitamin D, and 3,250 IU/kg of vitamin E).

Blood samples from the coccygeal vessels were obtained on the first day of supplementation (d -29 , 29.1 ± 6.9 d prepartum), between d -18 and -10 relative to calving (d -15 , 15.0 ± 2.4 d prepartum), and on d 7 and d 56 of lactation. Blood β -carotene content was quantified as in Schweigert et al. (2007): a single-step denaturation and β -carotene extraction into organic solvent, followed by β -carotene measurement using iCheck, a portable spectrophotometer (BioAnalyt GmbH, Teltow, Germany). Blood concentrations were classified as high (≥ 3.5 $\mu\text{g/mL}$), intermediate (≥ 2 to < 3.5 $\mu\text{g/mL}$), or low (< 2 $\mu\text{g/mL}$; Schweigert and Imig, 2007).

Blood serum progesterone (P4) concentration was analyzed in samples from the coccygeal vessels obtained on d 21 and 42 of lactation. Serum was stored

Table 1. Number of observations used for analysis of response variables on treatments control and β -carotene, for primiparous and multiparous cows

Item	Control		β -carotene	
	Primiparous	Multiparous	Primiparous	Multiparous
Calving to first estrus	33	97	33	91
Calving to first service	31	94	29	87
Calving to conception	31	94	29	87
Colostrum density	31	95	26	97
Conception at first service	30	84	28	74
Day of peak	30	69	29	70
Difficult calvings	32	107	33	106
Linear SCC	33	95	32	89
Mastitis	33	95	32	89
Metritis	31	94	31	87
Milk	30	69	29	70
Milk % fat/% protein	31	89	31	84
Milk fat %	31	89	31	84
Milk protein %	31	89	31	84
P4 ¹ >1 ng/mL at 21 DIM	23	86	29	83
P4 >1 ng/mL at 42 DIM	28	83	31	78
Peak	30	69	29	70
Blood β -carotene d -29	33	109	33	108
Blood β -carotene d -15	20	41	18	46
Blood β -carotene d 7	30	95	32	103
Blood β -carotene d 56	29	75	31	73
Pregnancy at 150 DIM	33	95	33	87
Pregnancy at 90 DIM	33	98	33	91
Time of placenta release	32	107	33	106

¹P4 = progesterone.

at -20°C until assayed for P4 concentration by solid phase RIA using a Coat-A-Count Progesterone kit (Diagnostic Products Corporation, Los Angeles, CA). The intraassay coefficients of variation for P4 assays were 0.14 and 1.68%, for low and high samples, and the interassay coefficients of variation were 9.93 and 2.12%, for low and high samples, respectively. Cows with luteal activity on the day of sampling were defined as serum progesterone concentration above 1 ng/mL.

Immediately before the expected calving date, cows were moved to sand-bedded maternity pens. Calving was classified by farm personnel as unassisted or assisted (manual extraction by 1 or 2 farm employees with or without assistance of a veterinarian). Neither cesarean section nor fetotomy were performed. The incidence of twins was 1.06% of parturitions in both treatments. Colostrum density was determined with a thermo lactodensimeter (Incoterm, Porto Alegre, Brazil) at 20°C . The time between postpartum placenta release (min) and calf delivery was recorded up to 12 h. The fetal membranes still attached after 12 h were considered retained placenta.

Cows were milked 3 times per d starting at 0400, 1200, and 2000 h. Milk yield of each cow was recorded daily from 20 to 109 DIM, and one sample was obtained between 15 and 42 DIM (30.1 ± 8.3). Milk samples were preserved in 2-bromo-2-nitropropane-1,3-diol, and

the concentrations of solids and SCC were measured by infrared analysis (Bentley 2000, Bentley Instruments Inc., Chaska, MN). Milk fat to protein ratio greater than 1.5 was considered a predictor of ketosis (Heuer et al., 1999). A SCC greater than 200,000 cells/mL was considered as mastitis. The SCC was converted to a linear scale from 0 to 9 (linear SCC), the midpoint of each score represented by the following linear SCC values ($\times 1,000$ cells/mL): 12.5 for linear SCC 0, 25 for linear SCC 1, 50 for linear SCC 2, 100 for linear SCC 3, 200 for linear SCC 4, 400 for linear SCC 5, 800 for linear SCC 6, 1,600 for linear SCC 7, 3,200 for linear SCC 8, and 6,400 for linear SCC 9.

