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Original Articles

Effects of Peripartum β-Carotene Supplementation on Serum Vitamin Concentrations and Passive Immunity in Japanese Black Cattle

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Summary : The effects of peripartum supplementation with β-carotene on serum vitamin concentrations and passive immunity were assessed in Japanese Black cattle. Nineteen pregnant cows were assigned to supplemented (n=10) or control (n=9) group. Supplemented cows received 300 mg of β-carotene once daily from 4 weeks prepartum through 4 weeks postpartum. All cows were immunized twice with enterotoxigenic *E. coli* vaccine at 8 and 4 weeks before calving. Serum β-carotene concentrations in supplemented cows increased up to calving ($P<0.001$) and higher than those in control cows on week 0 ($P<0.05$) and 1 ($P<0.1$). Treatment did not affect concentrations of serum retinol and α-tocopherol in cows. Serum β-carotene concentrations in calves from supplemented dams tended to be higher than those in calves from control dams on week 4, but no significant difference was observed between 2 groups. β-Carotene feeding did not influence serum antibody titer to K99 in dams and their calves as well as growth performance of calves. These results suggest that 300 mg of β-carotene supplementation did not affect serum vitamin concentrations except β-carotene in dams and their calves and showed no effects on passive immunity.

Key words : β-Carotene, Colostrum, Japanese Black cattle, K99 antibody titer, Vitamin.

Introduction

It has been well-known that β-carotene and certain carotenoids possess provitamin A activities. More recently, β-carotene and other carotenoids have been reported to possess antioxidant activities and play important roles in animal health by modulating the cellular and noncellular host defense systems (Chew 1995). Blood lymphocyte proliferation in response to mitogens during the peripartum period increased in cows supplemented with β-carotene compared with control animals (Michal *et al.* 1994). β-Carotene enhanced mitogen-induced proliferation of peripartum bovine blood mononuclear cells *in vitro* (Daniel *et al.* 1991). In addition to cell-mediated immune response, dogs fed β-carotene had higher concentrations of plasma IgG (Chew *et al.* 2000). Dietary lutein, a different carotenoid, increased plasma IgG concentrations in cats (Kim *et al.* 2000a). The production of IgG increased in dogs fed lutein after the antigenic challenge (Kim *et al.* 2000b).

Plasma β-carotene concentrations in dairy cows decreased during the peripartum period and supplemental β-carotene increased plasma β-carotene (Michal *et al.* 1994). In our previous study (Katamoto *et al.* 2003), serum concentrations of β-carotene were lower than the desirable concentrations in Japanese Black cattle housed in tie stall barns and fed mainly low quality of forage or hay. In addition, the efficacy of β-carotene supplementation on immune response in Japanese Black cattle has not been fully evaluated. The objectives of this study were to determine the effects of peripartum maternal supplementation with β-

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carotene on serum vitamin concentrations and serum antibody titers to enterotoxigenic *Escherichia coli* (*E. coli*) vaccine in dams and their calves of Japanese Black cattle.

Materials and methods

Animals

A total of 19 pregnant Japanese Black cows including one nulliparous heifer were used. They had been raised on 8 farms in Hyogo prefecture and were randomly divided into supplemented (n=10) or control (n=9) group. The average ages of supplemented and control cows were 6.5 years old (range 2.1-14.9) and 6.5 years old (range 3.8-10.2), respectively. The main roughage source was rice straw, rye grass straw, sudan grass hay, fescue grass straw, oats hay or cane top. All cows were supplemented with barley grain, wheat bran or commercial concentrates.

Experimental procedures

Supplemental cows received 300 mg of β-carotene (Kanotin®, 10 % beadlet; Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan) once daily from 4 weeks prepartum through 4 weeks postpartum. All cows were inoculated twice subcutaneously in the side of neck region with 5 ml of enterotoxigenic *E. coli* vaccine (Imocolibov®; Rhone Meriux, Inc., France) at 8 and 4 weeks before calving. Blood samples were collected from the jugular vein at -8, -4, 0, 1 and 4 weeks postpartum in cows. In addition, blood samples were obtained from their calves at birth, 1 and 4 weeks of age. Serum was harvested and stored at -20℃ until the analysis of vitamins with high performance liquid chromatography (HPLC) analysis and the determination of antibody titers. Colostrum samples were also collected from 10 supplemented and 8 control cows within 24 h after calving and stored at -20℃ until HPLC analysis. Calves were weighed at birth and 4 weeks of age, and daily gain was calculated for each calf by subtracting the body weight (BW) at birth from the BW at 4 weeks of age and then dividing by the number of days. In addition, calves were examined daily for clinical disorders.

