ABSTRACT

Little is known about transfer of dietary β-carotene into colostrum, its absorption by the calf, and its effects on retinol and α-tocopherol in the calf when the dam’s dietary vitamin A is adequate. Our objective was to assess the effect of β-carotene supplementation during the close-up dry period on the colostrum and calf. The study was conducted on a large commercial dairy farm in Indiana during early summer of 2015. Ninety-four multiparous Holstein cows were blocked by calving data, parity, and previous production, and then randomly assigned to either control or β-carotene (BC) treatments. While locked in headgates each morning, each cow received a topdress of β-carotene (Rovimix, DSM Nutritional Products, 8 g/d; provided 800 mg β-carotene) or carrier from 21 d before expected calving until calving. Colostrum was collected within 2 h of parturition. Calf blood samples were obtained within 2 h of birth before receiving the dam’s colostrum, at 24 h after birth, and at 7 d and 60 d of age. Blood serum was analyzed for β-carotene, retinol, α-tocopherol, and other metabolites and enzymes. Colostrum was analyzed for β-carotene, retinol, α-tocopherol, colorimetry profile, and milk components. Data were analyzed using mixed-effects models in SAS (SAS Institute Inc.). Calf serum β-carotene data were analyzed using the FREQ procedure. Colostrum β-carotene was higher for BC cows. Colostrum from BC cows had increased a* [measures red (positive) to green (negative)] and b* [measures yellow (positive) to blue (negative)] colorimeter values, indicating that β-carotene altered colostrum color toward red and yellow. Supplementation did not affect colostral or calf IgG concentrations. Colostrum color indices were correlated with IgG concentrations as well as concentrations of β-carotene, retinol, and α-tocopherol. Before receiving colostrum, the concentration of β-carotene in calf serum was below the detectable threshold of 0.05 μg/mL. At 24 h of age, the number of calves with detectable β-carotene concentrations increased, with more calves from BC cows (52.1%) having detectable concentrations than calves from cows in the control group (6.1%). No differences in concentrations of retinol or α-tocopherol were observed in calf serum. Supplementation of β-carotene to cows decreased activities of gamma-glutamyl transpeptidase and glutamate dehydrogenase in calf serum. In pregnant cows already receiving adequate vitamin A, supplementation of β-carotene increased concentration of β-carotene in colostrum, altered colostrum color, and increased serum β-carotene in calves at birth.

Key words: β-carotene, transition cow, colostrum, color

INTRODUCTION

Achieving adequate intake of colostrum soon after birth is a critical factor for calf survival, health, and growth (Godden, 2008). Because of low placental transfer, calves are born with low vitamin A status and depend on colostrum to receive vitamin A (NRC, 2001). Increased concentrations of retinol in colostrum improve vitamin A status of newborn calves (Puvogel et al., 2005). Although it is assumed that the β-carotene found in colostrum could be used as a source of vitamin A for the calf, data on dynamics of β-carotene, retinol, and α-tocopherol in the cow and newborn calf are limited.

In addition to its role as a precursor to vitamin A, β-carotene also functions as an antioxidant. Because of their high metabolic rate after birth, calves have an increased load of reactive oxygen species that can damage cellular structures, particularly their naive immune system (Lykkesfeldt and Svendsen, 2007). Whether supplemental β-carotene provided to the dam during late gestation would increase β-carotene in the colostrum is uncertain, as Aragona et al. (2021) found no difference in colostral β-carotene between control and supplemented cows. However, it has been demonstrated that calves born from dams supplemented with β-carotene prepartum had a lesser incidence of diarrhea, greater serum IgG, and greater retinol concentrations.
(Lothammer, 1978). It is not known whether similar effects would occur in a herd where vitamin A status of prepartum cows is adequate.

A common perception is that color of colostrum is associated with quality and nutrient composition. However, few quantitative data are available to support that supposition. Gross et al. (2014) used colorimetry to determine that colostral IgG concentration was correlated with the relative lightness parameter L*. At an IgG concentration of 50 mg/mL, color measurement had a negative predictive value of 87.9% (Gross et al., 2014). Those authors concluded that colostrum color could be used to estimate IgG concentration as well as concentrations of fat, protein, and lactose. Because we expected colostrum color to be affected by β-carotene fed to cows (Calderón et al., 2007), we sought to determine a quantitative measure of color and relate it to colostrum quality and nutrient content. We also developed a simple colostrum color scale to estimate β-carotene concentration on farm.

Our hypothesis was that supplemental β-carotene provided to cows as a topdress during the close-up period would increase β-carotene in colostrum and provide metabolic benefits to the newborn calves. Our 2-part objective was (1) to determine the effects of prepartal supplementation of β-carotene on colostrum composition, concentration of retinol and α-tocopherol, quality, and color, and (2) to determine concentrations of β-carotene, retinol, α-tocopherol, IgG, and metabolites and enzymes in serum of newborn calves fed their dam’s colostrum.

