



CASE STUDY: Effect of Supplemental β -Carotene on Yield of Milk and Milk Components and on Reproduction of Dairy Cows

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ABSTRACT

A well-managed, high-producing commercial dairy herd was used to test the impact of supplementing β -carotene (425 mg/d per cow) to lactating multiparous Holstein cows with normally low serum β -carotene ($< 3 \mu\text{g/mL}$). Milk production was not affected by treatment (43.83 and 43.65 kg/d for the β -carotene and control diets). There was no difference because of treatment in overall 3.5% FCM (43.23 vs. 42.24 kg/d for the β -carotene and control diets) but early-lactation cows (0 to 100 DIM) and mature cows (3+ lactation) tended to produce more 3.5% FCM when supplemented with β -carotene ($P < 0.10$). Milk fat percentage was higher ($P < 0.05$) with supplemental β -carotene (3.28 and 3.18% for β -carotene and control cows), especially in early-lactation cows and mature cows. Overall milk fat yield (kg/d) was unaffected by treatment but early-lactation and mature cows supplemented with β -carotene tended to produce more milk fat than control cows. Milk true protein percentage and yield

were not affected by treatment. Milk urea N levels were higher for cows supplemented with β -carotene (16.06 vs. 15.72 mg/dL for β -carotene-supplemented and control cows; $P < 0.05$). Somatic cell count was not affected by treatment. Overall pregnancy rate was unaffected by treatment, but after 105 d of β -carotene supplementation, pregnancy rate was 22% for β -carotene-supplemented cows compared with 11% for control cows. β -Carotene supplementation increased percentage of milk fat and tended to increase 3.5% FCM and milk fat yield in early-lactation and mature cows.

Key words: β -carotene, milk yield, milk component, reproduction

INTRODUCTION

Dietary β -carotene is recognized as the major precursor of vitamin A. However, β -carotene also functions separately as an antioxidant and can directly enhance immunity, with possible reproductive and mammary benefits (Chew, 1993). The NRC (2001) concluded that data were insufficient to establish a β -carotene

requirement for dairy cattle. Responses to β -carotene supplementation have been inconsistent (Table 1), in part because of the wide variation in serum β -carotene status (Weiss, 1998). A large proportion of serum samples from the 1996 National Animal Health Monitoring System study of US dairy herds (National Animal Health Monitoring System, 1996) contained less than $3.0 \mu\text{g/mL}$ β -carotene, the suggested level at which supplementation is beneficial (Frye et al. 1991). LeBlanc et al. (2004) found the mean serum β -carotene concentration of 1,828 samples from peripartum (± 1 wk) Holstein cows from 20 Canadian herds to be $1.12 \mu\text{g/mL}$ (SD = 0.78).

Some studies found supplemental β -carotene to have a positive effect on milk yield, immunity, and digestive function. Heat-stressed cows supplemented with 400 mg β -carotene increased milk yield by 11% (Arechiga et al., 1998). Oldham et al. (1991) supplemented cows with 300 mg β -carotene and increased milk yield by 6.4%, with this difference approaching significance. Rakes et al.

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Table 1. Previous responses to β -carotene supplementation

Study	β -Carotene (mg/d)	Milk yield	Milk fat (%)	SCC	Mastitis	Reproduction
Arechiga et al. (1998)	400	\uparrow 6 to 11% ($P < 0.05$)	—	—	—	35.4 vs. 21.1% pregnancy rate at 120 DIM when ≥ 90 d of β -carotene supplemented ($P < 0.05$)
Oldham et al. (1991)	300	\uparrow 6.4% (NS)	\downarrow 4.6% ($P < 0.05$)	NS	NS	—
Rakes et al. (1985)	300	NS	NS	Lower (NS)	—	Smaller cervix diameters at 21 and 28 DIM ($P < 0.05$)
Wang et al. (1988a)	600	—	—	—	—	NS
Wang et al. (1988b)	300	NS	—	—	\downarrow 84% ($P < 0.01$)	NS
Akordor et al. (1986)	400	NS	—	—	—	NS
Marcek et al. (1985)	300	—	—	—	—	Ovarian cysts; NS
Bindas et al. (1984)	600	NS	—	NS	—	NS

(1985) supplemented cows with 300 mg β -carotene and numerically lowered SCC content of milk without significantly improving milk production. Wang et al. (1988b) found that cows supplemented with 300 mg β -carotene required fewer clinical mastitis treatments, whereas Oldham et al. (1991) did not reduce the incidence of mastitis.