HPLC analysis

Retinol, α-tocopherol and β-carotene concentrations in serum were determined by HPLC as previously described (Katamoto *et al.* 2003). In addition, retinol, α-tocopherol and β-carotene concentrations in colostrum were measured by HPLC using a modification of the procedure of Katamoto *et al.* (1998). Briefly, 0.5 ml of colostrum was placed into a centrifuge tube together with 0.5 ml of phosphate buffer (pH 7.4, 0.01 M), 1.5 ml of 6 % pyrogallol solution in ethanol and 1 ml of ethanol, and was preincubated for 2 min at 70℃. After preincubation, 0.4 ml of 60 % KOH was added to the solution mixture, and the samples were saponified at 70℃ for 30 min. After cooling, 2.5 ml of distilled water and 5 ml of hexane were added to the tube, which was vigorously shaken for 5 min. The mixture was centrifuged at 1,000 rpm for 5 min and 3 ml of the hexane layer was removed and evaporated under nitrogen at 37℃. The residue was dissolved in 0.5 ml of methanol by sonication and filtered through Cosmonice Filter S (pore size 0.5 μm, Nacalai Tesque, Inc., Kyoto, Japan), and 40 μl was injected into the HPLC column. The instruments used in this assay were as previously described (Katamoto *et al.* 2003). All-*trans* retinol, α-tocopherol and β-carotene were used as external standards.

K99 antibody titers

Enterotoxigenic *E. coli* vaccine which contains K99, FY and 31A pili antigens protects newborn calves by passive immunity. Protection is provided by antibodies from vaccinated dams through the colostrum and milk. Serum K99 antibody titers in dams and their calves were determined using a bacterial agglutination test by Scientific Feed Laboratory Co., Ltd. Tokyo, Japan.

Statistical analysis

Data was analyzed by two-way repeated measures analysis of variance (ANOVA). Differences between sampling weeks within a treatment and between treatment groups within a sampling period were determined by paired and unpaired Student's *t*-test, respectively. Incidence of clinical disorder was analyzed using the chi-square test.

Results

Serum vitamin concentrations

Mean serum concentrations ($\pm$ SEM) of β-carotene in all cows were 142.7 $\pm$ 26.7 μg/dl prior to supplementation (Fig. 1). Serum β-carotene concentrations in cows supplemented with β-carotene increased up to calving ($P<0.001$) and higher than those in control cows on week 0 ($P<0.05$) and 1 ($P<0.1$). Control cows showed the lowest serum β-carotene concentrations on week 1. Mean serum concentrations of retinol were 17.1 $\pm$ 1.1 μg/dl prior to supplementation. Serum retinol concentrations in both supplemented and control cows were the lowest on week 0. No significant differences in serum retinol concentrations were detected between supplemented and control cows throughout the experimental period. Mean serum concentrations of α-tocopherol were 230 $\pm$ 26.3 μg/dl prior to supplementation. Like serum β-