MATERIALS AND METHODS

Description of Commercial Farm

The University of Illinois Institutional Animal Care and Use Committee approved all procedures involving animals. The research trial was conducted on a large commercial dairy farm in northern Indiana, which was described previously (Prom et al., 2022). The herd consisted of approximately 7,000 cows in milk and 675 dry cows. The herd had an average 21-d pregnancy rate of 18% and an average 305-d mature equivalent milk yield of ~14,000 kg. The farm was selected for its large herd size, strict adherence to protocols, well-maintained computer records, and commitment to research. The facilities of particular interest included headlocks in the close-up pens, headlocks in the fresh pens, sanitary maternity area, and headlock with stand-alone milking setup in maternity area. The 2 dry-cow freestall barns, which included the maternity area, and calf raising facilities were located on separate sites near the main dairy site. The calf ranch was located approximately halfway between the two. Upon calving, cows were milked in the maternity area. Twice daily, newly calved cows were taken by stock trailer to the fresh cow pens at the main dairy site. Once daily, new calves were taken by stock trailer to the calf ranch, where they were housed in individual hutches.

The diet and β-carotene supplementation procedures were described in Prom et al. (2022). Briefly, 94 prepartum Holstein cows in the close-up pens were locked in headlocks in the morning and fed either a control (CON; n = 47) or a β-carotene-supplemented topdress (BC; n = 47). The number of cows was determined previously (Prom et al., 2022) based on detection of differences in milk production. Cows were blocked by expected calving dates and parity, and then balanced by previous lactation milk yield. Cows then were assigned randomly (coin toss) to either the BC treatment or CON treatment. The topdress was a mixture of carrier (50 g of cracked corn, 50 g of dry molasses flakes, 50 g of shredded beet pulp, and 25 g of yeast culture) either without or with β-carotene (8 g/d of Rovimix, DSM Nutritional Products; supplied 800 mg/d of β-carotene). Supplementation was stopped at calving. Both CON and BC cows were housed in each of the 2 close-up pens. The close-up diet fed to the cows contained 0.81 mg/kg of DM of β-carotene, 6,600 IU/kg of DM of retinol, and 140 IU/kg of DM of α-tocopherol. Effects of β-carotene on the cow were reported previously (Prom et al., 2022). Research staff were not blinded to treatments but farm staff and veterinarians were. No protocol was developed from the study.

Colostrum

Within 2 h of calving, each cow was milked with a bucket milker in the maternity headlock. Colostrum was stirred and then samples were placed into 2 15-mL plastic conical Falcon tubes (Becton Dickinson) and 2 60-mL flip-top milk sampling vials (Thermo Scientific). The remaining colostrum was then used to feed to the calf as described in the following section. The colostrum was immediately assigned a color score on a 1 to 4 scale established for this study, with a score of 1 being almost white (the color of milk) and 4 having the color of orange juice (Figure 1).

Calves: Management and Sampling

All calves of the cows on trial were given 3.78 L of colostrum from their dam within 2 h of birth. Dam-specific colostrum was administered regardless of co-
lostrum quality. If the cow produced less than 3.78 L of colostrum, additional colostrum was fed from a cow assigned to the same treatment. This extra colostrum was stored in the refrigerator at 3°C by the staff according to farm protocols and marked with the treatment and cow identification. Colostrum was stored for up to 3 d. All other care following calving, such as navel dipping and second colostrum feeding (from 6 to 12 h after the initial feeding), was done by the farm employees per their protocols. Heifer calves received the same color ear tag as their dam, whereas all bull calves received a different color tag.

Blood was sampled from the jugular vein before colostrum feeding within 2 h of birth (d 0), at 24 h ± 6 h (h 24) following birth, at 7 d of age (d 7), and again at 60 d of age (d 60). Samples were collected into two 10-mL red-gray stopper evacuated serum separation tubes and one 10-mL lavender Hemogard (BD Vacutainer) closure evacuated K$_2$EDTA whole blood tube. The whole blood tubes were placed on ice in a dark cooler, and the serum tubes were placed in dark pockets or wrapped with aluminum foil until centrifuged and frozen (<2 h).

Following the first 2 colostrum feedings, the calves were fed and cared for according to the farm’s protocols. Twice daily, they were given pasteurized whole milk supplemented with milk replacer when needed, along with starter and water. No supplemental β-carotene was given to the calves. Calves were weaned at 56 d of age but not moved to group pens until approximately 70 d of age. The d 60 samples were after weaning and were meant to represent a time period where the calves were under heightened stress from weaning and their changing diet.

Once weekly, the calves were assigned a health score by the research team according to the metrics developed by the University of Wisconsin (https://www.vetmed.wisc.edu/fapm/wp-content/uploads/2020/01/calf_respiratory_scoring_chart.pdf). The health scores included fecal, respiratory, nasal, ocular, and ear.