Cows were kept under routine herd reproductive management practices. Transrectal ultrasonography (Aloka SSD 500, 5-MHz linear transducer, Tokyo, Japan) of all cows was performed by the herd veterinarian at 35.1 ± 4.3 DIM. Cows were then examined by transrectal ultrasonography at 2-wk intervals and received prostaglandin $\text{F}_{2\alpha}$ (500 μg of cloprostenol, i.m.; Ciosin, Schering-Plough, Cotia, Brazil) when a corpus luteum was detected or GnRH (100 μg of Fertagyl, i.m.; Schering-Plough) when a follicle >10 mm was detected, until insemination after a 60-d voluntary waiting period. Purulent secretion or presence of liquid in the uterus was defined as metritis. Estrus detection was performed visually twice per day for 1 h. Inseminations

were performed 12 h after the manifestation of the first signs of natural or prostaglandin $F_{2\alpha}$ -induced estrus. Pregnancy diagnosis by ultrasonography was performed from d 25 to 38 after insemination, and confirmation was performed 14 d later.

Statistical Analyses

Continuous variables were analyzed with the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with a model containing the fixed effects of parity (primiparous cows, multiparous cows), block within parity, treatment (control, β -carotene), and the interaction of parity and treatment. Frequency distributions were analyzed with the GENMOD procedure of SAS using logistic regression for binomial data. Within parity, nonparametric estimates of the survivor function for variables calving to first estrus, calving to first service, calving to conception, and time of placenta release were computed using the product-limit method of the Kaplan-Meier method, by the LIFETEST procedure of SAS. Survival curves were generated. Comparison of the survival curves between treatments used rank tests for homogeneity: log-rank and Wilcoxon test. Blood β -carotene concentration at 7 and 56 DIM was also analyzed with the MIXED procedure of SAS without taking treatment into account, with a model containing the fixed effects of event (difficult calving, retained placenta, milk fat to protein ratio, metritis, mastitis, P4 at 21 DIM, P4 at 42 DIM, conception at first service, pregnancy at 90 DIM, and pregnancy at 150 DIM), sampling day (7 and 56

DIM), and the interaction of event and sampling day. Statistical significance, trends, and weak trends were considered at $P \leq 0.05$, $P > 0.05$ to $P \leq 0.10$, and $P > 0.10$ to $P \leq 0.15$, respectively.

RESULTS

The blood concentration of β -carotene at the start of the prepartum transition period (d -29) was similar between treatments (Tables 2 and 3). Blood concentration of β -carotene for control cows was lower on d -15 than on d -29 relative to calving, whereas it was higher for the β -carotene-supplemented cows (Table 3). Blood β -carotene concentration of supplemented cows peaked at 3.3 $\mu\text{g/mL}$ on d -15. Blood concentrations were lowest in both treatments immediately after calving, although they were increased by supplementation. Primiparous cows had lower blood β -carotene concentration than multiparous cows only at d -29 (Table 3). The prepartum supplementation of β -carotene increased β -carotene concentration in blood around calving, but the difference between treatments decreased with advanced DIM.

No evidence was observed for positive responses to β -carotene supplementation in colostrum density, milk yield, and milk fat concentration (Table 4). However, β -carotene supplementation tended to increase milk protein concentration. A trend was observed for a decreased proportion of primiparous cows with a milk fat to protein ratio greater than 1.5 when β -carotene was supplemented (Table 5). A weak trend was observed

Table 2. Proportion of cows with blood β -carotene content high (≥ 3.5 $\mu\text{g/mL}$), intermediate (≥ 2 to < 3.5 $\mu\text{g/mL}$), or low (< 2 $\mu\text{g/mL}$) at d -29 and -15 prepartum, and at d 7 and 56 postpartum, on control or β -carotene treatments

Item	Control	β -carotene	Est ¹	SE ²	Odds ratio	95% CI ³		P-value
Day -29 ⁴								
High	28.2	29.1	-0.04	0.26	0.96	0.57	1.60	0.87
Intermediate	66.9	64.5	0.10	0.25	1.11	0.68	1.81	0.68
Low	4.9	6.4	-0.27	0.52	0.76	0.27	2.10	0.60
Day -15 ⁵								
High	4.92	48.4	-2.93	0.65	0.05	0.02	0.19	<0.01
Intermediate	49.2	45.3	0.83	0.37	1.17	0.58	2.36	0.02
Low	45.9	6.3	2.68	0.59	12.72	4.11	39.42	<0.01
Day 7								
High	0.8	5.2	-1.91	1.08	0.15	0.02	1.22	0.08
Intermediate	23.2	45.9	-1.03	0.27	0.36	0.21	0.61	<0.01
Low	76.0	48.9	1.20	0.27	3.31	1.95	5.63	<0.01
Day 56								
High	3.8	9.6	-0.98	0.61	0.38	0.11	1.24	0.11
Intermediate	59.6	63.5	-0.16	0.29	0.85	0.48	1.48	0.57
Low	36.5	26.9	0.45	0.30	1.56	0.87	2.81	0.14

¹Parameter estimate generated with the GENMOD procedure of SAS using logistic regression for binomial data. Control is zero.