Supplemental β -carotene may enhance rumen function. In vitro growth of rumen bacteria and cellulose digestion has been increased with the addition of β -carotene in the presence of safflower oil (Hino et al., 1993). Diets designed for high production typically contain significant amounts of lipids, which increases the free radical burden in the rumen (Andrews et al., 2006). Other dietary antioxidants have increased fiber digestion when fed with diets containing high levels of unsaturated fat in continuous culture (Vázquez-Añón and Jenkins, 2007). Perhaps β -carotene may perform a positive role as an antioxidant in the rumen.

Benefits of supplemental β -carotene may be related to the conversion of circulating β -carotene to vitamin A, specifically in the uterus and ovaries (Schweigert, 2003). Cows that ovulated during the first follicular

wave postpartum had a higher mean plasma β -carotene concentration than anovulatory cows (2.97 ± 0.24 vs. 1.53 ± 0.14 $\mu\text{g/mL}$) at 3 wk prepartum (Kawashima et al., 2009). Pregnancy rate at 120 d postpartum in heat-stressed cows supplemented with 400 mg β -carotene/d for ≥ 90 d was increased (35.4 vs. 21.1%; Arechiga et al., 1998). Others have seen no positive reproductive responses to β -carotene supplementation (Marcek et al., 1985; Akordor et al.,

1986; Wang et al., 1988a,b), possibly because of season or β -carotene status (Weiss, 1998).

The objective of this trial was to determine if supplementing β -carotene to lactating multiparous Holstein cows in a commercial herd with normally low serum β -carotene (< 3 $\mu\text{g/mL}$) but adequate vitamin A supplementation (8,400 IU/kg) would affect the synthesis of milk components, milk yield, and reproduction.

Table 2. Overview of cow characteristics by treatment [AU10: Spell out ME305 in col. 1, bottom row]

Item	Control	β -Carotene
Total cows	249	266
Total observations	16,952	16,996
Cow lactation no.		
1	0	1
2	113	108
3	67	76
4	39	48
5	15	14
6	7	7
7	4	8
8	4	4
Mean lactation no.	3.02 ± 1.29	3.21 ± 1.35
Mean DIM	149 ± 83	143 ± 78
Mean previous ME305 milk, kg	$12,879 \pm 2,811$	$13,233 \pm 2,808$

MATERIALS AND METHODS

Animals

Treated and control cows were housed in a free-stall barn in separate pens in a side-by-side study. The study was 120 d in length, beginning January 18, 2008, and ending May 16, 2008. Cows were fed a TMR once per day and had ad libitum access to the TMR and water. Cows were milked 3 times per day. All procedures were in accordance with the guidelines presented in Guidelines for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. **[AU1: Please provide a year here and add citation to References.]**

Experimental Treatments

Half the cows ($n = 266$) received supplemental β -carotene (425 mg/d per cow) and half did not ($n = 249$). The basal ration consisted of corn silage, alfalfa-grass haylage, high-moisture shelled corn, and a commercial feed blend. Cows were fed monensin at 299 mg/d per cow (Rumensin 80, Elanco Animal Health, Greenfield, IN).

Measurements and Analytical Methods

Daily pen DMI were monitored and recorded. The nutrient analyses of the diets consumed were calculated using CPM Dairy 3.08. **[AU2: Please provide the name and location of the supplier. If online only, provide a URL.]** Before beginning the study and every 4 wk throughout the study, all forages were sampled and analyzed via chemical analysis at Cumberland Valley Analytical Services (Hagerstown, MD). Samples were analyzed for DM [forages: 105°C for 3 h per National Forage Testing Association **[AU3: Please add to References.]** (2002) recommendations; grains: method 930.15, AOAC, 2000], ash (AOAC, 2000, method 942.05, modified with a 0.5-g sample weight and a 535°C furnace temperature), ether extract (AOAC, 1990, method 920.39,

Table 3. Ingredient composition of diets by treatment (% of DM)
[AU11: In a footnote, provide the trade name, supplier, and supplier's location for the Cargill rumen bypass fat.]

