Beta-Carotene for the Transition Cow and Calf – More Than Provitamin A

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Summary
Beta-carotene serves as the major Vitamin A (retinol) precursor for ruminants, but as a carotenoid can be found in many tissues serving special roles as a local source of vitamin A and as a direct antioxidant for transition cows. Specific roles for β-carotene (vs. retinol) include: follicular source of vitamin A, and antioxidant maintenance in the cow, colostrum, and calf. Beta-carotene (BC) also acts as an antioxidant and can increase the function of lymphocytes and phagocytes, and therefore may be beneficial during immunocompromised states (periparturient period), or in mammary gland infections. The BC metabolite, retinoic acid, is needed for cell differentiation and can also regulate gene expression. Dietary sources of BC include variable and decreasing contributions from vegetative plants, and direct supplementation via BC "beadlet" sources.

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Introduction
Dietary BC is the major precursor of vitamin A with an activity of 400 IU per milligram for ruminants. Vitamin A is needed for eyesight, growth, reproduction, and maintenance of epithelial tissues. The activity of vitamin A is measured in retinol equivalents (1 IU of vitamin A equals 0.3 µg of all-*trans* retinol). Signs of vitamin A deficiency include: abortion, retained placenta, reduced immune function, and calf morbidity and mortality (NRC, 2001). Dietary BC is absorbed with fat and converted to retinol by intestinal enzymes. In ruminants, much BC is also absorbed and stored directly. Guernsey and Jersey cattle convert less BC to retinol in the enterocyte, resulting in higher circulating levels and more excretion in milk.

BC also functions separately from vitamin A as an antioxidant and can directly enhance immunity with possible reproductive and mammary benefits (Chew, 1993). The National Research Council (NRC, 2001) concluded that data was insufficient to establish a BC requirement for dairy cattle, but recommended that additional dietary vitamin A be considered with low forage diets, high corn silage diets, diets with low quality forages, and situations with high pathogen loads or reduced immunocompetence.

Responses to BC supplementation have been inconsistent (Table 1) in part due to the wide variation in serum BC status (Weiss, 1998; deOndarza et al, 2009). Most BC is found in vegetative plants and concentrations decrease with plant maturity. Most grains and fermented feeds contain minimal levels of BC because of heat damage and breakdown during storage (Pickworth, et al, 2012). A serum BC level of 3.0 µg/ml has been suggested as the level in which supplementation is beneficial (Frye et al.1991). A large proportion of serum samples from the 1996 NAHMS study of U.S. dairy herds (NAHMS, 1996) contained less than 3.0 µg/ml BC (Herdt and Seymour). LeBlanc et al. (2004) found mean serum BC concentration of 1828 samples from peripartum (+/- 1 wk) Holstein cows from 20 Canadian herds to be 1.12 µg/ml (SD=0.78). Stage

of lactation greatly affects serum BC levels (Kawashima, 2009a), with the lowest occurring immediately pre-calving (Figure 1).

Production Responses

Although the mode of action is not well understood (improved antioxidant/immune status?) some studies have found supplemental BC to positively effect milk yield (Table 1). Heat-stressed cows supplemented with 400 mg BC increased cumulative milk yield by 11% (Arechiga et al., 1998). Oldham et al. (1991) supplemented 300 mg BC and increased milk yield by 6.4% with this difference approaching significance. However, others have not seen production responses with supplemental BC (Bindas et al., 1984, Rakes et al., 1985, Wang et al., 1988b).

Immunity

Chew et al. (1982) reported that cows with lower plasma vitamin A, BC, and total retinol equivalents had more mastitis. Chew (1983) supplemented 300 mg BC and 53 KIU. vitamin A, or 80 KIU. vitamin A, or 53 K.I.U. vitamin A, or no supplement from 30 days before calving to 70 DIM. In this study, BC had a positive effect on immune response. Rakes et al. (1985) supplemented 300 mg BC and numerically lowered SCC content of milk, and Wang et al. (1988b) required fewer clinical mastitis treatments in cows supplemented with 300 mg BC.

Other researchers have not found indications that BC improved immune function. Oldham et al. (1991) did not reduce the incidence of mastitis with supplemental BC. Bindas et al. (1984) found that supplementing 600 mg of BC per day had no effect on SCC. LeBlanc et al. (2004) could not relate serum BC concentrations with either retained placenta or mastitis. However, they did find that when there was a 100 ng/ml increase in serum retinol concentration during the last week prior to calving, there was a 60% reduction in clinical mastitis in early lactation.