²Standard error of the estimate.

³Profile likelihood 95% confidence intervals for odds ratio.

⁴29.1 $\pm$ 6.9 d prepartum.

⁵15.0 $\pm$ 2.4 d prepartum.

Table 3. Blood β -carotene content ($\mu\text{g/mL}$) at d -29 and -15 prepartum, and at d 7 and 56 postpartum, for primiparous (Prim) and multiparous (Mult) cows on control or β -carotene treatments

Item	Control		β -carotene		SEM	<i>P</i> -value ¹		
	Prim	Mult	Prim	Mult		Par	Treat	Par $\times$ Treat
Day -29^2	2.71	3.33	2.73	3.19	0.113	0.01	0.59	0.52
Day -15^3	2.51	2.73	3.19	3.33	0.283	0.51	<0.01	0.71
Day 7	1.55	1.71	2.02	2.08	0.104	0.32	<0.01	0.64
Day 56	2.26	2.29	2.38	2.54	0.094	0.30	0.06	0.50

¹Effects of parity (Par), treatment (Treat), and the interaction of Par and Treat.²29.1 $\pm$ 6.9 d prepartum.³15.0 $\pm$ 2.4 d prepartum.**Table 4.** Milk somatic cell count, colostrum density, and milk yield and solids content for primiparous (Prim) and multiparous (Mult) cows on control of β -carotene treatments

Item	Control		β -carotene		SEM	<i>P</i> -value ¹		
	Prim	Mult	Prim	Mult		Par	Treat	Par $\times$ Treat
Linear SCC, 1 to 9	2.18	3.43	2.31	3.09	0.334	<0.01	0.77	0.50
Colostrum density, g/L	1,056	1,056	1,053	1,057	1.9	0.30	0.54	0.31
Milk % fat/% protein	1.25	1.34	1.19	1.32	0.042	0.01	0.44	0.65
Milk fat, %	3.65	3.78	3.54	3.87	0.109	0.04	0.95	0.38
Milk protein, %	2.94	2.85	2.97	2.95	0.037	0.12	0.09	0.36
Milk, ³ kg	3,105	3,667	3,104	3,522	71.2	<0.01	0.32	0.33
Peak, ⁴ kg	45.5	53.5	46.7	52.9	0.93	<0.01	0.79	0.34
Day of peak, ⁵ d	75.3	61.9	71.3	68.0	3.65	0.03	0.78	0.18

¹Effects of parity (Par), treatment (Treat) and the interaction of Par and Treat.²Milk yield from d 20 to 109 of lactation.³Maximum daily yield.⁴Day postpartum of maximum yield.**Table 5.** Proportion of health and reproductive events for primiparous cows on control of β -carotene treatments

Item	Control	β -carotene	Est ¹	SE ²	Odds ratio	95% CI ³	<i>P</i> -value
Difficult calvings, %	18.8	24.2	-0.33	0.61	0.72	0.22 2.38	0.59
Mastitis, ⁴ %	12.1	15.6	-0.29	0.72	0.74	0.18 3.07	0.68
Metritis, ⁵ %	6.5	9.7	-0.44	0.95	0.64	0.10 4.15	0.64
Milk % fat/% protein >1.5, ⁶ %	22.6	6.4	1.44	0.84	4.23	0.8 22.29	0.08
P4 >1 ng/mL at 21 DIM, ⁷ %	21.7	13.8	0.55	0.74	1.74	0.41 7.38	0.46
P4 >1 ng/mL at 42 DIM, ⁷ %	35.7	29.0	0.31	0.56	1.36	0.45 4.06	0.58
Conception at first service, %	50.0	42.9	0.29	0.53	1.33	0.47 3.76	0.59
Pregnancy at 90 DIM, ⁸ %	42.4	39.4	0.13	0.50	1.13	0.42 3.03	0.80
Pregnancy at 150 DIM, ⁸ %	60.6	57.6	0.13	0.50	1.13	0.42 3.03	0.80

¹Parameter estimate generated with the GENMOD procedure of SAS using logistic regression for binomial data. Control is zero.²Standard error of the estimate.³Profile likelihood 95% confidence interval for odds ratio.⁴Cows with SCC >200,000/mL at first test 30.1 $\pm$ 8.3 DIM.⁵Purulent secretion or presence of liquid in uterus at ultrasound exam 40.8 $\pm$ 9.7 DIM.⁶Ratio of milk fat content to milk protein content at first test 31.3 $\pm$ 9.6 DIM.⁷Blood progesterone (P4) content >1 ng/mL at 21 and 42 DIM.⁸Cows pregnant at 90 and 150 DIM.