### Sample Analysis and Storage

Within 2 h of blood collection, the 2 serum tubes were centrifuged with a HN-SII centrifuge (Damon IEC) at 1,300 × g for 15 min. The serum was pipetted into 5-mL polystyrene tubes (Globe Scientific) and stored at −20°C in the on-farm chest freezer. The whole blood was used for on-farm β-carotene analysis using iEx vials and an iCheck reader (BioAnalyt), which was validated for bovine whole blood by Raila et al. (2012). The remaining whole blood was pipetted into 5-mL polystyrene tubes and stored at −20°C. Periodically throughout the study, the frozen samples were trans-
ported on ice by car back to the University of Illinois (Urbana, IL) where they were again stored at −20°C. Serum samples were taken on ice to the Diagnostic Center for Population and Animal Health at Michigan State University (East Lansing, MI) for HPLC analysis of retinol, α-tocopherol, and β-carotene. Other samples were sent to the University of Illinois College of Veterinary Medicine Diagnostic Laboratory (Urbana, IL) for automated enzymatic analysis of metabolites and enzymes. These metabolites included creatinine, urea N, total protein, albumin, globulin, Ca, P, Na, K, Cl, Mg, glucose, total alkaline phosphatase, aspartate aminotransferase, gamma-glutamyl transpeptidase (GGT), total bilirubin, creatine phosphokinase, total cholesterol, glutamate dehydrogenase (GLDH), bicarbonate, triglycerides, and anion gap. Assays for nonesterified fatty acid and BHB (Wako Diagnostics) were performed in-house.

Following centrifugation, the serum was checked for total protein using a digital refractometer (Misco). Serum samples obtained at d 0 and h 24 were sent on ice to Prairie Diagnostic Services (Saskatoon, Saskatchewan, Canada) to be analyzed for IgG concentration. Whole blood from the calves was tested for concentration of β-carotene and then stored at −20°C.

The colostrum in the 2 Falcon tubes was immediately frozen at −20°C and later transported to the on-campus freezer. One of the tubes was taken to the Diagnostic Center for Population and Animal Health for vitamin analysis, then sent on ice to Animal Diagnostic Services for IgG analysis, whereas the second tube remained in storage. The first flip-top vial of colostrum immediately had a Broad Spectrum Microtab II preservative tablet (D&F Control Systems Inc.) added and was then refrigerated at 4°C. Twice weekly, the vials with the preservative tablets were sent to Dairy Lab Services (Dubuque, IA) to be analyzed for fat, protein, SCC, lactose, other solids, TS, and MUN. The second flip-top vial was used for β-carotene analysis with the iCheck and Brix reading with a Misco refractometer, and then was refrigerated at 4°C. No preservative tablet was added to these samples as it would alter the color of the colostrum. Twice weekly, these vials were taken back to the University of Illinois, where the color was analyzed using a colorimeter (Konica Minolta) as described by Arkfeld et al. (2016). Colorimetry is similar to spectrophotometry, but is modified to account for human perception of color. Colorimeter results were on the CIELAB color space scale of L*a*b* (ASTM International, 2016), where L* measures relative lightness (0 = black to 100 = white), a* measures red (positive) to green (negative), and b* measures yellow (positive) to blue (negative).

### Statistical Analysis

Cow or calf was considered the experimental unit because treatments were applied individually to cows during feeding. Cows from both treatments were housed in the same pen, and pen was replicated so that environmental effects could be adequately accounted for in the statistical models. The collected data were divided into calf serum and colostrum data sets with each then analyzed using models developed in SAS 9.4 (SAS Institute Inc.). No specific criteria were established a priori for removal of samples. The PROC UNIVARIATE was used on each combination of variable, time point, and treatment assignment to examine residual plots and Shapiro-Wilk test statistics for normality. Outliers greater than 5 standard deviations from the median were removed. Following deletion of extreme outliers, the Brown-Forsythe test in PROC GLM was used to test each variable for equal variances. Transformations were not performed to obtain normality, but were performed where necessary to achieve homogenous variances. No transformations were necessary for the data reported here.

Continuous variables were used to construct mixed-effects models for a complete randomized design in PROC MIXED for each variable. The treatment, parity, pen numbers, and associated interactions were included in the model as fixed effects. We found no treatment by pen or 3-way interactions, so the terms were removed from the model. Parity was included in the model due to known milk yield and vitamin differences by parity. Of the 94 cows used for the trial, 61 were beginning their second lactation (29 for BC and 31 for CON), 26 were beginning their third lactation (15 for BC and 12 for CON), and 7 were beginning their fourth lactation (3 for BC and 4 for CON); additionally, the latter 2 parities were grouped as ≥3. The time point was included in the model for serum data and analyzed as a repeated measure. Several covariance structures were tested, and an autoregressive (1) structure was used for the calf data based on the Akaike information criterion. Model residuals were examined for normality and heteroscedasticity. For analysis of colostrum characteristics by color score, linear and quadratic contrasts were used to evaluate treatment means. Pearson correlations between variables were inspected using PROC CORR. Correlations of r = 0.25 to r = 0.59 are discussed as “moderate,” and those with r ≥ 0.60 are referred to as “strong.” For calf serum β-carotene data, PROQ FREQ was used. Statistical tests were deemed significant when P < 0.05 and as trending toward significance when 0.05 < P < 0.10. Data are available from the corresponding author by reasonable request.
RESULTS

