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Effect of Beta-carotene Supplementation on Plasma Carotene Content and Fertility of Lactating Crossbred Sahiwal Cows

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ABSTRACT

Background: Beta-carotene (BETA) is a precursor of retinol (Vitamin A) and positively influenced on reproductive efficiency in cows. The aim of the study was to determine the effect of orally supplementation of beta-carotene on plasma carotene content and fertility rate of lactating crossbred Sahiwal cows.

Methods: Twenty-four disease-free lactating crossbred Sahiwal cows with a close date of calving were randomly divided into two homogeneous groups for Control group (CONT; n = 12) and Beta-carotene group: 500 mg/cow/d (BETA; n = 12) from 7 days post partum (dpp) until 105 dpp thereafter on concentrations of beta-carotene and selected protein and energy parameters in plasma were determined. In addition, effects on fertility rate were studied.

Result: Beta-carotene concentrations increased in plasma of beta-carotene (BETA-group) supplemented cows compared to control (CONT-group) cows ($p<0.001$). In BETA-group cows, urea concentration in blood plasma decreased significantly compared to cows of CONT-group ($p<0.001$) but did not differ in total protein, albumin, glucose and total cholesterol content in cows of both groups. Occurrence of first post partum estrus decreased ($p<0.001$) in beta-carotene (BETA-group: 74 dpp) cows compared to control (CONT-group: 81 dpp) cows. In this study, it was observed that the overall conception rate was higher ($p<0.001$) in BETA-group: 83.33% (cows pregnant: 10/12) and lesser in the CONT-group: 50.00% (cows pregnant: 6/12). The results of this study indicated that oral supplementation with beta-carotene increased the concentration of beta-carotene and decreased the concentration of urea in plasma and cows with higher blood plasma beta-carotene content improved fertility over cows with lower blood plasma beta-carotene content.

Key words: Beta-carotene, Conception rate, Crossbred Sahiwal cows, Plasma concentrations, Post partum.

INTRODUCTION

Prolonged post partum estrus negative impacts on future reproductive and productive performance, including by a longer calving interval and a higher negative economic impact (Lee and Kim, 2007; Hussain *et al*., 2009). Detection of post partum estrus is one of the most important factors affecting fertility in dairy cows. However, the rate of post partum estrus detection is below 50% in many commercial dairy farms (Unalan, 2016; Bilen and Mecitoglu, 2021). Fertility of lactating dairy cows has reportedly been decreased over the last decades and appears to be multifactorial including improper and deficient nutrition is one of the reasons behind this problem. The nutritional deficiency causes several infertility conditions which constitutes about 2/3rd of reproductive problems occurring in lactating crossbred cows (Tewari *et al*., 2014). For normal development and activity of reproductive organs, feeding of balanced ration is of utmost importance. Proper blood-vitamin constituents are good indicators for normal reproductive and health status of individual animals. Beta-carotene in particular is currently being investigated for potential reproductive benefits (Purohit *et al*., 2008; Madureira *et al*., 2020).

Beta-carotene (BETA) belongs to the family of carotenoids, phytochemical pigments naturally synthesized in fruits, vegetables, plants, algae and photosynthetic bacteria. Specifically, BETA is a precursor of retinol (Vitamin A), a fat-soluble vitamin, involved in cellular division and differentiation, bone development and reproductive function (Lopez-Flores *et al*., 2020). Vitamin A (VA) cannot be synthesized by animals, therefore beta-carotene is the principal natural precursor of vitamin A in cattle and it is mainly provided by forages. Beta-carotene is either absorbed intact or metabolized in the intestinal mucosa and the resulting retinol is absorbed. Beta-carotene is transported with fat in the lymphatic system and temporarily stored in the liver. Besides being a vitamin A precursor, beta-carotene plays a role in reproductive efficiency in cows (Kaewlamun *et al*., 2011).

There are limited studies in India on the impact of beta-carotene supplemental diet especially on indigenous cows.