Table 6. Proportion of health and reproductive events for multiparous cows on control of β -carotene treatments

Item	Control	β -carotene	Est ¹	SE ²	Odds ratio	95% CI ³		P-value
Difficult calvings, %	25.2	18.9	0.37	0.33	1.45	0.75	2.79	0.26
Mastitis, ⁴ %	38.9	28.1	0.49	0.32	1.63	0.88	3.03	0.12
Metritis, ⁵ %	31.9	35.6	-0.17	0.31	0.85	0.46	1.57	0.60
Milk % fat/% protein >1.5, ⁶ %	29.2	30.9	-0.08	0.33	0.92	0.48	1.76	0.80
P4 >1 ng/mL at 21 DIM, ⁷ %	17.4	25.3	-0.47	0.38	0.62	0.29	1.31	0.21
P4 >1 ng/mL at 42 DIM, ⁷ %	33.7	38.5	-0.21	0.33	0.81	0.43	1.55	0.53
Conception at first service, %	27.4	33.8	-0.30	0.35	0.74	0.37	1.46	0.38
Pregnancy at 90 DIM, ⁸ %	23.5	24.2	-0.04	0.34	0.96	0.49	1.88	0.91
Pregnancy at 150 DIM, ⁸ %	40.0	41.4	-0.06	0.30	0.94	0.52	1.71	0.85

¹Parameter estimate generated with the GENMOD procedure of SAS using logistic regression for binomial data. Control is zero.

²Standard error of the estimate.

³Profile likelihood 95% confidence interval for odds ratio.

⁴Cows with SCC >200,000/mL at first test 27.3 $\pm$ 8.1 DIM.

⁵Purulent secretion or presence of liquid in uterus at ultrasound exam 39.7 $\pm$ 8.5 DIM.

⁶Ratio of milk fat content to milk protein content at first test 29.6 $\pm$ 8.3 DIM.

⁷Blood progesterone (P4) content >1 ng/mL at 21 and 42 DIM.

⁸Cows pregnant at 90 and 150 DIM.

for a reduction in the incidence of SCC greater than 200,000 cells/mL in multiparous cows supplemented with β -carotene (Table 6).

The supplementation of β -carotene was associated with a reduction in the time of placenta release for multiparous cows (Figure 1), which reduced the proportion of cows with retained placenta 12 h postcalving from 29.9 to 21.7%. For primiparous cows, in which the proportion of cows with retained placenta was 15.4% across both treatments, β -carotene supplementation did not affect placenta release (Figure 2).

The supplementation of β -carotene did not affect the proportion of cows pregnant at first service, at 90

and 150 DIM, or the proportion of cows with serum progesterone content above 1 ng/mL at 21 and 42 DIM (Tables 5 and 6). In addition, no response was observed in the intervals from calving to first estrus (Figures 3 and 4) or from calving to conception (Figures 5 and 6). An unexpected trend was found for multiparous cows supplemented with β -carotene to have an extended calving to first service interval (Figure 7), but this interval was similar between supplemented and non-supplemented primiparous cows (Figure 8).

Independent of supplementation, blood β -carotene concentration postpartum was higher in cows that had improved reproduction and decreased mastitis

Figure 1. Survival curve for time postcalving of placenta release for multiparous cows on control (O, n = 107) or β -carotene ($\blacktriangle$, n = 106) treatments. Median and 95% confidence interval were 392 min (340 to 440) for β -carotene and 490 min (395 to 540) for control. LogRank $P = 0.05$, and Wilcoxon $P = 0.04$.

Figure 2. Survival curve for time postcalving of placenta release for primiparous cows on control (O, n = 33) or β -carotene ($\blacktriangle$, n = 33) treatments. Median and 95% confidence interval were 388 min (285 to 440) for β -carotene and 375 min (290 to 425) for control. LogRank $P = 0.87$, and Wilcoxon $P = 0.86$.

Figure 3. Survival curve for calving to first estrus for multiparous cows on control (○, $n = 97$) or β -carotene (▲, $n = 91$) treatments. Median and 95% confidence interval were 38 d (34 to 43) for β -carotene and 44 d (37 to 50) for control. LogRank $P = 0.55$, and Wilcoxon $P = 0.23$.

(Table 7). A trend was also present for increased blood β -carotene concentration in cows with milk fat to protein ratio greater than 1.5, and cows with serum progesterone greater than 1 ng/mL at 21 and 42 DIM (Table 7). The relationship between these variables and blood β -carotene concentration was consistent across sampling days, and no treatment by day interaction was detected. No difference in blood β -carotene concentration postpartum was detected for cows with or

Figure 5. Survival curve for calving to conception for multiparous cows on control (○, $n = 94$) or β -carotene (▲, $n = 87$) treatments. LogRank $P = 0.82$, and Wilcoxon $P = 0.75$.

without retained placenta, metritis, or difficult calving (Table 7).