Reproduction

Dietary BC levels have been linked to fertility as evidenced by higher concentrations of BC in the ovary, particularly the corpus luteum (Chew et al., 1984). Schweigert (2003) postulates that BC is converted to retinol specifically in the uterus and ovaries. Graves-Hoagland et al. (1988) found plasma BC to be positively related to progesterone production by corpus luteum cells. Cows that ovulated during the first follicular wave postpartum had a higher mean plasma BC concentration than anovulatory cows three weeks prepartum (Kawashima et al., 2009a). In a follow-up study, Kawashima et al (2009b) supplemented BC during the close-up period (500 mg/d or 2000 mg/d in two different experiments) and increased the number of ovulating cows at the first follicular wave postpartum. Pregnancy rate at 120 d postpartum in heat-stressed cows supplemented with 400 mg BC/d for $\geq$ 90 d was increased (35.4% vs. 21.1%), (Arechiga et al., 1998). Rakes et al. (1985) found that supplementing 300 mg of BC for the first 100 DIM reduced days to first estrus and reduced cervix diameters at 21 and 28 DIM ($P<0.05$). Lotthammer (1978, 1979) found that supplemental BC improved conception rates, uterine involution, and ovulation and reduced incidence of cystic ovaries and early embryonic death.

Inaba et al. (1986) reported that cows with ovarian cysts had significantly lower plasma concentrations of BC than cows without ovarian cysts. Goto et al. (1989) determined that

plasma BC concentration was related to embryo quality in superovulated Japanese Black cattle. Plasma BC concentrations above 200 μg/dl tended to improve numbers of corpus lutea and total recovered embryos and significantly improved the numbers of normal transferable embryos. DeBie et al (2016) found that supplemental BC improved follicular health irrespective of negative energy balance.

Others have seen no positive reproductive responses to BC supplementation in dairy cattle (Bindas et al., 1984, Marcek et al., 1985, Wang et al., 1988a, Wang et al., 1988b) possibly due to season or initial BC status (Weiss, 1998). Greenburg et al. (1986) concluded that BC did not improve reproduction in beef heifers.

There are reports of improved reproduction with supplemental BC in other species. Schweigert (2001) found that supplemental vitamin A (4000 IU) and BC (100 ppm) increased BC levels in the adrenals and corpus lutea of gilts. Besenfelder et al. (1996) supplemented 40 mg BC to rabbits that were assumed to have sufficient vitamin A status (20,000 IU vitamin A per kg of feed). They hypothesized that the improved reproductive performance (higher numbers of corpus lutea, fewer ovarian cysts, more oocytes and embryos) was simply related to the resulting higher levels of serum vitamin A following BC supplementation.

Colostrum Quality and Calf Issues

Calves are born with minimal vitamin A liver stores, making ingestion of colostrum with high vitamin A and BC concentration imperative, as both have proven to be important for proper immune function. Kehoe et al. (2007) found that the BC concentration of colostrum from cows sampled across Pennsylvania varies from 0.1 to 3.4 μg/g. Torsein et al (2011) found that calves born with serum levels below 0.25 ug/ml (up to 40% of the calves in high-mortality herds) were 5.3 times more likely to die than calves with higher serum BC levels.

Supplementing 1 g of BC per day increased BC concentration in colostrum compared to control (3.1 mg/L vs 1.44 mg/L, respectively; Kaewlamun et al., 2011). Concentration of colostral BC was also increased in cows supplemented with 800 mg BC during the closeup period (Prom et al., 2016). The number of calves with detectable BC concentrations was higher for calves receiving maternal colostrum from dams supplemented with BC, compared to calves born from control fed dams. Oliveira et al (2015) saw an increase in BC blood concentrations when supplementing 1.2 g/cow per day, but researchers did not measure colostral BC concentration.

Recently, Aragona et al. (2017) fed 700 mg BC/cow per day for 4 wk prepartum to determine effects on colostrum quality and calf performance. Colostral IgG concentration increased (82.7 vs 57.6 g/L for BC and control fed cows, respectively) although colostrum yield was reduced in BC-supplemented cows. Calves born from cows supplemented with BC gained 0.44 g/g DMI compared to calves born from cows not supplemented with BC that gained 0.32 g/g DMI (*P*= 0.03).