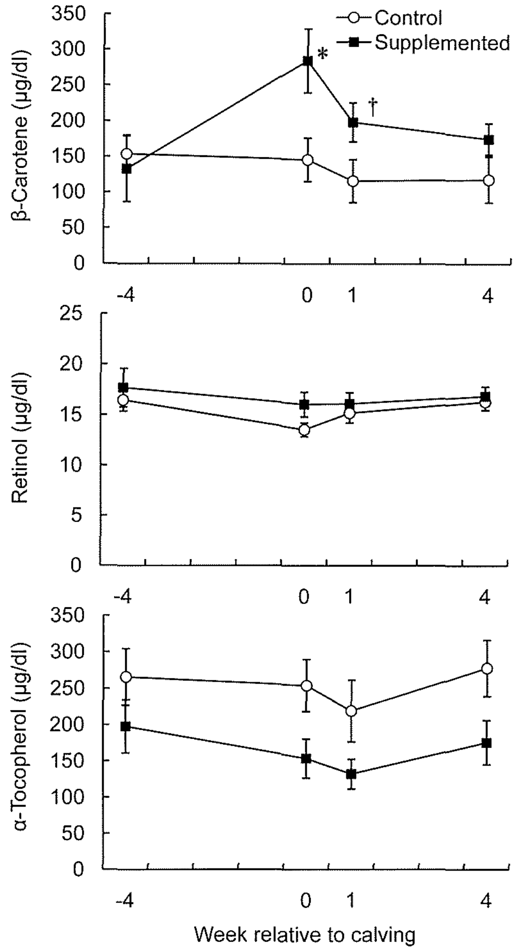

Fig. 1. Mean (± SEM) serum concentrations of β-carotene, retinol and α-tocopherol in control (n=9) and supplemented cows (n=10) with β-carotene from 4 weeks prepartum through 4 weeks postpartum. Statistical significance is as follows : *P<0.05, †P<0.1 compared with control cows.

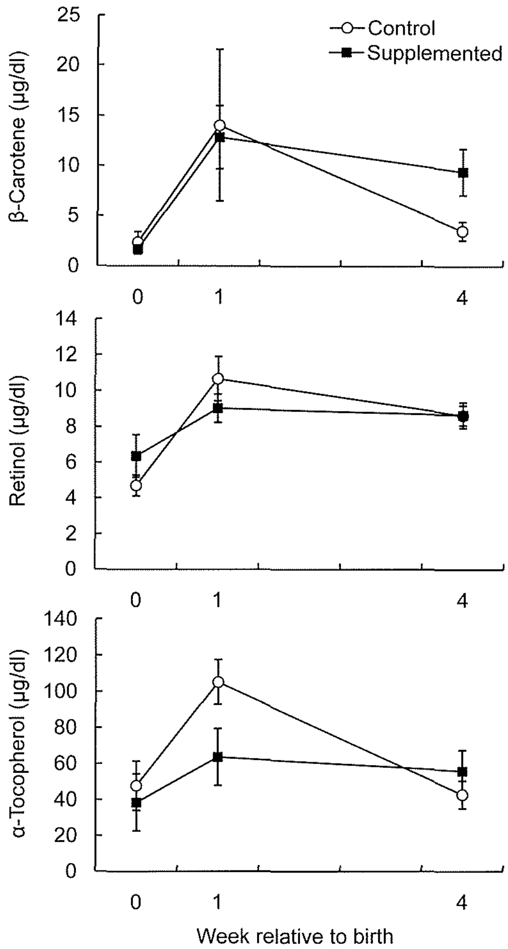

Fig. 2. Mean (± SEM) serum concentrations of β-carotene, retinol and α-tocopherol in calves from control dams (n=9) and ones from supplemented dams (n=10). No significant differences were observed between 2 groups at any measuring time.

carotene concentrations in control cows, serum α-tocopherol concentrations in both groups gradually decreased up to week 1. No significant differences in serum α-tocopherol concentrations were observed between 2 groups. In all newborn calves, serum concentrations of β-carotene, retinol and α-tocopherol were extremely low when compared to those in their dams (Fig. 2). Serum β-carotene concentrations in both groups of calves peaked on week 1 and gradually decreased through week 4. On week 4, the concentrations in calves from supplemented dams tended to be higher than those in calves from control dams. However, no significant differences in the concentrations were detected between 2 groups. As with β-carotene, serum concentrations of retinol and α-tocopherol in both groups of calves peaked on week 1 and decreased through week 4, but no significant differences were observed between 2 groups.

Colostrum vitamin concentrations

Vitamin concentrations in colostrum were shown in Fig. 3. β-Carotene and retinol concentrations in supplemented cows tended to be higher than those in control cows. However, the differences between supplemented and control cows were not significant because of their large variations. In addition, no significant differences were observed between 2 groups in α-tocopherol concentrations.