DISCUSSION

The lower concentration of β -carotene in blood at d -29 for primiparous compared with multiparous cows suggests that heifers were more deficient in this compound than lactating cows. The farm routinely used some green chop Tifton grass as a source of long fiber for lactating cows (8.4% of diet DM during the ex-

Figure 4. Survival curve for calving to first estrus for primiparous cows on control (○, $n = 33$) or β -carotene (▲, $n = 33$) treatments. Median and 95% confidence interval were 39 d (33 to 51) for β -carotene and 34 d (30 to 43) for control. LogRank $P = 0.34$, and Wilcoxon $P = 0.42$.

Figure 6. Survival curve for calving to conception for primiparous cows on control (○, $n = 31$) or β -carotene (▲, $n = 29$) treatments. Median was 125 d for β -carotene and 97 d for control. LogRank $P = 0.77$, and Wilcoxon $P = 0.65$.

Figure 7. Survival curve for calving to first service for multiparous cows on control ($\circ$, $n = 94$) or β -carotene ($\blacktriangle$, $n = 87$) treatments. Median and 95% confidence interval were 70 d (65 to 79) for β -carotene and 64 d (61 to 70) for control. LogRank $P = 0.09$, and Wilcoxon $P = 0.07$.

Figure 8. Survival curve for calving to first service for primiparous cows on control ($\circ$, $n = 31$) or β -carotene ($\blacktriangle$, $n = 29$) treatments. Median and 95% confidence interval were 65 d (56 to 69) for β -carotene and 61 d (57 to 67) for control. LogRank $P = 0.73$, and Wilcoxon $P = 0.87$.

periment), whereas heifers were fed on corn or sorghum silages as the only forage source before the prepartum transition period. Beta-carotene deficiency may be an issue not only for lactating cows, but also for heifers. Although parity had an effect on blood β -carotene content before supplementation begun prepartum, content at d -29 was similar between supplemented and non-supplemented cows. Parturition apparently eliminated the parity effect on blood β -carotene concentration at calving. The blood β -carotene concentration being lowest shortly after calving (d 7) is in agreement with other reports (Folman et al., 1987; Kaewlamun et al.,

2011). This has been attributed to the uptake of the compound by the mammary gland for colostrogenesis, especially during the last week of gestation (Johnston and Chew, 1984; Michal et al., 1994; Chawla and Kaur, 2004). The oxidation of β -carotene by an increased oxidative stress and the physiological decrease in DMI around calving may also contribute to a decreased blood β -carotene level (Goff et al., 2002).

Blood β -carotene concentration of supplemented cows peaked at d -15 , 2 wk after the start of the supplementation. The mean concentration of $3.3 \mu\text{g/mL}$ (± 0.17 SEM) for supplemented cows was lower than

Table 7. Blood β -carotene content ($\mu\text{g/mL}$) at 7 and 56 d postcalving according to events (+ = occurrence; – = nonoccurrence)

Item	Day 7		Day 56		SEM	P-value		
	+	–	+	–		Event	Day	Event $\times$ day
Difficult calvings	1.84	1.93	2.42	2.39	0.083	0.48	<0.01	0.36
Retained placenta	1.87	1.90	2.33	2.38	0.082	0.66	<0.01	0.92
Milk % fat/% protein $>1.5^1$	1.96	1.90	2.55	2.31	0.091	0.13	<0.01	0.37
Metritis ²	1.89	1.88	2.36	2.36	0.080	0.99	<0.01	0.91
Mastitis ³	1.67	1.96	2.08	2.44	0.080	<0.01	<0.01	0.69
P4 >1 ng/mL at 21 DIM ⁴	2.14	1.84	2.39	2.34	0.092	0.07	<0.01	0.20
P4 >1 ng/mL at 42 DIM ⁴	2.02	1.82	2.40	2.32	0.081	0.10	<0.01	0.49
Conception at first service	2.01	1.84	2.53	2.28	0.079	0.01	<0.01	0.56
Pregnancy at 90 DIM ⁵	1.96	1.85	2.48	2.25	0.081	0.01	<0.01	0.79
Pregnancy at 150 DIM ⁵	2.05	1.82	2.49	2.30	0.079	0.04	<0.01	0.44

¹Ratio of milk fat content to milk protein content at first test 31.3 ± 9.6 DIM.

²Purulent secretion or presence of liquid in uterus at ultrasound exam 40.1 ± 9.2 DIM.

³Cows with SCC $>200,000/\text{mL}$ at first test 29.1 ± 8.3 DIM.

⁴Blood progesterone (P4) content >1 ng/mL at 21 and 42 DIM.