Colostrum

Mean parity, mean previous lactation milk yield, mean initial BCS, and number of days dry were not different between the treatment groups. One colostrum sample was lost so that n = 47 for CON and 46 for BC. The results for colostrum analysis are shown in Table 1. Colostrum overall was of high quality, averaging 78.3 mg/mL and 25.1% for IgG and Brix, respectively. Maternal dietary treatment affected β-carotene (P < 0.01), a* (P = 0.01), b* (P < 0.01), color score (P < 0.01), and SCC (P = 0.03). The concentration of fat tended (P = 0.08) to be greater for colostrum from BC cows. Concentrations of retinol, α-tocopherol, IgG, protein, lactose, other solids, total solids, and MUN were not affected by maternal diet. Values for L* and Brix also were not affected by maternal diet.

Color score was significantly increased by β-carotene supplementation, as shown in Table 1. The mean color score for BC cows was 3.2, whereas it was 2.4 for CON cows. A visual representation of each color score, along with the mean β-carotene concentration in the colostrum for each score, is shown in Figure 1. No cows on the trial had a colostrum color score of 1. The mean β-carotene of colostrum scored 2 was 0.62 ± 0.15 μg/mL, whereas for scores 3 and 4 the concentration increased to 0.96 ± 0.16 μg/mL and 1.62 ± 0.18 μg/mL, respectively.

The treatment by parity interaction was significant for fat percentage. For BC-supplemented cows, fat percentage increased with parity, as cows in parity 2 and parity ≥3 had fat percentages of 3.46 ± 0.46 and 4.64 ± 0.48, respectively. However, fat percentages for CON cows were 3.62 ± 0.46 and 3.36 ± 0.48 for parity 2 and parity ≥3 cows. Significant effects of parity were detected for retinol, L*, b* (trend, P = 0.07), color score, IgG, and Brix, with all but L* being higher for parity ≥3 than for parity 2. The L* was lower for parity ≥3 than for parity 2.

We compared colostrum β-carotene concentrations determined with the rapid on-farm test with those determined in the laboratory with HPLC (Figure 2). As shown in Figure 2, the relationship was poor, with an R² of only 0.03.

Correlations among the key composition variables in colostrum with P ≤ 0.01 are shown in Table 2. Colostral β-carotene had moderate positive correlations with retinol (r = 0.42), α-tocopherol (r = 0.47), Brix (r = 0.28), a* (r = 0.48), b* (r = 0.54), and fat percentage (r = 0.44). Retinol was correlated with α-tocopherol (r = 0.31), Brix (r = 0.27), a* (r = 0.32), and fat percentage (r = 0.34). α-Tocopherol was moderately correlated with Brix (r = 0.55), IgG (r = 0.37), a* (r = 0.39), b* (r = 0.28), fat percentage (r = 0.45), and protein percent-
The Brix value was strongly correlated with IgG and protein (r = 0.73 and 0.64, respectively), and less strongly with L* (r = −0.34), a* (r = 0.30), and b* (r = 0.47). Concentration of IgG was negatively correlated with L* (r = −0.28), and positively correlated with b* (r = 0.51) and protein percentage (r = 0.68). Value L* was negatively correlated with a* (r = −0.42). Value a* was correlated with b* (r = 0.41). Value b* was correlated with protein percentage (r = 0.27).

Characteristics of colostrum by color score are shown in Table 3. Values of L* decreased linearly, whereas those of a* and b* increased linearly, as color score increased from 2 to 4. Concentrations of β-carotene, retinol, α-tocopherol, IgG, and Brix increased linearly as color score increased. Concentrations of fat and protein were not different among color scores. The concentration of lactose was lower for colostrum color score 3, whereas the SCC was greater for score 3.

**Calf Serum**

Two cows fed BC and one cow from the CON had twins, so the number of calves was 49 for CON and 48 for BC. β-Carotene concentration in calf serum could not be analyzed with PROC MIXED due to many of the observations having nondetectable concentrations.

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**Table 2.** Correlations among retinol, α-tocopherol, β-carotene, and other variables in colostrum from cows fed control (CON; n = 47) or β-carotene supplemented (BC; 800 mg/d; n = 46) diets prepartum (values from top to bottom are correlation coefficient, P-value, and n)†