Item	Control	β -Carotene
Corn silage	33.16	33.13
Mixed alfalfa-grass haylage	20.27	20.11
High-moisture shelled corn	13.73	13.75
Cornmeal	1.15	1.16
Soybean meal 48	8.20	8.23
Corn gluten feed	5.07	5.09
Corn distillers grains	4.64	4.66
Corn germ meal	4.07	4.09
Blood meal	1.48	1.49
Bakery product	0.35	0.35
Molasses	1.33	1.33
Dextrose	1.70	1.70
Energy Booster ¹	0.37	0.37
Cargill rumen bypass fat	0.98	0.98
Fat mixer	0.30	0.30
Sodium bicarbonate	0.52	0.52
Calcium carbonate	1.39	1.40
Alimet ²	0.06	0.06
Smartamine M ³	0.04	0.04
Salt	0.44	0.44
Dicalcium phosphate	0.13	0.13
Diamond V XP yeast culture ⁴	0.03	0.03
Urea	0.17	0.17
Magnesium oxide	0.21	0.21
Potassium magnesium sulfate	0.09	0.09
Trace minerals-vitamins	0.11	0.11
β -Carotene	0	0.05

¹Energy Booster (MS Specialty Nutrition, Dundee, IL).

²Alimet (Novus International Inc., St. Louis, MO).

³Smartamine M (Adisseo USA Inc., Alpharetta, GA).

⁴Diamond V XP (Diamond V Corporation, Cedar Rapids, IA).

Table 4. Nutrient analysis of diets by treatment

Item	Control diet	β -Carotene diet
CP, % of DM	17.88	17.89
Soluble CP, % of CP	38.29	38.22
RUP, % of CP	36.03	36.18
Methionine, % of MP	2.42	2.42
Lysine, % of MP	6.75	6.75
NE _p , Mcal/kg	1.78	1.78
ADF, % of DM	16.92	16.86
NDF, % of DM	29.96	29.90
Forage NDF, % of DM	21.69	21.60
Lignin, % of DM	2.53	2.53
NFC, ¹ % of DM	41.42	41.42
Sugar, % of DM	5.81	5.82
Starch, % of DM	25.32	25.33
Ether extract, % of DM	5.47	5.47
Calcium, % of DM	0.98	0.98
Phosphorus, % of DM	0.36	0.37
Magnesium, % of DM	0.33	0.33
Potassium, % of DM	1.18	1.18
Sulfur, % of DM	0.26	0.26
Sodium, % of DM	0.40	0.40
Chlorine, % of DM	0.53	0.53
Iron, ppm	195.22	211.80
Zinc, ppm	88.50	88.69
Copper, ppm	24.11	24.14
Manganese, ppm	70.75	70.87
Selenium, ppm	0.46	0.46
Cobalt, ppm	1.42	1.43
Iodine, ppm	0.76	0.76
Vitamin A, kIU/kg	8.43	8.45
Vitamin D, kIU/kg	1.56	1.58
Vitamin E, IU/kg	38.85	38.98

¹Nonfiber carbohydrates.

modified with anhydrous ether extraction, boiled 20 min, rinsed 20 min), CP (AOAC, 2000, method 990.03), soluble CP (Krishnamoorthy et al., 1982), ADF (AOAC 2000, method 973.18, modified with Whatman

Table 5. Production and component least squares means (\pm SE) of all cows by treatment

Item	Control		β -Carotene		P-value	Observations
	Least squares mean	SE	Least squares mean	SE		
Milk, ¹ kg/d	43.65	0.56	43.83	0.55	0.8150	33,011
3.5% FCM, ¹ kg/d	42.24	0.56	43.23	0.57	0.2166	1,248
Fat, %	3.18	0.03	3.28	0.03	0.0461	1,261
Fat, ¹ kg/d	1.41	0.02	1.46	0.02	0.1180	1,248
Protein, %	2.99	0.01	2.99	0.01	0.6968	1,261
Protein, ¹ kg/d	1.32	0.02	1.34	0.02	0.5270	1,248
Milk urea N, mg/dL	15.72	0.11	16.06	0.11	0.0335	1,261
SCC, \times 1,000	170.1	29.89	140.6	30.73	0.4912	1,261

¹Covariate = previous ME305, kg ($P < 0.0001$).