⁵Cows pregnant at 90 and 150 DIM.

the 7.5 $\mu\text{g/mL}$ 2 wk before calving of Kaewlamun et al. (2011). Those authors supplemented prepartum cows with 1 g/d of β -carotene for 6 wk; blood β -carotene was 3 $\mu\text{g/mL}$ 8 wk before calving (at dry-off), and 6.5 $\mu\text{g/mL}$ with 2 wk of supplementation. The lower blood concentration value in our study may have been the result of a shorter duration of the supplementation period or of a supplementation period that was initiated closer to the active functioning of the mammary gland in preparation for the next lactation. However, blood β -carotene concentration was lowest immediately after calving in both experiments, although it was increased by the supplement in both experiments.

The supplementation of β -carotene before calving did not affect milk yield in early lactation in this experiment, similarly to the observations of others (Kaewlamun et al., 2012). However, Aréchiga et al. (1998) reported an increase in milk yield when cows under heat stress were supplemented with β -carotene from 15 to 170 DIM. Oldham et al. (1991) also observed a trend for increased milk yield when cows were supplemented with β -carotene from -75 d prepartum to 42 d postpartum. These results suggest that to elicit a positive milk yield response to β -carotene, the supplementation may need to occur in early lactation. However, we observed a trend for increased milk protein concentration for cows supplemented with β -carotene, although the mechanism for this response cannot be elucidated based on our data.

The trend for decreased proportion of primiparous cows with a milk fat to protein ratio greater than 1.5 when β -carotene was fed, suggests that the supplement had a beneficial effect on the incidence of ketosis in first lactation cows (Heuer et al., 1999). Although previous studies have not detected differences in plasma NEFA and glucose in response to β -carotene supplementation before calving (Kawashima et al., 2010; Kaewlamun et al., 2012), the large numerical difference in the frequency of high milk fat to protein ratio between supplemented and nonsupplemented primiparous cows (22.6% vs. 6.4%) suggests that this finding may be worthy of further study. A weak trend for decreased proportion of multiparous cows with SCC greater than 200,000 cells/mL in response to β -carotene supplementation was also observed. Bian et al. (2007) and Wang et al. (2013) also observed positive effects of β -carotene supplementation before calving on the incidence of mastitis in dairy cows. A possible mechanism for the beneficial effects of β -carotene on mastitis incidence may be related to its action as an antioxidant, leading to improved immune function (Tjoelker et al., 1988; Michal et al., 1994). A positive effect of the supplement on immune function is also indicated by the reduction in the time

of placenta release in multiparous cows. Michal et al. (1994) observed that the supplementation of dairy cows with β -carotene before and after calving improved the phagocytic activity and killing ability of blood neutrophils and induced higher blood lymphocyte proliferation in response to mitogenic agents, which translated to a lower incidence of retained placenta and metritis.

Despite the reduction in placenta retention time, β -carotene supplementation did not affect uterine health or the DIM at postpartum ovulation. The precalving supplementation of β -carotene was only capable of affecting a response variable event occurring at calving (placenta release), or the therapeutic procedures adopted by the farm were capable of masking a supplementation effect on reproductive tract status. An increase in plasma hydroxyproline concentration, suggestive of a more complete uterine involution (Kaewlamun et al., 2011), and a decreased incidence of puerperal metritis in cows with retained placenta (Wang et al., 2013), have been reported in response to the prepartum supplementation of β -carotene.

The supplementation of β -carotene did not affect the intervals from calving to first estrus and from calving to conception, the proportion of cows pregnant at first service, at 90 and 150 DIM, and the proportion of cows with plasma progesterone content above 1 ng/mL at 21 and 42 DIM. The similarity in the duration of the interval from calving to first detected estrus suggests that the supplement did not induce advantages in estrous behavior. Kaewlamun et al. (2011) also did not detect a favorable response in reproductive performance of dairy cows to the prepartum supplementation of β -carotene. The supplementation of β -carotene postpartum may be required to elicit an increase in plasma levels in early lactation, possibly inducing a positive response in reproductive performance. The β -carotene has improved the pregnancy rate of dairy cows when it was supplemented for at least 90 d (Aréchiga et al., 1998) or 105 d (De Ondarza et al., 2009) postpartum.

Although no effect was detectable of the prepartum supplementation of β -carotene on conception rate, cows that conceived at first service and were pregnant at 90 and 150 DIM had higher blood β -carotene concentration postpartum than nonpregnant cows. Cows with serum progesterone content greater than 1 ng/mL at 21 and 42 DIM also had higher blood concentration of β -carotene than cows showing no luteal function. Kawashima et al. (2009) also showed a positive relationship between plasma β -carotene concentration before calving and ovulation in early lactation. Cows with low SCC had higher blood β -carotene concentration postpartum than cows with SCC higher than 200,000 cells/mL. In agreement with this finding, Chew et

al. (1982b) reported a negative relationship between California Mastitis Test scores and plasma β -carotene concentration in dairy cows. These results suggest that β -carotene may have a role in immune-related diseases and fertility in dairy cows. High blood β -carotene concentration postpartum was also related to increased milk fat to protein ratio, suggesting that increased body fat mobilization increased β -carotene content in blood, probably as the result of β -carotene incorporation in triglycerides of bovine adipose tissue (Patterson, 1965; Arias et al., 2009).