Antibody titer to K99

Serum antibody titer to K99 in both groups of cows increased after vaccination (P<0.001) and remained constant from week 0 through 4 (Fig. 4). Both groups of calves showed the highest antibody titer to

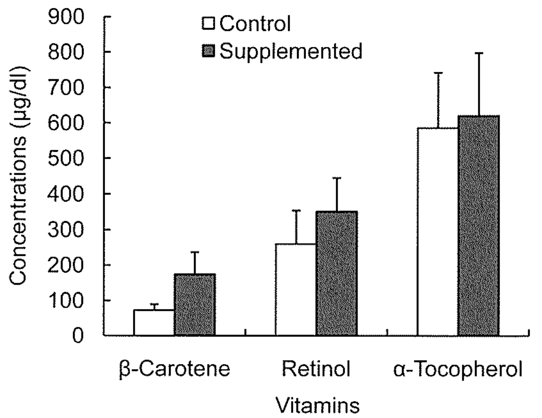

Fig. 3. Mean (± SEM) colostrum concentrations of β-carotene, retinol and α-tocopherol in control (n=8) and supplemented cows (n=10). No significant differences were observed between 2 groups.

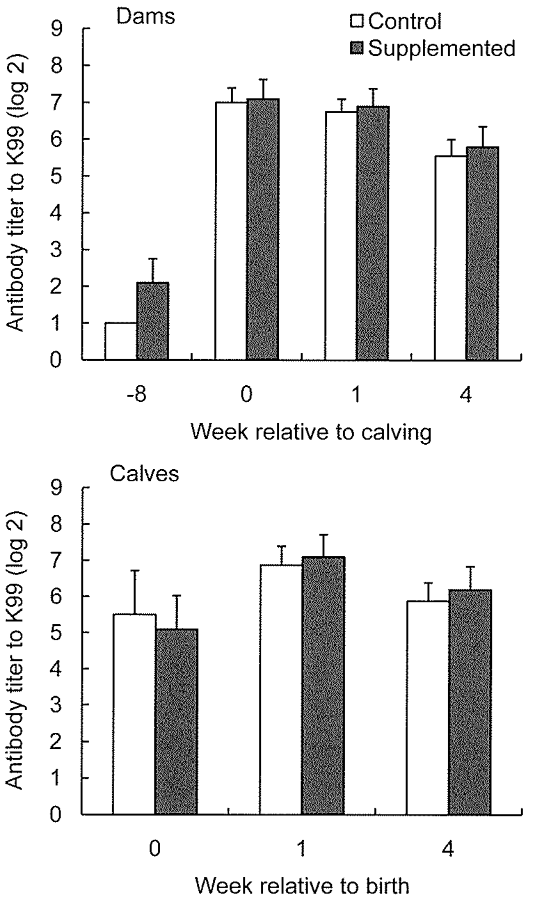

Fig. 4. Mean (± SEM) serum K99 antibody titers in dams and their calves. Open column shows control dams (n=9) and their calves. Dotted column shows supplemented dams (n=10) and their calves. Values are means of log 2 titer. No significant differences were observed between 2 groups in dams or calves.

Table 1. Body weight (BW) at birth and average daily gain (ADG) during 4 weeks after birth in calves

		Treatment	
		Control	Supplemented
Birth BW, kg	Female	22.10 ± 0.70 (n=5)	21.25 ± 0.74 (n=8)
	Male	22.33 ± 2.59 (n=3)	27.50 ± 2.50 (n=2)
ADG, kg/day	Female	0.52 ± 0.05 (n=5)	0.55 ± 0.04 (n=8)
	Male	0.44 ± 0.09 (n=3)	0.60 ± 0.16 (n=2)

Data represent the means ± SEM.

K99 on week 1. Supplementation of diet with β-carotene did not show any significant effect on antibody titer to K99 in both cows and their calves throughout study period.

Growth performance of calves

Diarrhea occurred in calves (60 %) from supplemented dams and ones (33 %) from control dams during experimental period, but there was no statistical difference between 2 groups. BW at birth and average daily gain (ADG) in calves from supplemented dams were not significantly different from those of calves from control dams (Table 1).

Discussion

In the study by Michal *et al.* (1994), Holstein cows supplemented with 600 mg of β-carotene had higher concentrations of plasma β-carotene and retinol than control cows, but not for those supplemented with 300 mg of β-carotene. Additionally, mean plasma β-carotene and retinol concentrations in all cows prior to supplementation were 230.9 μg/dl and 45.8 μg/dl, respectively. In the present study, supplementation with 300 mg of β-carotene increased serum β-carotene concentrations, but did not affect serum retinol concentrations. These differences may be attributed to the different β-carotene and retinol status, *i.e.* lower concentrations of β-carotene and retinol in serum of cows used in this study and/or the difference in breed of cattle. For example, Holsteins convert carotene to retinol efficiently, while Guernseys are much less efficient (Frye *et al.* 1991). The concentrations of serum β-carotene in control cows as well as serum retinol and α-tocopherol in supplemented and control cows were the lowest at calving or 1 week after calving. The decrease in these vitamin concentrations during peripartum period may be related to the decreased intake of vitamins due to decreased feed intake or the transfer of vitamins from the blood pool to the colostrum and milk pool (Weiss *et al.* 1990). The concentrations of serum β-carotene, retinol and α-

