Effects of Processing Methods on Beta-carotene Contents in Forages and Supplementation of Synthetic Beta-carotene on Cows

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Abstract Changes of beta-carotene contents were determined in four consecutive cuts of freshly harvested mixture of timothy and ladino clover and two cuts of round baled silages from the same meadows. Under aerobic conditions, losses of beta-carotene in medium and low moisture wilted silages and hay during drying were also investigated. Furthermore, the effects of feeding wilted silages with and without beta-carotene supplements in 4 groups of cows and subsequently in their newborn calves were likewise evaluated. In the first and second cuts of freshly harvested forages, the beta-carotene content decreased linearly as timothy matured. In the first cut of round baled silage, beta-carotene content also diminished as the grasses matured. Losses of beta-carotene in wilted silages and hay during drying were considerable. In groups 3 and 4 of cows, serum beta-carotene concentrations decreased before parturition but increased two weeks postpartum. After parturition, only cows that received medium moisture wilted silages of high carotene level (119 mg/kg dry matter) showed significantly higher levels compared to those with low moisture low carotene (36 mg/kg dry matter). Cows supplemented with 500 mg/day of synthetic beta-carotene had elevated carotene level much greater than control cows. Moreover, colostrum of supplemented cows showed higher level of beta-carotene than controls. The average days to third ovulation and conception days in cows showing lower level of serum beta-carotene after parturition were rather increased. The dietary intake of beta-carotene derived from forages and supplementation of synthetic beta-carotene influenced the levels of beta-carotene in the serum and colostrum of cows, and eventually reflected to the serum of newborn calves.

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Key words : beta-carotene, forage, supplementation, calf, dairy cattle

Intensive forms of dairy cattle production demand a high input of a range of nutrients including vitamins. Among the carotenoids, beta-carotene is the most active biological form. It had long been thought that the only function of carotene in the nutrition of cattle was its role as a precursor of vitamin A. In

fact, however, recent researches conducted in the U.S. and Europe suggest that feeding supplemental beta-carotene may help in maintaining normal reproduction in dairy cows by preventing deficiency of this nutrient^{3,12,15)}. While there are dissimilar results regarding the specific role of beta-carotene in cows^{1,4,18)},

these discrepancies could be attributed to the beta-carotene content or nutritional elements in the total ration. Additional studies are therefore needed to clarify the physiological role of beta-carotene in cows. Carotene level in forage varies over a very wide range depending on plant species, climatic conditions, harvesting time and the length and conditions of storage^{8,9,14)}. Information on the beta-carotene status of forages in Hokkaido is scarce¹¹⁾. In addition, there is a limited number of systematic studies to corroborate the effects of processing methods on carotene level in forage under practical conditions and the effects of ingestion of these experimentally processed feeds on cows and their colostrum during parturition, and eventually on their new born calves.

The purposes of this study were: 1) to investigate effects of harvesting and processing methods on carotene contents in field-cured forage, and 2) to evaluate effects of synthetic beta-carotene supplementation on cows fed with medium and low moisture silages of high and low beta-carotene contents.

Materials and Methods

Forages: Fresh green mixture of timothy-ladino clover in same meadow were consecutively cut four times from June 1 to November 10, 1988 in Konsen area, eastern Hokkaido. For each cut, the mix forages were harvested daily, moisture content measured, dried then stored until analysis for their constituents. Analyses of beta-carotene content and vegetation were done every sampling day for the freshly cut green mixture. The round baled silage, about 600 kg of fresh weight with 70 to 75% moisture, was processed from June 14 to September 13 with two cuts. In the first cut, those forages sampled on July 14 were at their earliest growth stage of internode elongation and those on August 1 at full blooming in timothy. Second cut baled silage was harvested with intervals of 29 to 72 days from the

same meadow of the first cut. In both cuts, beta-carotene contents in dry matter were analyzed. The wilted silages were classified as medium and low moisture. For the medium moisture silage, the original forage was harvested at heading stage and ensiled within the day of cutting, while the low moisture silage was harvested at full blooming, dried, then ensiled 4 days later. During the drying process, loss of beta-carotene was determined morning and afternoon. Preliminary drying by microwave radiation of fresh forages and silages before analysis of beta-carotene was patterned after PARK *et al.*¹⁴⁾. Fifty grams of each sample was radiated for 2.5 minutes (Toshiba ER-601 S, oscillating wave: 2,450 MHz, rated high frequency output: 600 W). After radiation, samples were dried at 40°C for 24 hours, ground through a 0.5 mm screen and vacuum packed in aluminum foil covered containers to minimize destruction of carotenoids then stored at -20°C until chemical analysis. Prior to ensiling, silage materials were subjected to determination of moisture content, and then ensiled in an air tight silo. For hays, loss of beta-carotene during drying was also determined twice a day until baled.