CONCLUSIONS

The supplementation of β -carotene before calving increased blood concentration in periparturient dairy cows, but the difference in blood concentration between supplemented and nonsupplemented cows was reduced as DIM advanced. The prepartum supplementation of multiparous cows with β -carotene was associated with a lower incidence of retained placenta, but no responses were detectable in milk yield or reproductive performance.

ACKNOWLEDGMENTS

Authors are grateful to DSM Nutritional Products (São Paulo, Brazil), the Brazilian Federal Agency for Postgraduate Education (CAPES), and the Minas Gerais Foundation for Research Support (FAPEMIG) for funding this project; to the members of Grupo do Leite of the Federal University of Lavras, Bruno Gonzales and João Paulo Costa Gonçalves; to Cecília Rodrigues Alves Silveira from São Paulo State University (Jaboticabal, UNESP) for assistance during data collection; and to Kristina Weld from the University of Wisconsin–Madison for suggestions in text editing.

REFERENCES

- Akordor, F. Y., J. B. Stone, J. S. Walton, K. E. Leslie, and J. G. Buchanan-Smith. 1986. Reproductive performance of lactating Holstein cows fed supplemental β -carotene. *J. Dairy Sci.* 69:2173–2178.
- Aréchiga, C. F., C. R. Staples, L. R. McDowell, and P. J. Hansen. 1998. Effects of timed insemination and supplemental beta-carotene on reproduction and milk yield of dairy cows under heat stress. *J. Dairy Sci.* 81:390–402.
- Arias, E., A. Gonza, A. Shimada, A. Varela-Echavarría, F. Ruiz-Lo, A. During, and O. Mora. 2009. β -Carotene is incorporated or mobilized along with triglycerides in bovine adipose tissue in response to insulin or epinephrine. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 93:83–93.
- Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci.* 85:2173–2179.
- Bian, S. B., R. Elliott, I. Immig, and D. F. Sun. 2007. The influence of beta-carotene supplementation on post-partum disease and subsequent reproductive performance of dairy cows in China. *J. Anim. Feed Sci.* 16(Suppl. 2):370–375.
- Bindas, E. M., F. C. Gwazdauskas, R. J. Aiello, J. H. Herbein, M. L. McGilliard, and C. E. Polan. 1984. Reproductive and metabolic characteristics of dairy cattle supplemented with β -carotene. *J. Dairy Sci.* 67:1249–1255.
- Borel, P., J. Draï, H. Faure, V. Fayol, C. Galabert, M. Laromiguière, and G. Le Moel. 2005. Recent knowledge about intestinal absorption and cleavage of carotenoids. *Ann. Biol. Clin. (Paris)* 63:165–177.
- Castillo, C., J. Hernandez, A. Bravo, M. Lopez-Alonso, V. Pereira, and J. L. Benedito. 2005. Oxidative status during late pregnancy and early lactation in dairy cows. *Vet. J.* 169:286–292.
- Chauveau-Duriot, B., D. Thomas, J. Portelli, and M. Doreau. 2005. Carotenoids content in forages: Variation during conservation. *Renc. Rech. Ruminants.* 12:117.
- Chawla, R., and H. Kaur. 2004. Plasma antioxidant vitamin status of periparturient cows supplemented with α -tocopherol and β -carotene. *Anim. Feed Sci. Technol.* 114:279–285.
- Chew, B. P., L. L. Hollen, J. K. Hillers, and M. L. Herlugsen. 1982b. Relationship between vitamin A and β -carotene in blood plasma and milk and mastitis in Holsteins. *J. Dairy Sci.* 65:2111–2118.
- Chew, B. P., and J. S. Park. 2004. Carotenoid action on the immune response. *J. Nutr.* 134:257S–261S.
- Chew, B. P., H. Rasmussen, M. H. Pubols, and R. L. Preston. 1982a. Effects of vitamin A and β -carotene on plasma progesterone and uterine protein secretions in gilts. *Theriogenology* 18:643–654.
- De Ondarza, M. B., J. W. Wilson, and M. Engstrom. 2009. Case study: Effect of supplemental β -carotene on yield of milk and milk components and on reproduction of dairy cows. *Prof. Anim. Sci.* 25:510–516.
- Drackley, J. K. 1999. Biology of dairy cows during the transition period: The final frontier? *J. Dairy Sci.* 82:2259–2273.
- Folman, Y., I. Ascarelli, D. Kraus, and H. Barash. 1987. Adverse effect of β -carotene in diet on fertility of dairy cows. *J. Dairy Sci.* 70:357–366.
- Goff, J. P., K. Kimura, and R. L. Horst. 2002. Effect of mastectomy on milk fever, energy, and vitamins A, E, and β -carotene status at parturition. *J. Dairy Sci.* 85:1427–1436.
- Heuer, C., Y. H. Schukken, and P. Dobbelaar. 1999. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. *J. Dairy Sci.* 82:295–304.
- Holden, J. M., A. L. Eldridge, G. R. Beecher, I. M. Buzzard, S. Bhagwat, C. S. Davis, L. W. Douglass, S. Gebhardt, D. Haytowitz, and S. Schakel. 1999. Carotenoid content of US foods: An update of the database. *J. Food Compos. Anal.* 12:169–196.
- Johnston, L. A., and B. P. Chew. 1984. Peripartum changes of plasma and milk vitamin A and β -carotene among dairy cows with or without mastitis. *J. Dairy Sci.* 67:1832–1840.
- Kaewlamun, W., M. Okouyi, P. Humblot, D. Remy, M. Techakumphu, C. Duvaux-Ponter, and A. A. Ponter. 2012. Effects of a dietary supplement of β -carotene given during the dry period on milk production and circulating hormones and metabolites in dairy cows. *Rev. Med. Vet. (Toulouse)* 163:235–241.
- Kaewlamun, W., M. Okouya, P. Humblot, M. Techakumphu, and A. A. Ponter. 2011. Does supplementing dairy cows with β -carotene during the dry period affect postpartum ovarian activity, progesterone, and cervical and uterine involution? *Theriogenology* 75:1029–1038.
- Kawashima, C., K. Kida, F. J. Schweigert, and A. Miyamoto. 2009. Relationship between plasma carotene concentration during the peripartum period and ovulation of the first follicular wave postpartum in dairy cows. *Anim. Reprod. Sci.* 111:105–111.
- Kawashima, C., S. Nagashima, K. Sawada, F. J. Schweigert, A. Miyamoto, and K. Kida. 2010. Effect of β -carotene supply during close-up dry period on the onset of first postpartum luteal activity in dairy cows. *Reprod. Domest. Anim.* 45:e282–e287.