tocopherol in calves were extremely low at birth in accordance with previous studies (Nonnecke *et al.* 1999; Zanker *et al.* 2000), suggesting these vitamins may not cross the placenta, and therefore colostrum is the primary source of these vitamins for the calves after birth. Treatment did not affect β-carotene concentrations in colostrum or serum concentrations in calves from supplemented dams. These results suggest that maternal supplementation of β-carotene is an insufficient way to elevate blood β-carotene concentrations in neonatal calves. Therefore, we should take into consideration supplementing milk with higher β-carotene content to provide sufficient β-carotene for calves.

In this study, the immunization of cows with the maternal enterotoxigenic *E. coli* vaccine enhanced the passive immunity levels in calves against K99 *E. coli* as reported previously (Kohara *et al.* 1997). However, supplementation of β-carotene had no effect on antibody titer to K99 in dams and their calves. Cattle fed β-carotene had increased mitogen-induced lymphocyte proliferation during the peripartum period, whereas preformed vitamin A did not produce a similar response (Michal *et al.* 1994). Piglets injected with β-carotene showed enhanced mitogen-induced lymphocyte proliferation (Hoskinson *et al.* 1992). β-Carotene supplementation enhanced cell-mediated and humoral immune response in the canine (Chew *et al.* 2000) but not in the feline (Chew *et al.* 1998). Uptake of β-carotene into the subcellular organelles of lymphocytes suggests possible cell regulatory and protective functions in several species (Chew *et al.* 1991 ; Chew *et al.* 1993 ; Chew *et al.* 1998). The discordance between our data and the literature data could be due to differences in the experimental protocols used, *e.g.* the number of animals, the route of administration, the level of supplementation as well as the species and breed of animals. Although commercially recommended dosage of β-carotene preparations was used, peak β-carotene concentrations in supplemented cows on week 0 was 284.5 $\pm$ 44.9 μg/dl. Desirable blood β-carotene concentrations are considered to be in excess of 300 μg/dl in dairy cattle (Herdt & Stowe 1991). Therefore, it appears that the level of β-carotene supplementation used in this study may be insufficient for modulating immune response as well as elevating blood β-carotene or retinol levels. Further studies are needed to clarify the supplemental benefits of carotenoids on the health or immune system in cattle.

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分娩前後のβ-カロテン補給が黒毛和種牛の血清ビタミン濃度および受動免疫に及ぼす効果

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要　約

分娩前後におけるβ-カロテン補給が，黒毛和種牛の血清ビタミン濃度および受動免疫に及ぼす影響について調べた．19頭の妊娠雌牛を補給群（n=10）または対照群（n=9）に分けた．補給群は分娩4週間前より分娩4週間後まで，1日1回300 mgのβ-カロテンが与えられた．全ての雌牛に分娩8および4週間前の2回，毒素原性大腸菌ワクチンが接種された．補給群の血清β-カロテン濃度は分娩時にかけて上昇し（P<0.001），分娩時（P<0.05）および分娩1週間後（P<0.1）において対照群に比べ高かった．β-カロテン補給は雌牛の血清レチノールおよびα-トコフェロール濃度に影響を与えなかった．補給群の母牛から生まれた子牛の血清β-カロテン濃度は，対照群の母牛から生まれた子牛に比べ，生後4週間において高い傾向にあったが有意差は認められなかった．β-カロテン補給は，母牛およびその子牛のK99に対する血清抗体価および子牛の発育に影響しなかった．以上の結果から，300 mgのβ-カロテン補給は，母牛および子牛においてβ-カロテン以外の血清ビタミン濃度に影響を与えず，また受動免疫にも影響しないことが示唆された．

キーワード：β-カロテン，初乳，黒毛和種牛，K99抗体価，ビタミン