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The objective of this study was to determine the effect of oral supplementation of beta-carotene on plasma carotene content and fertility rate of lactating crossbred Sahiwal cows. We hypothesized that cows with higher plasma beta-carotene content will have improved fertility than cows with lower plasma beta-carotene content.

MATERIALS AND METHODS

Study location

In late April 2019, this study was conducted at the *Gowshala* (dairy farm), Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, which is situated in the eastern part of Uttar Pradesh, extends between 23045'N to 28030'N and 80045'E to 84030'E. Varanasi city is located roughly in the centre of the north Gangetic alluvial plain on the left of Ganga river at an altitude above of the sea level 128.93 meters. That means it is under a subtropical climate and is often subjected to extreme weather and the average rainfall is about 110 cm annually.

Selection of cows (n = 24) and experimental design

Twenty-four disease-free lactating crossbreed Sahiwal cows with the beginning date of calving were randomly located in individual pens to form two homogeneous experimental groups: (i). Control (CONT; n = 12; live weight = 418.14±14.21 kg) and (ii). Beta-carotene (BETA; n = 12; live weight = 419.98±14.69kg), with similar BCS between treatment groups, respectively and did not differ significantly. The experimental groups received a basal diet twice daily containing wheat straw (*Triticum aestivum*) *ad lib* and green lucerne (*Medicago sativa*) as a green fodder in a mixed-ration. Diets of lactating cows were formulated as per the nutritional requirements of NRC (2001). The ingredient composition of the concentrate mixtures feed during the experiment are shown in Table 1.

Beta-carotene supplementation

Supplements of beta-carotene were purchased from DSM Nutritional Products India Pvt. Ltd., Mumbai, India. The BETA-cows were orally supplemented with beta-carotene (500 mg/cow/day, mixed with concentrate mixture) during the entire experimental period (Table 1), which lasted from 7 days after calving to 105 days postpartum period (dpp).

Table 1: Ingredient composition (%) of concentrate mixture offered to the animal under different dietary treatment groups.

Ingredients	Concentrate mixture	
(kg/100 kg)	Control	Beta-carotene
Barley	10	10
Maize	20	20
Arhar chuari	14	14
Wheat bran	16	16
Mustard cake	14	14
Cottonseed cake	23	23
Mineral mixture	2	2
Common salt	1	1
BETA-carotene (mg/cow/day)	-	500

Blood sampling and analyses

Blood samples were obtained at days 0, 45 and 85 dpp from the jugular vein (10 ml) of each cow. Blood was collected in the morning access to feed and water and was placed immediately on ice. The first blood sample was collected using puncture into sterile glass flasks (5 ml), containing ethylenediamine tetraacetic acid (EDTA) as an anticoagulant, whereas, the other sample was collected without using anticoagulant. The (EDTA) blood samples were used for determination of biochemical blood parameters. Blood plasma concentrations of total protein, albumin, urea, glucose and total cholesterol were determined by colorimetric techniques using kits from diamond diagnostic company and measured by spectrophotometer in the UV range 240nm.

Blood samples were analysed for beta-carotene using the iCheck™ procedure (iCheck CAROTENE, BioAnalyt GmbH, Germany). This is a hand-held portable spectrophotometer (Hamann *et al*., 2019). The blood content of beta-carotene was classified as deficient (<1.5 mg/L), marginal (≥1.5 mg/L; <3.5 mg/L), or optimal (≥3.5 mg/L) according to Schweigert and Immig (2007). Blood analyses were done after the first morning milking. This is a single step separation method, done using direct whole blood and without the use of centrifugation where beta-carotene is extracted into an organic fluid within a vial (Fig 1).

Evaluation of reproductive parameters

The voluntary waiting period after calving was 45 days post partum (dpp). Detection of estrus was performed twice a day, morning and evening, for at least 30 min. The following parameters were evaluated: estrous response, as number of cows in estrus/number of total cows treated *100 and the conception rate was evaluated as the number of cows conceived/number of total cows mated *100. In addition, during the remainder of the day, any cows that showed estrus behaviour were reported to the inseminators by the farm workers. Artificial insemination was performed by well trained inseminators. Pregnancy diagnosis was performed by a veterinarian on cows which did not return to estrus after insemination at about 30 days post-insemination by palpation per rectum. Pregnancy was confirmed again between 60 and 90 days post-insemination using palpation per rectum.