| Item† | Retinol | α-Tocopherol | Brix | IgG | L*  | a*  | b*  | Fat % | Protein, % | SCC |
|-------|---------|--------------|------|-----|-----|-----|-----|-------|------------|-----|
| β-Carotene | 0.42 | 0.47 | 0.28 | 0.18 | −0.08 | 0.48 | 0.54 | 0.44 | 0.07 | 0.19 |
| <0.01 | <0.01 | <0.01 | 0.07 | 0.44 | <0.01 | <0.01 | <0.01 | 0.54 | 0.08 |
| 93 | 93 | 93 | 93 | 93 | 93 | 93 | 88 | 85 | 88 |
| Retinol | 0.31 | 0.27 | 0.06 | 0.003 | 0.48 | 0.23 | 0.34 | 0.03 | −0.10 |
| <0.01 | 0.01 | 0.53 | 0.98 | <0.01 | 0.03 | <0.01 | 0.79 | 0.36 |
| 93 | 93 | 93 | 93 | 93 | 93 | 93 | 88 | 85 | 88 |
| α-Tocopherol | 0.55 | 0.37 | 0.11 | 0.39 | 0.28 | 0.45 | 0.30 | 0.13 |
| <0.01 | <0.01 | 0.30 | <0.01 | <0.01 | <0.01 | 0.23 |
| 93 | 93 | 93 | 73 | 93 | 93 | 88 | 85 | 88 |
| Brix | 0.73 | 0.34 | 0.30 | 0.47 | 0.18 | 0.64 | 0.006 |
| <0.01 | <0.01 | <0.01 | <0.01 | 0.09 | <0.01 | 0.95 |
| 93 | 93 | 93 | 73 | 93 | 93 | 88 | 85 | 88 |
| IgG | 0.28 | 0.19 | 0.51 | 0.17 | 0.68 | 0.06 |
| <0.01 | 0.10 | <0.01 | 0.10 | <0.01 | 0.59 |
| 93 | 73 | 93 | 88 | 93 | 88 |
| L* | −0.42 | −0.25 | 0.24 | −0.18 | −0.02 |
| <0.01 | 0.02 | 0.02 | 0.10 | 0.80 |
| 73 | 93 | 88 | 85 | 88 |
| a* | 0.41 | 0.12 | 0.03 | 0.22 |
| <0.01 | 0.32 | 0.80 | 0.06 |
| 73 | 70 | 68 | 70 |
| b* | 0.22 | 0.32 | 0.05 |
| 0.04 | <0.01 | 0.06 |
| 88 | 88 | 88 |
| Fat % | 0.27 | −0.04 |
| 0.01 | 0.72 |
| 85 | 87 |
| Protein % | −0.06 |
| 0.57 |

†L* measures relative lightness (0 = black to 100 = white); a* measures red (positive) to green (negative); and b* measures yellow (positive) to blue (negative).
Consequently, PROC FREQ was used to investigate whether there were any treatment differences for the number of calves having concentrations above the detection threshold of 0.05 μg/mL. Results are shown in Figure 3. Treatment was significant for calves at 24 h (\(P < 0.01\)) and d 7 (\(P = 0.047\)). At 24 h, 89.3% of calves found to be above the β-carotene detection threshold were from β-carotene-treated cows. Similarly, at d 7, 85.7% of calves above the threshold were from cows supplemented with β-carotene. At d 0, only one calf (from a control cow) had detectable β-carotene concentrations, and at d 60, no calves did. The maximal number of calves with β-carotene concentrations above the threshold occurred at d 7 (\(n = 28\)).

Means for serum constituents by dam’s treatment are presented in Table 4. For metabolites, the only significant overall treatment effect was for albumin (trend, \(P = 0.09\)), which was slightly greater for calves from BC dams. Treatment by parity interactions or trends were noted for α-tocopherol, glucose, BHB, cholesterol, and the albumin-to-globulin ratio, which are shown in Table 5. The minerals Ca, P, Na, and Cl were all greater for

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**Table 3.** Least squares means (± SE) of color components and composition for colostrum with different color scores from cows fed control (\(n = 47\)) or β-carotene-supplemented (800 mg/d; \(n = 46\)) diets prepartum

| Variable | Colostrum color score | \(P\)-value |
|----------|-----------------------|-------------|
|          | 2                     | 3           | 4           | Linear | Quadratic |
| Color component\(^{1}\) | | | | | |
| \(L^*\) | 82.1 ± 0.68           | 78.8 ± 1.00 | 75.5 ± 1.62 | <0.01  | 0.96 |
| \(a^*\) | -0.70 ± 0.71          | 0.90 ± 0.82 | 3.23 ± 0.92 | <0.01  | 0.62 |
| \(b^*\) | 21.3 ± 0.58           | 25.2 ± 0.85 | 28.1 ± 1.37 | <0.01  | 0.64 |
| Composition | | | | | |
| β-Carotene, μg/mL | 0.62 ± 0.15 | 0.96 ± 0.16 | 1.62 ± 0.18 | <0.01  | 0.25 |
| Retinol, ng/mL | 4.004 ± 569 | 4.307 ± 620 | 5.578 ± 700 | 0.03  | 0.37 |
| α-Tocopherol, μg/mL | 2.48 ± 0.34 | 3.07 ± 0.39 | 3.89 ± 0.50 | 0.01  | 0.77 |
| IgG, mg/mL | 71.9 ± 2.15 | 86.6 ± 3.15 | 90.9 ± 5.08 | <0.01  | 0.22 |
| Brix, % | 23.7 ± 0.40 | 26.3 ± 0.59 | 28.7 ± 0.96 | <0.01  | 0.86 |
| Fat, % | 3.55 ± 0.42 | 3.57 ± 0.46 | 3.58 ± 0.60 | 0.96  | 0.98 |
| Protein, % | 15.18 ± 0.24 | 16.05 ± 0.36 | 15.82 ± 0.81 | 0.46  | 0.33 |
| Lactose, % | 2.61 ± 0.08 | 2.21 ± 0.12 | 2.64 ± 0.22 | 0.89  | 0.01 |
| SCC/mL × 1,000 | 667 ± 143 | 1,208 ± 211 | 725 ± 374 | 0.88  | 0.08 |