Animals: A total of 22 pregnant cows were grouped into four by feeding treatment. Group 1: Four cows were fed with medium moisture silage, containing beta-carotene 119 mg/kg of dry matter, and supplemented with 500 mg of synthetic beta-carotene (Rovimix beta-carotene 10%, Nippon Roche K.K., Tokyo). Group 2: Seven cows were fed with same silage as in Group 1 without beta-carotene supplementation, as control. Group 3: Four cows were fed with low moisture silage, containing beta-carotene 36.4 mg/kg, and supplemented with 500 mg of synthetic beta-carotene. Group 4: Seven cows were fed with same silage as in Group 3 without carotene supplementation, as control. The average body weight after parturition, number of calvings and daily milk yield for 16 weeks postpartum

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of all cows were 643 kg, 3.4 calvings and 29.8 kg. Their average daily intakes of forages before and after parturition were 11.8 kg and 12.5 kg in dry matter during experimental period. Vitamin A was not contained in concentrate.

To quantify the beta-carotene levels in the blood of cows, jugular blood samples were biweekly obtained 4 weeks before and 16 weeks after parturition. On the day of parturition, blood was collected immediately after delivery from dams and new born calves, then analyzed. Seven days postpartum, colostrum was collected every milking time in bucket milk. Beta-

carotene contents in forage, serum and milk samples were analyzed by the High Performance Liquid Chromatography (Shimazu LC-6 A with STR ODS column, Tokyo), using the method validated by UENO¹⁸⁾ with some modifications in the composition of mobile fluid as follows : methanol : acetonitrile : chloroform = 47 : 47 : 6. Recovery of reproductive organs after parturition was monitored by regular rectal palpation, and first days of ovulation and conception were evaluated.

Results

In first and second cuts of fresh timothy-

Table 1. Analysis of the constituents in 4 cuts of fresh green grasses harvested in the same meadow

No. of cuts	Sampling date	Fresh sample		Sample after drying		TDN
		Beta-carotene	Vegetation Legume ratio	Crude protein	Crude Fiber	
	Month Day	mg/kg	%	%	%	%
First-cut	June 8	358.2	24.0	16.8	21.1	78.7
	June 18	307.5	13.3	16.6	25.4	79.4
	June 23	250.1	11.6	15.5	26.6	79.4
	(Average)	305.3	16.3	16.3	24.4	79.2
Second-cut	July 13	417.7	57.2	19.2	22.8	76.1
	July 20	348.5	56.6	19.1	22.4	76.7
	July 26	365.9	39.5	20.0	21.4	76.3
	Aug. 2	299.0	32.2	17.4	22.3	76.6
	Aug. 9	238.1	33.6	16.1	25.6	76.3
	(Average)	333.8	43.8	18.4	22.9	76.4
Third-cut	Aug. 15	486.0	59.4	20.4	23.7	69.3
	Aug. 30	225.3	46.1	21.7	25.0	69.7
	Sep. 6	317.7	29.4	19.6	24.5	69.2
	Sep. 15	192.2	26.4	18.1	24.0	68.9
	Sep. 20	331.3	43.5	18.7	23.7	69.1
	Sep. 27	250.9	51.3	20.2	23.4	69.4
	(Average)	300.6	42.7	19.8	24.1	69.3
Fourth-cut	Oct. 6	202.7	46.6	19.9	20.9	69.0
	Oct. 11	294.3	55.0	20.0	20.7	69.4
	Oct. 18	319.6	53.9	19.4	19.4	69.7
	Oct. 25	224.5	48.8	18.3	18.6	70.2
	Nov. 1	306.2	53.1	18.6	18.6	70.7
	(Average)	269.5	51.5	19.2	19.6	69.8

TDN was determined by the digestion trial using four wethers.