Evaluating Beta-Carotene Status and Targeting Supplementation

Because the actual BC content of diets varies and BC status was usually unknown in previous research, it can be difficult to evaluate BC supplementation strategies. Mean serum BC can now be assessed on the farm using the iEx™ system, a single step denaturation and BC extraction into organic solvent followed by BC measurement using iCheck® (BioAnalyt GmbH, Germany), a portable spectrophotometer (Schweigert et al., 2007). Routine BC measurements can be used to evaluate herd status and to recommend specific supplementation strategies in the field.

Conclusions

Beta-carotene (BC) serves as a major retinol precursor in ruminants. Research has shown also that BC can be transported and metabolized directly by the ovary and other tissues, serves as an antioxidant (unlike retinol), and is the major source of vitamin A for the newborn calf. Many trials have shown positive responses in milk production, reproduction, colostrum quality, and immunocompetence in BC-supplemented cows, even when vitamin A levels are adequate. Variability in these observed responses may be due to initial BC status

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Figure 1. Herd Whole Blood β-carotene Means from North American Dairy Herds, 2018

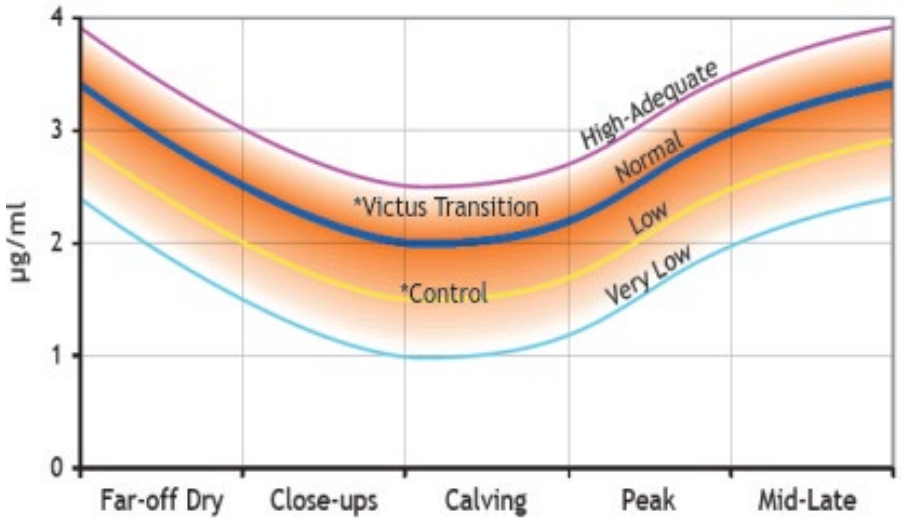

Figure legend: Plot of BC in whole blood (ug/ml) vs. stage of lactation

Table 1. Production Responses to Beta-Carotene Supplementation

Study	Beta-carotene (mg/d)	Milk Yield	Milk Fat %	SCC	Mastitis	Reproduction
Arechiga et al. (1988)	400	↑ 6-11% (P<0.05)	--	--	--	35.4% vs. 21.1% pregnancy rate at 120 DIM when ≥90 d BC supplement (P<0.05)
Oldham et al. (1991)	300	↑ 6.4% (NS)	↓ 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. (1888a)	600	--	--	--	--	NS
Wang et al. (1988b)	300	NS	--	--	↓ 84% (P<0.01)	NS
Marcek et al. (1986)	300	--	--	--	--	Ovarian cysts - NS
Bindas et al. (1984)	600	NS	--	NS	--	NS
deOndarza et al (2009)	400	↑ 2.3%	↑ 3.1% (P<0.05)	Lower NS		23 vs. 18% 21d Preg rate when > 63d BC supplement (P<0.05)

Table 2. Colostrum Responses to Beta-Carotene Supplementation

Study	Beta-carotene (mg/d)	IgG, mg/ml	BRIX%	Colostrum BC, mg/L	Calf serum BC, ug/ml	Calf Serum IgG, mg/L
Kaewlamun et al (2011)	1000/14d			↑215% (P<0.01)		
Prom et al (2014)	800/21d	↑ 3.4% (NS)	↑ 2.0% (NS)-	↑239% (P<0.01)	↑566% (P<0.05)	--
Aragona et al (2017)	700/28d	↑ 43% (P<0.05)	--	--	NS	↑41% (P<0.01)