\(^{1}\)\(L^*\) measures relative lightness (0 = black to 100 = white); \(a^*\) measures red (positive) to green (negative); and \(b^*\) measures yellow (positive) to blue (negative).
Calves from BC cows than for those from CON cows (Table 4). Activity of GGT was lower for calves from BC dams than for those from CON. Concentration of Mg and activity of creatine phosphokinase tended \((P = 0.08)\) to be affected by the interaction of treatment by parity (Table 5).

As shown in Table 4, the effect by time point was significant for all calf serum variables (data not shown). Time-related changes followed known patterns of change over age and diet. Concentrations or activities of retinol, \(\alpha\)-tocopherol, cholesterol, and GLDH increased with age, whereas those of nonesterified fatty acids, creatinine, bilirubin, Ca, and anion gap decreased. Others showed variable responses with age. Treatment by time interactions or trends for triglycerides, bilirubin, aspartate aminotransferase, GGT, and GLDH are shown in Table 6.

### DISCUSSION

#### Colostrum

Supplementation of \(\beta\)-carotene to cows during the last 3 wk before parturition increased \(\beta\)-carotene concentration in colostrum, in contrast to a recent study that also explored prepartal BC supplementation (Aragona et al., 2021). We demonstrated that \(\beta\)-carotene is associated with colostrum color. Our results indicate that colostrum from BC cows was more red and yellow than the colostrum from CON cows. Other carotenoids most likely contribute to colostrum color as well (Calderón et al., 2007) and should be investigated further.

Supplementation of cows with \(\beta\)-carotene did not affect IgG concentration or Brix value of colostrum, similar to another recent study (Aragona et al., 2021).

#### Table 4. Least squares means for serum samples for calves from cows fed control (CON; \(n = 49\)) or \(\beta\)-carotene supplemented (BC; \(800 \text{ mg/d; } n = 48\)) diets prepartum