934-AH glass microfiber filters with 1.5- μ m particle retention used in place of a fritted glass crucible), NDF (Goering and Van Soest, 1970, modified without sodium sulfite and using Whatman 934-AH glass microfiber filters with 1.5- μ m particle retention used in place of a fritted glass crucible), lignin (Goering and Van Soest, 1970), starch (Holm et al., 1986), and sugar (Dubois et al., 1956). Nonfiber carbohydrate was calculated as the difference between 100 and the sum of CP (minus NDF-bound CP), NDF, ether extract, and ash. Analyses of Ca, P, Mg, K, Na, Fe, Zn, Cu, and Mn were conducted using a Perkin-Elmer 3300 XL ICP [AU4: Please spell out ICP.] (Perkin-Elmer, Shelton, CT) according to method 985.01 of AOAC (2000). Sulfur was analyzed using a Leco S-144DR Sulfur Combustion Analyzer (Leco, St. Joseph, MI). Chloride ion was extracted with 1% nitric acid and analyzed using a Corning 925 Chloride Analyzer (CIBA-Corning, Medfield, MA).

Daily milk production of individual cows was recorded using an AFI2000 V1.28 system. [AU5: Please provide a supplier and location] Milk was sampled on wk 11, 13, 15, and 17 of the study. Milk samples were preserved with Bronolab-W II (D&F Control Systems Inc., Dublin, CA), a liquid preservative containing the active ingredient Bronopol, and sent to Dairy One Milk Laboratories (Ithaca, NY). Milk was analyzed for fat, true protein, SCC, and milk urea

N (MUN) with an infrared analyzer (MilkoScan 4000, Foss Electric, Hillerød, Denmark).

Pregnancy rate (percentage of cows that were eligible to become pregnant and became pregnant) was evaluated for control compared with β -carotene-supplemented cows for seven 21-d periods from the beginning of the trial (January 18, 2008), using Dairy Comp 305 software (Valley Ag Software, Tulare, CA). Mean serum β -carotene was assessed on 10 random cows per pen (DIM = 110 \pm 34) at d 60 and 120 as described by Schweigert et al. (2007) using the iEx system, a single-step denaturation, and β -carotene extraction into organic solvent, followed by β -carotene measurement using iCheck (BioAnalyt GmbH, Teltow, Germany), a portable spectrophotometer.

Statistical Analysis

Before beginning the study, cow groups were balanced based on lactation number, DIM, and milk production during the 12 d before the beginning of the study. All data from cows on study for more than 2 wk were analyzed using JMP statistical software (SAS Institute, Cary, NC) to determine if milk production and milk component production were affected by treatment. Milk yield, percentage of milk fat, kilograms of milk fat, percentage of protein, kilograms of protein, 3.5% FCM, MUN, and SCC were analyzed using the REML model-fitting protocol of JMP, and

subsequent multiple 2-sample comparisons were performed with Tukey's honest significant differences test. The model fit used DIM (by category), lactation number (by category), and pen (treatment vs. control) as independent factors. Previous ME305 [AU6: Please spell out ME305] milk (kg) was used as a covariate in the statistical models.

Pair-wise comparisons between the control and treatment groups within lactation category (parity 2 or 3+) and within DIM category (0 to 100, 101 to 200, and 201+ DIM) were subsequently recalculated using the contrast analysis of JMP, which uses Student's *t*-test. Student's *t*-test does not adjust for multiple comparisons and is less conservative than Tukey's honest significant differences test. Significant differences from the contrast analysis indicate trends in the data. The effect of treatment on pregnancy rate was evaluated using a chi-square approximation (Wilcoxon/Kruskal-Wallis test, JMP software).

RESULTS AND DISCUSSION

Animals

The 2 groups of cows used for the study were very similar in lactation number (3.02 and 3.21 for the control and treatment groups) and DIM (149 and 143 for the control and treatment groups; Table 2). Mean serum β -carotene assessed on d 60 of the study in 10 random cows per

Table 6. Production and component least squares means (\pm SE) according to parity and DIM by treatment