Table 2. Analysis of beta-carotene contents in the first and second cuts

First Cut Baled Silage				
Sampling Date	Beta- ¹⁾ carotene	Crude ¹⁾ protein	Growth ²⁾ stage	Sampling Date
Month Day	mg/kg	%		Month
June 14	308.5	16.1	Vegetative	August
June 20	235.1	14.9	Heading	August
July 3	172.7	11.6	Heading	August
July 3	172.7	11.6	Heading	September
July 11	121.7	9.6	Full heading	September
July 18	82.9	9.4	Full heading	September
July 26	93.5	8.7	Blooming	September
August 1	59.4	8.7	Full blooming	September

¹⁾ On dry matter basis.²⁾ Growth stage of timothy.

ladino clover, the average concentration of beta-carotene decreased linearly as the mix forages matured, while this tendency was vague in third and fourth cuts (Table 1). As the percentage of legume in vegetation increased, the higher beta-carotene concentration was noted.

In the first cut round baled silage, beta-carotene substantially decreased as harvesting date was delayed (Table 2). However, higher levels were noted with forages harvested at heading stage than at blooming or full blooming in timothy. In the second cut, the correlation of beta-carotene contents with the intervals from 1st cut was not significant. Among its constituents, percentage of crude protein in dry matter was shown to be positively correlated to carotene content in the first cuts. During drying, beta-carotene drastically decreased while tracing a regression curve (Fig. 1). Low moisture silage (40%) dried for four days after cutting showed a significant reduction of carotene concentration on ensiling (30 mg/kg in dry matter), while medium moisture silage (65%) ensiled within the day of cutting maintained higher beta-carotene level (305 mg/kg). However, the reduction rate of carotene concentration within a day after cutting was not significantly

different between medium and low moisture silages (41% vs. 39%). Hay baled 6 days after cutting showed a decreased carotene concentration to almost one eighth of the original fresh grass (163 mg/kg original vs. 22 mg/kg in hay on baling). A regression curve for the loss of carotene content in low moisture silage and hay is depicted in Fig. 1. The average beta-carotene concentrations in different forages at feeding were, 176 ± 54 mg/kg (average $\pm$ S.D.) in medium moisture silage, 24.6 ± 6 mg/kg in low moisture silage and 4.4 ± 2.2 mg/kg in hay.

Serum beta-carotene of groups 3 and 4 cows decreased during late pregnancy and was shown to be lowest a week after parturition (Fig. 2). Two weeks later, it increased and showed a maximum level between 8 to 12 weeks but those in group 4 remained at a low level, less than 200 μ g/dl. Highest level was observed in group 1 cows. The beta-carotene level in colostrum decreased rapidly after first milking. Levels on the day of parturition (day 0), 2, 4 and 6 days in group 3 and 4 cows were as follows, 247 ± 158 μ g/dl, 23 ± 6 μ g/dl, 8.7 ± 4.6 μ g/dl, 14.0 ± 6.9 μ g/dl and 148 ± 35 μ g/dl, 23 ± 20 μ g/dl, 2.9 ± 1.6 μ g/dl, 5.2 ± 3.0 μ g/dl, respectively. Milk from cows fed with low moisture silage supplemented with beta-carotene in the ration contained higher carotene than control

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of round baled silages in relation to the interval of harvesting

Second Cut Baled Silage				
	Interval From 1st Cut	Beta- ¹⁾ carotene	Crude ¹⁾ protein	Growth ²⁾ stage
Day	days	mg/kg	%	
1	48	187.2	19.6	Heading
1	42	187.4	19.4	Heading
1	29	160.0	21.0	Heading
13	72	103.9	14.8	Full heading
13	64	57.4	14.7	Full heading
13	57	59.7	16.1	Full heading
13	49	85.1	17.2	Heading
13	43	131.9	18.2	Heading

cows, 4 days postpartum. Calves delivered from dams in group 1 showed higher level of serum carotene than those of control cows (Table 3). Three out of 7 cows in group 4 and one of 4 in group 3 experienced stillbirths but

none in cows with high carotene silage. No specific degeneration was observed organoleptically in stillborn calves.

There was no difference in the average days to first ovulation in all groups. But average

Fig. 1. Loss of beta-carotene in wilted silages and hay during drying.

Regression curve

$$Y_s = 252 - 174 (\ln X) + 21.1 (\ln X)^2 \quad R^2 = 0.921$$

$$Y_h = 197 - 123 (\ln X) + 12.3 (\ln X)^2 \quad R^2 = 0.970$$

X : (Days after cutting) + 1

Day 0 : Day of cutting fresh forage

Fig. 2. Changes of serum beta-carotene in 4 groups of cows

○ : Four cows in group 1 ● : Seven cows in group 2

□ : Four cows in group 3 ■ : Seven cows in group 4

Points represent mean values, with their standard errors represented by vertical lines.