- LeBlanc, S. J., T. H. Herdt, W. M. Seymour, T. F. Duffield, and K. E. Leslie. 2004. Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. *J. Dairy Sci.* 87:609–619.
- Michal, J. J., L. R. Heirman, T. S. Wong, B. P. Chew, M. Frigg, and L. Volker. 1994. Modulatory effects of dietary β -carotene on blood mammary leukocyte function in periparturient dairy cows. *J. Dairy Sci.* 77:1408–1421.
- Morales, A., A. Rosas, A. González, A. Antaramian, A. Varela-Echavarría, A. Shimada, and O. Mora. 2006. Cloning of the bovine β -carotene-15, 15'-oxygenase and expression in gonadal tissues. *Int. J. Vitam. Nutr. Res.* 76:9–17.
- Oldham, E. R., R. J. Eberhart, and L. D. Muller. 1991. Effects of supplemental vitamin A or β -carotene during the dry period and early lactation on udder health. *J. Dairy Sci.* 74:3775–3781.
- Patterson, D. S. P. 1965. Plasma carotenoids and fat mobilization in stall-fed cattle. *Nature* 206:1069.
- Schweigert, F. J., F. Engalbert, R. Mothes, A. Hurtienne, and I. Immig. 2007. Cooperative European study for the validation of a novel cow-side β -carotene assay in serum and blood. Page 162 in Program of 13th Int. Conf. on Production Disease in Farm Animals, Leipzig, Germany.
- Schweigert, F. J., and I. Immig. 2007. Rapid assessment of β -carotene status. *Int. Dairy Top.* 6:15–17.
- Tjoelker, L. W., B. P. Chew, T. S. Tanaka, and L. R. Daniel. 1988. Bovine vitamin A and β -carotene intake and lactational status. 1. Responsiveness of peripheral blood polymorphonuclear leukocytes to vitamin A and β -carotene challenge in vitro. *J. Dairy Sci.* 71:3112–3119.
- Wang, D., M. Garcia, R. S. Bisinotto, N. Martinez, F. S. Lima, L. F. Greco, J. H. Shin, A. M. M. DiCalaça, A. L. Ranieri, B. L. Artiga, E. K. Ganda, G. C. Gomes, L. F. V. Becker, S. C. Soares, V. S. Rezende, M. A. Engstrom, J. E. P. Santos, and C. R. Staples. 2013. Effect of supplementing vitamin E and β -carotene to prepartum Holstein cattle on health and reproductive responses. *J. Dairy Sci.* 96 (E-Suppl. 1):W76. (Abstr.)