Item	Control		β -Carotene		P-value ¹
	Least squares mean	SE	Least squares mean	SE	
Milk, ^{2,3} kg/d					
Parity 2	44.42	0.84	44.05	0.85	0.75
Parity 3+	42.88	0.76	43.62	0.71	0.47
0 to 100 DIM	47.78	0.57	48.52	0.56	0.35
101 to 200 DIM	43.65	0.56	43.59	0.55	0.94
201+ DIM	39.52	0.57	39.40	0.56	0.88
3.5% FCM, ^{2,3,4} kg/d					
Parity 2	43.88	0.82	43.84	0.89	0.98
Parity 3+	40.60	0.76	42.62	0.71	0.05
0 to 100 DIM	48.33	0.88	50.39	0.80	0.08
101 to 200 DIM	43.58	0.74	43.49	0.77	0.94
201+ DIM	34.81	1.02	35.80	1.04	0.50
Fat, %					
Parity 2	3.19	0.05	3.25	0.05	0.35
Parity 3+	3.18	0.05	3.30	0.04	0.04
0 to 100 DIM	3.15	0.06	3.30	0.05	0.06
101 to 200 DIM	3.15	0.05	3.25	0.05	0.18
201+ DIM	3.24	0.07	3.28	0.07	0.65
Fat, ^{2,3,4} kg/d					
Parity 2	1.47	0.03	1.48	0.03	0.90
Parity 3+	1.36	0.03	1.45	0.03	0.02
0 to 100 DIM	1.62	0.04	1.73	0.03	0.03
101 to 200 DIM	1.46	0.03	1.47	0.03	0.82
201+ DIM	1.16	0.04	1.19	0.04	0.67
Protein, ³ %					
Parity 2	2.99	0.02	2.99	0.02	0.84
Parity 3+	2.98	0.02	3.00	0.02	0.40
0 to 100 DIM	2.92	0.02	2.96	0.02	0.11
101 to 200 DIM	2.99	0.02	3.01	0.02	0.40
201+ DIM	3.05	0.02	3.00	0.02	0.18
Protein, ^{2,3,4} kg/d					
Parity 2	1.38	0.02	1.36	0.03	0.65
Parity 3+	1.27	0.02	1.32	0.02	0.13
0 to 100 DIM	1.49	0.02	1.53	0.02	0.21
101 to 200 DIM	1.37	0.02	1.36	0.02	0.79
201+ DIM	1.11	0.03	1.12	0.03	0.79
Milk urea N, mg/dL					
Parity 2	15.69	0.16	16.25	0.17	0.02
Parity 3+	15.75	0.15	15.86	0.14	0.60
0 to 100 DIM	15.48	0.19	16.02	0.17	0.04
101 to 200 DIM	15.92	0.15	16.12	0.17	0.39
201+ DIM	15.75	0.22	16.03	0.22	0.38

¹Student's *t*-test contrast analysis does not adjust for multiple comparisons. Significant differences indicate likely trends in the data.

²Covariate = previous ME305, kg ($P < 0.0001$). [AU12: Please spell out ME305.]

³DIM category interactions with treatment were significant ($P < 0.05$).

⁴Lactation category interactions with treatment were significant ($P < 0.05$).

pen (DIM = 108 \pm 37) was 2.47 \pm 0.98 and 3.56 \pm 1.93 μ g/mL for the control and treatment groups. Mean serum β -carotene assessed on d 120 of the study in 10 random cows per pen (DIM = 108 \pm 33) was 1.71 \pm 0.55

and 2.75 \pm 1.34 μ g/mL and for the control and treatment groups, respectively.

Experimental Diets

The diet was typical of high-production diets fed in the northeastern United States. Diet nutrient analyses and diet ingredient compositions were

Table 7. Pregnancy rate by treatment for seven 21-d periods from trial start

Day β -carotene received	Date	Control			β -Carotene		
		Pregnant eligible	Pregnant	Pregnancy rate	Pregnant eligible	Pregnant	Pregnancy rate
0	1/18/08	67	15	22	47	8	17
21	2/8/08	66	14	21	51	10	20
42	2/29/08	64	13	20	60	11	18
63	3/21/08	62	17	27	73	18	25
84	4/11/08	55	14	25	67	16	24
105	5/2/08	51	6	12	62	14	23
126	5/23/08	54	5	9	61	12	20
Total		419	84	20 (mean)	421	89	21 (mean)

calculated from means of daily ingredients offered in the TMR (Tables 3 and 4). Daily pen DMI were excellent throughout the study for both groups, with 24.0 ± 0.78 kg/d per cow consumed by control cows and 24.2 ± 0.78 kg/d per cow consumed by β -carotene-supplemented cows.

Milk Yield and Milk Components

Overall milk production was excellent throughout the study, with means of 43.65 and 43.83 kg/d for the control and β -carotene-supplemented cows. Milk production was not affected ($P = 0.82$) by β -carotene supplementation (Table 5).