| Variable                      | Trt1 | Trt Parity | Trt × Parity | Time | Trt × Time |
|-------------------------------|------|------------|--------------|------|------------|
| IgG, \(\text{mg/dL}\)        | 43.4 | 43.2       | 2.7          | 0.95 | 0.79       | 0.82 | — | — |
| Vitamin \(\beta\)-Carotene, \(\text{mg/mL}\) | —    | —          | —            | —    | —          | —    | — | — |
| Vitamin \(\alpha\)-Tocopherol, \(\text{mg/mL}\) | —    | —          | —            | —    | —          | —    | — | — |
| Metabolite                    |      |            |              |      |            |      |    |
| Glucose, \(\text{mg/dL}\)    | 77.6 | 82.0       | 2.1          | 0.13 | 0.11       | 0.09 | <0.01 | 0.83 |
| Nonesterified fatty acid, \(\text{mmol/L}\) | 0.435 | 0.457 | 0.036 | 0.67 | 0.15 | 0.23 | <0.01 | 0.93 |
| BHB, \(\text{mg/dL}\)        | 58.4 | 62.6       | 4.5          | 0.52 | 0.49       | 0.63 | — | — |
| Cholesterol, \(\text{mg/dL}\) | 45.1 | 45.2       | 1.1          | 0.90 | 0.23       | 0.03 | <0.01 | 0.67 |
| Triglycerides, \(\text{mg/dL}\) | 17.9 | 18.5       | 1.2          | 0.17 | 0.11       | 0.30 | <0.01 | 0.01 |
| Bilirubin, \(\text{mg/dL}\)  | 0.41 | 0.45       | 0.015        | 0.05 | 0.52       | 0.78 | <0.01 | 0.06 |
| Creatinine, \(\text{mg/dL}\) | 1.68 | 1.78       | 0.056        | 0.25 | 0.28       | 0.46 | <0.01 | 0.75 |
| Urea N, \(\text{mg/dL}\)     | 12.2 | 12.3       | 0.24         | 0.70 | 0.88       | 0.66 | <0.01 | 0.95 |
| Total protein, \(\text{g/dL}\) | 5.88 | 5.84       | 0.05         | 0.63 | 0.20       | 0.68 | <0.01 | 0.33 |
| Albumin, \(\text{g/dL}\)     | 2.58 | 2.63       | 0.017        | 0.09 | <0.01      | 0.27 | <0.01 | 0.48 |
| Globulin, \(\text{g/dL}\)    | 3.27 | 3.18       | 0.056        | 0.15 | 0.75       | 0.47 | <0.01 | 0.54 |
| Albumin:globulin              | 0.98 | 0.99       | 0.012        | 0.45 | 0.39       | 0.04 | <0.01 | 0.97 |
| Minerals                      |      |            |              |      |            |      |    |
| Ca, \(\text{mg/dL}\)         | 10.8 | 11.1       | 0.06         | 0.01 | <0.01      | 0.14 | <0.01 | 0.24 |
| P, \(\text{mg/dL}\)          | 7.16 | 7.45       | 0.08         | 0.01 | 0.24       | 0.22 | <0.01 | 0.85 |
| Mg, \(\text{mg/dL}\)         | 2.15 | 2.19       | 0.02         | 0.21 | 0.02       | 0.08 | <0.01 | 0.87 |
| Na, \(\text{mmol/L}\)        | 135.8 | 138.0 | 0.62 | 0.02 | 0.02       | 0.28 | <0.01 | 0.14 |
| K, \(\text{mmol/L}\)         | 5.08 | 5.15       | 0.04         | 0.17 | 0.06       | 0.19 | <0.01 | 0.35 |
| Cl, \(\text{mmol/L}\)        | 96.4 | 97.6       | 0.42         | 0.05 | 0.09       | 0.68 | <0.01 | 0.27 |
| HCO3\(^{-}\), \(\text{mmol/L}\) | 20.7 | 21.1       | 0.21         | 0.28 | 0.55       | 0.87 | <0.01 | 0.44 |
| Anion gap, \(\text{mmol/L}\) | 24.2 | 24.5       | 0.25         | 0.41 | 0.13       | 0.43 | <0.01 | 0.28 |
| Enzymes                       |      |            |              |      |            |      |    |
| Alkaline phosphatase, \(\text{U/L}\) | 235 | 242      | 11           | 0.59 | 0.05       | 0.22 | <0.01 | 0.11 |
| Aspartate aminotransferase, \(\text{U/L}\) | 41.8 | 40.8 | 1.0 | 0.48 | 0.05 | 0.88 | <0.01 | 0.10 |
| Gamma-glutamyltransferase, \(\text{U/L}\) | 441 | 312      | 31           | <0.01 | 0.97 | 0.54 | <0.01 | <0.01 |
| Creatine phosphokinase, \(\text{U/L}\) | 194 | 214      | 12           | 0.23 | 0.80       | 0.08 | <0.01 | 0.36 |
| Glutamate dehydrogenase, \(\text{U/L}\) | 35.8 | 26.4      | 4.2          | 0.12 | 0.40       | 0.67 | <0.01 | 0.06 |

\(^{1}\text{Trt} = \text{treatment.}\)

\(^{2}\text{Tested only in 24-h sample.}\)

\(^{3}\text{Samples were mostly below the test threshold so they were entered as missing samples.}\)

\(^{4}\text{Only 0 d and 24 h were tested.}\)

\(^{5}\text{Only 0 d was tested.}\)
Although we detected no treatment effect on IgG or Brix, colostrum was of high quality overall, averaging 78.3 mg/mL and 25.1% for IgG and Brix, respectively. Cows administered BC tended to have increased colostrum fat percentage, from 3.49% in the CON cows to 4.05% in the BC cows, but this was primarily due to parity ≥3 cows fed BC. No other components of colostrum were altered by β-carotene supplementation. Aragona et al. (2021) also reported a trend for greater fat concentration in colostrum of cows supplemented with β-carotene before calving. Although not a direct economic effect for dairy producers, improved nutrient supply for the neonate would be a benefit. This increased amount of fat in the colostrum could indicate that the cow was in a more positive energy balance and could partition more energy toward colostrum production, although the regulation of colostrum composition is poorly understood. Calves from BC dams had greater triglycerides at 24 h of age, likely as a response to the greater fat intake from colostrum.

Use of a handheld on-farm device to measure β-carotene in colostrum would be more practical than intensive laboratory procedures such as HPLC. We compared results from the 2 techniques. Although the technique has been validated for blood (Raila et al., 2012), we determined that the rapid test did not reliably predict colostral β-carotene concentration. The rapid test was used as described for blood samples without optimization for colostrum. Additional research would be required to attempt to improve the predictive ability of the rapid on-farm test for colostrum, but its success seems unlikely based on our results.