Table 3. Analysis of the constituents of serum and body weight of new born calves delivered by dams fed with medium and low moisture silages with and without beta-carotene supplementation

Group	Silage intake of dams	No. of calves (Stillborn)	Serum constituents				Body weight	
			Beta-carotene		Retinol		Day 0	Day 7
			Day 0	Day 7	Day 0	Day 7		
						μg/dl	μg/dl	kg
		Medium moisture high carotene silage						
Grp. 1	With suppl.	4 (0)	4.7±0.2	60.5±26.0	5.9±1.2	12.2±4.5	41.5±4.0	43.9±4.3 ¹⁾
Grp. 2	Without suppl.	7 (0)	3.7±4.3	23.7±10.1	5.9±2.0	14.2±5.5	49.2±4.1	49.0±4.0
		Low moisture low carotene silage						
Grp. 3	With suppl.	4 (1)	UD	9.8±1.7	9.5±3.6	16.8±1.9	46.7±3.3	46.3±0.3
Grp. 4	Without suppl.	7 (3)	UD	5.8±3.4	7.2±1.2	19.3±1.1	45.8±3.1	46.7±1.3

Stillborn calves were excluded in the statistical analysis.

Beta-carotene content was 119±18.4 mg/kg in dry matter in medium moisture silage (61%), 36.4±5.1 mg/kg in low moisture silage (40%).

UD : Beta-carotene level was undetected.

With suppl. : 500 mg of synthetic beta-carotene, Rovimix (Beta-carotene 10%) was added daily to concentrate.

Without suppl. : No beta-carotene additive as control.

1) Mean±SD.

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Table 4. Days required from calving to ovulation, uterine involution and conception and occurrence of reproductive disorders

Group of dams	No. of cows	Ovulation		Uterine Involution	Conception	Retained placenta	Uterine metritis
		First	Third				
days						No. of cows	
Grp. 1	4	20.5±5.0 ¹⁾	61.7±10.6 ^a	39.8±6.3	91.3±17.8	0	0
Grp. 2	7	25.8±6.9	66.0±12.7	41.4±8.0	100.6±36.5	0	0
Grp. 3	4	24.8±4.4	68.0±2.4	42.0±5.6	103.0±24.1	2	1
Grp. 4	7	24.8±5.8	84.3±12.3 ^b	43.3±5.8	119.7±47.5	1	2

¹⁾ Mean±SD.

^{a, b)} Values with different superscripts are significantly different (P<0.05).

days to third ovulation and conception days in group 4 cows were rather increased than cows in other three groups, although differences were not significant (Table 4).

Discussion

Beta-carotene is easily susceptible to oxidative degradation in the laboratory analysis. But studies have shown that this could be minimized. As reported by PARK *et al.*¹⁴⁾ among 12 drying methods, microwave-radiated grass retained the highest concentration of beta-carotene. KOBAYASHI *et al.*¹¹⁾ concurred with this finding and attributed it to enzyme inactivation. Therefore, to attain the minimum degradation, preliminary microwave radiation was adopted in fresh green samples and wilted silages prior to drying for analysis of carotene content.

The average range of beta-carotene in dry matter varied among the different forages tested, *i.e.*, 192-486 mg/kg in freshly harvested green mix forages, 57-308 mg/kg in medium moisture silage and round bale silages, 15-54 mg/kg in low moisture silage and 2-10 mg/kg in hay. These variations are in accord with previous reports^{3,10,11,14)} and these could be due to a number of factors influencing carotene contents in forages which include species, cultivar, stage of growth, fertilizer treatments, pesticides and climate, especially the intensity of sun radiation^{8,14)}. KALAC and McDONALD¹⁰⁾ reviewed the changes and destructions of