Milk fat percentage was higher ($P < 0.05$) for cows fed β -carotene, with means of 3.28% for cows fed β -carotene and 3.18% for cows on the control diet (Table 5). This difference was especially true for early-lactation cows (0 to 100 DIM; $P = 0.06$) and cows in their third or greater lactation ($P = 0.04$; Table 6). Lotthammer (1978) concluded that β -carotene deficiency was related to a lower milk fat percentage. A higher milk fat percentage in response to β -carotene supplementation makes sense if it has a positive effect on the rumen cellulolytic bacteria, as seen in vitro by Hino et al. (1993). Another possible mode of action could be that supplemental β -carotene altered rumen biohydrogenation and reduced the formation of *trans*-10 isomers in

the rumen, resulting in less milk fat depression, as vitamin E has been shown to do (Bell et al., 2006; Pottier et al., 2006)

Overall, 3.5% FCM was not affected by β -carotene supplementation (Table 5; $P = 0.22$), but based on the Student's *t*-test contrast analysis, early-lactation cows (0 to 100 DIM) tended to produce more 3.5% FCM when supplemented with β -carotene (50.39 vs. 48.33 kg/d; $P = 0.08$) and cows in their third or greater lactation produced more 3.5% FCM when supplemented with β -carotene (42.62 vs. 40.60 kg/d; $P = 0.05$; Table 6). Although the contrast analysis does not adjust for multiple comparisons and significant differences ($P < 0.05$) indicate only trends in the data, it may point out that early-lactation and mature cows were more responsive to supplemental β -carotene, similar to heat-stressed cows (Arechiga et al., 1998).

Overall milk fat production (kg/d) was unaffected by treatment ($P = 0.12$; Table 5). When cows were separated according to stage of lactation and lactation number, cows between 0 and 100 DIM supplemented with β -carotene produced 1.73 kg/d milk fat, whereas control cows produced 1.62 kg/d ($P = 0.03$; Table 6). Cows in their third lactation or greater also produced more milk fat (kg/d) when supplemented with β -carotene (1.45 vs. 1.36 kg/d; $P = 0.02$; Table 6). Milk true protein percentage and milk true protein production (kg/d) were

not affected by β -carotene supplementation ($P > 0.50$; Table 5), regardless of DIM or lactation number (Table 6).

Overall MUN (mg/dL) levels were 2% higher for cows supplemented with β -carotene (16.06 vs. 15.72 mg/dL for β -carotene-supplemented and control cows; $P = 0.03$; Table 5), with the effect being greatest in second-lactation cows ($P = 0.02$) and early-lactation cows (0 to 100 DIM; $P = 0.04$; Table 6). The reason for any increase in MUN levels attributable to β -carotene supplementation is not clear.

Somatic cell count was not affected by treatment ($P = 0.49$; Table 5). This response is contrary to those of Rakes et al. (1985) and Wang et al. (1988b) but is comparable with that of Oldham et al. (1991), in which the incidence of mastitis was not reduced with β -carotene supplementation.

Reproduction

Pregnancy rates (%) for seven 21-d periods during the 21 wk from the beginning of the trial are shown in Table 7. Mean pregnancy rate was numerically higher with β -carotene supplementation (20 vs. 21% for the control vs. treatment group) but this difference was not significant ($P = 1.0$). However, for the last two 21-d periods, pregnancy rate was 11% for the control cows compared with 22% for cows supplemented with β -carotene. This might be expected because cows fed β -carotene beginning shortly after calving became eligible

for breeding after approximately four 21-d periods. Cows eligible for breeding before this time in the study began β -carotene supplementation later in their lactation. This may indicate that an extended period of β -carotene supplementation is necessary to have a positive impact on reproductive efficiency. Arechiga et al. (1998) found that β -carotene supplementation (400 mg/d) did not improve overall reproductive function but that those cows supplemented for ≥ 90 d did have a higher pregnancy rate at 120 DIM (35.4 vs. 21.1%). A particular stress occurring at the end of this trial may also have triggered a response to β -carotene supplementation. A larger multisite study would be helpful to better understand the impact of β -carotene supplementation on reproduction.

IMPLICATIONS

The positive response in milk fat percentage to supplemental β -carotene is interesting. Further studies are needed to determine the mode of action of β -carotene in milk fat synthesis and the effect of β -carotene on the fiber-digesting bacteria in the rumen. It would be beneficial to conduct further *in vitro* and *in vivo* studies supplementing β -carotene in diets varying in rumen available fat, fiber, and starch. This study also suggests possible reproductive benefits from β -carotene supplementation. Future studies with greater cow numbers and multiple sites are needed to prove this.

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