In this experiment, we evaluated the use of a simple color scoring system for colostrum. The results for mean color characteristics showed that colostrum became more red and yellow as color score increased, which provides a quantifiable aspect to color. We expected that β-carotene would be greater in colostrum with higher color score, but the increases of retinol, α-tocopherol, IgG, and Brix were not anticipated. Our results agreed with those of Gross et al. (2014) and indicated that more highly colored colostrum provided more IgG than lighter-colored colostrum. In addition, retinol and α-tocopherol also were greater as the red and yellow colors increased in colostrum. Thus, our simple color score scheme should be evaluated further as a practical method to predict higher-quality colostrum. Because all of our colostrum was of relatively high IgG concentration, it will be important to evaluate use of the color score with a wider range of colostrum qualities.

| Table 5. Treatment by parity interactions (P < 0.10) for variables measured in blood of calves from cows fed control (CON; n = 49) or β-carotene supplemented (BC; 800 mg/d; n = 48) diets prepartum |
|---|---|---|---|---|---|---|
| Variable | CON Parity 2 | CON Parity ≥3 | BC Parity 2 | BC Parity ≥3 | SE | P-value |
| α-Tocopherol, μg/mL | 0.39 | 0.36 | 0.34 | 0.42 | 0.019 | 0.04 |
| Glucose, mg/dL | 82.3 | 72.8 | 81.8 | 82.1 | 2.9 | 0.09 |
| BHB, μmol/L | 63.5 | 53.4 | 53.0 | 72.2 | 6.4 | 0.03 |
| Cholesterol, mg/dL | 45.6 | 44.5 | 43.1 | 47.3 | 1.4 | 0.03 |
| Albumin:globulin | 0.97 | 0.99 | 1.02 | 0.97 | 0.015 | 0.03 |
| Mg, mg/dL | 2.21 | 2.10 | 2.20 | 2.18 | 0.028 | 0.08 |
| Creatine phosphokinase, U/L | 207 | 182 | 197 | 231 | 16 | 0.08 |

| Table 6. Treatment by time interactions for variables measured in blood of calves from cows fed control (CON; n = 49) or β-carotene supplemented (BC; n = 48) diets prepartum |
|---|---|---|---|---|---|---|---|
| Variable | CON 0 h | CON 24 h | CON 7 d | CON 60 d | BC 0 h | BC 24 h | BC 7 d | BC 60 d |
| Triglycerides, mg/dL | 13.4 | 20.1 | 12.0 | 27.4 | 14.2 | 26.4 | 14.5 | 23.8 | 1.5 | 0.01 |
| Bilirubin, mg/dL | 0.40 | 0.77 | 0.34 | 0.11 | 0.38 | 0.90 | 0.40 | 0.11 | 0.03 | 0.06 |
| Aspartate aminotransferase, U/L | 16.9 | 63.3 | 25.2 | 61.9 | 16.2 | 66.4 | 25.4 | 55.2 | 2.4 | 0.10 |
| Gamma-glutamyltransferase, U/L | 13 | 1.491 | 235 | 24 | 7 | 1.035 | 191 | 17 | 60 | <0.01 |
| Glutamate dehydrogenase, U/L | 4.4 | 8.6 | 17.6 | 112.3 | 3.2 | 9.2 | 17.9 | 75.2 | 8.2 | 0.06 |

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β-Carotene concentrations in serum of the calves were extremely low at all time points, with the highest single value being 0.23 μg/mL. The β-carotene benefit gained from colostrum peaked at 24 h, was greatly diminished by d 7, and was nonexistent at d 60. This finding conflicts with the recommendation given by Iwańska et al. (1986) that the best way to increase calf serum β-carotene concentrations is by supplementing the dam prepartum. The fact that only 1 CON calf had detectable β-carotene concentration at birth indicates that placental transfer of β-carotene is minimal and the calf relies on colostrum to obtain it.

Few significant treatment effects were observed for the panel of serum metabolites measured in calves. Of interest was the significantly greater concentrations of Ca, P, Na, and Cl in calves from BC cows. Although all were in the normal ranges for bovines, it is unexplained why the dam’s BC treatment would have influenced the concentrations of these minerals in blood of the calves. We found significant treatment effects for urea N and GGT, but both had a trend toward a treatment by parity interaction, and the biological relevance is uncertain.

Calves from dams fed BC had lower activities of GGT and GLDH in serum at 24 h of age. They also had lower GGT activities at all time points. Because these enzymes indicate tissue damage or remodeling, particularly in the liver (Kahn, 2010), it is possible that the greater β-carotene concentration in colostrum of BC-supplemented cows conferred some beneficial antioxidant effects on the young calf; however, with the exception of GGT, these did not persist beyond 24 h. To determine unequivocally effects of increased β-carotene on calves, further research should investigate the effects of direct supplementation of β-carotene to calves.

CONCLUSIONS

Supplementation of β-carotene to prepartum cows increased β-carotene in colostrum and affected colostrum color, with an increase in a* and b*, indicating that β-carotene caused colostrum to be more red and yellow. Colostrum from cows supplemented with β-carotene also had increased amounts of β-carotene and fat, but supplementation did not affect IgG concentration. Colostrum color was related to IgG concentration. Although calves from cows that received β-carotene were more likely to have detectable serum concentrations of β-carotene at 24 h and d 7, the response did not last. Supplementation of β-carotene to the dam resulted in lower serum activities of GGT and GLDH in calves, possibly indicating an antioxidant effect. Overall, β-carotene supplementation of the cow had modest effects on the calf, and direct supplementation should be investigated.

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