forage carotene and pointed these as the result of photochemical catalysis by chlorophyll, intrinsic haematin, especially peroxidase and cytochrome c, and by haem proteins present in chloroplasts and mitochondria. This may support the conclusion obtained from the results of the freshly harvested green mix forages and round baled silage, that as they matured, the beta-carotenes decreased. Substantial destruction of beta-carotene in silages during aeration¹¹⁾ and loss of 15 to 25% during 24 hour of air penetration⁸⁾ were reported. Conforming with these, beta-carotene of low moisture silage processed under practical operating conditions decreased from 230 mg/kg in its original fresh state to 140 mg/kg following day after cutting in the present study. To counteract such problem, KALAC and KYZLINK⁹⁾ suggested to minimize air penetration to prevent aerobic conditions. Furthermore, beta-carotenes were adversely affected during the drying periods, 4 days in low moisture silage and 6 days in hay, when once and twice rainfalls were respectively experienced, which may have caused the accelerated carotene degradation. Thus, it took longer days than usual for ensiling and baling, plunging the beta-carotene level to almost one eighth of the original. In wilted silages, mix forages ensiled within the day of cutting retained fairly higher beta-carotene level than those ensiled 4 days later. In the present experiment, considering factors that influenced the fluctuations of

beta-carotene level, aeration process had the most adverse effect.

The estimated average daily silage intake of all cows was 12 kg in dry matter and this corresponded to a total beta-carotene intake of 1,428 mg ($119 \text{ mg} \times 12$), in group 2 cows. This calculation exceeded that in group 3 with 932 mg ($36 \text{ mg} \times 12 + 500 \text{ mg}$). These results therefore indicate that supplementation of synthetic beta-carotene could be dispensed off in high carotene silage. According to NRC¹³⁾, 19 mg of carotene (7,600 IU of vitamin A)/100 kg of live weight has been suggested as the requirement for reproduction as well as lactation. It has also shown that feeding the diet containing 200-300 mg of beta-carotene supplemented with 50,000 IU of vitamin A should be recommended for lactating cows⁷⁾. Supplements of vitamin A were not added to the rations in the present study. Therefore, the average daily intake of beta-carotene in group 4 cows, which was estimated as 432 mg, might be critical level compared with those standards.

Several authors have reported reproductive disorder as a result of beta-carotene deficiency, LOTTHAMMER¹²⁾, INABA *et al.*^{5,6)} to mention a few. Furthermore, the beneficial effects of beta-carotene supplementation were cited by RAKES *et al.*¹⁵⁾ and UDA *et al.*¹⁷⁾, however WANG *et al.*¹⁸⁾ reported that supplementation did not alter progesterone level, LH patterns, estrous activity nor conception after PGF_{2α} administration. ASCARELLI *et al.*²⁾ determined the effect of dietary carotene in high yielding dairy cows and summarized that a high plasma carotene concentration is not a prerequisite for conception, however, under certain conditions which need defining, the carotene concentration appears to be a limiting factor. Although differences in conception days were not significant in the present study, average conception days after parturition was increased in group 4 cows. UDA *et al.*¹⁷⁾ attributed the decrease in serum beta-carotene at parturition to the abnormally large drain of carotene into the

colostrum. This could account therefore for the sudden decrease of colostrum beta-carotene after the first milking in the present study.

BLOCK and FARMER³⁾ proposed the scheme on the concentrations of beta-carotene in blood plasma as follows: more than 300 µg/dl was considered sufficient, 200-300 µg/dl suspect, 100-200 µg/dl critical and less than 100 µg/dl deficient. Based on these criteria, 200 µg/dl of serum beta-carotene in group 4 cows was evaluated critical. Hence, these cows secreted lower carotene in their colostrum, thus resulted in an even more critical serum level in their new born calves. However, beta-carotene level in serum of dams was not always reflected in the serum retinol of the new born calves.

When beta-carotene concentration is practically none as in concentrate feeds and/or limited as in some forages, addition or supplementation of synthetic beta-carotene is absolutely necessary to attain maximum beneficial effects of beta-carotene.

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調製条件が牧草中の β カロチン含量に及ぼす影響と 乳牛に対する β カロチン製剤の添加効果

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調製条件が牧草中の β カロチン含量に及ぼす影響を調査するとともに、乳牛への β カロチン製剤の添加効果を飼料中の β カロチン含量の関連で検討した。その結果、生草中の β カロチン含量は1番草および2番草で生育ステージが進むに従い減少し、牧草サイレージや乾草の調製過程で予乾や乾燥日数の経過に伴い激減した。血液中や初乳中の β カロチン濃度は β カロチンの摂取量を反映し、新生仔牛の血液中濃度にも影響した。低 β カロチン飼料給与牛では、分娩後第3回排卵までの日数や受胎日数が増加する傾向がみられたが、 β カロチン製剤の添加により改善しうることが示唆された。

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