Statistical analysis

Data related from the dpp concentrations of beta-carotene, selected protein and energy parameters in plasma and fertility were considered for statistical analysis. Data were compared using the student's t-test procedure of the IBM SPSS statistics software package (2012). Probability values $p<0.001$ were considered significant. All data given are means ± standard error of mean (SEM).

RESULTS AND DISCUSSION

Plasma beta-carotene content

The plasma beta-carotene content values at dpp for both control (CONT) and beta-carotene (BETA) group cows are reported in (Table 2). There was no significant difference between the CONT and BETA treatment groups before the start of the dietary beta-carotene supplementation. The beta-carotene status at initial period in both groups was found deficient with a narrow range from 0.55 to 1.51 mg/L and it was below the recommended level. In the BETA-group plasma beta-carotene content was significantly increased (P<0.001) at 45 dpp and plasma content of beta-carotene status was found to be marginal (2.87 mg/L) whereas in CONT-group the plasma content of beta-carotene level was found to be only 1.78 mg/L which was significantly lower than BETA-group. Further, the levels of plasma beta-carotene at 85 dpp in the BETA and CONT-groups were 3.89 and 2.64 mg/L, respectively. The increased ratios from 45 to 85 dpp was 1.02 mg/L in the BETA-group which was tended to be higher (P<0.001) than that of CONT-group which was 0.86 mg/L. The beta-carotene status at 85 dpp in BETA-group was found optimal when compared to the CONT-group cows. The plasma beta-carotene content in BETA-group cows can serve as an indicator showing whether the amount of beta-carotene in feed is sufficient. Our present findings are in accordance with the findings of Bhatnagar *et al*. (2020) and Ishida *et al*. (2018) who observed that supplementation of beta-carotene at the rate of 500 mg/animal/day significantly increased the plasma beta-carotene content in the beta-carotene group cows compared to control group cows.

Selected plasma protein and energy parameters

The selected plasma protein and energy parameters of both groups are presented in (Table 3), where the difference was significantly (P<0.001) found only in urea, while no differences were observed in total protein, albumin, glucose and total cholesterol content in both control (CONT) and beta-carotene (BETA) group cows. The total protein means were analogous and consistent in both groups. Albumin concentrations were constant over time and glucose values declined throughout the study, especially in CONT-group. It was also observed that the cholesterol content was decreased in the experimental period, especially in BETA-group cows though not significant. Whereas the trends of the various parameters were similar to those found by Trombetta *et al*. (2010) when fed diet supplemented with beta-carotene diet. A similar effect was also found by Meza-Herrera *et al*. (2017), who reported that beta-carotene supplementation did not resulted in significant differences between treatments for total protein, glucose and total cholesterol across time, such differences favored the beta- group, except the urea concentration of the serum which continued to be at higher level in the control group animals.

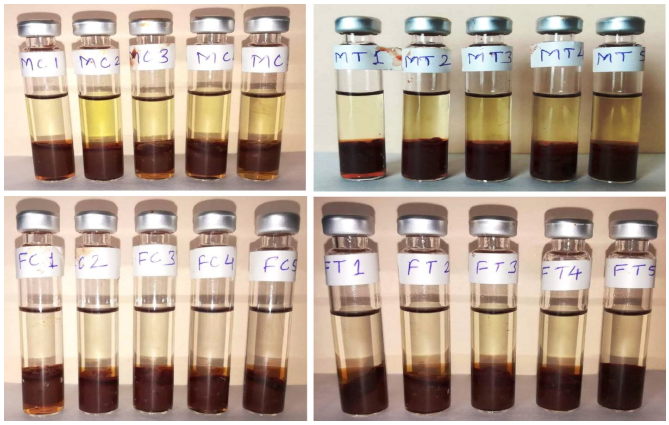

Fig 1: iCheck™ ampoules with sampled blood, absorbing beta-carotene into solution for analyses by spectrophotometer.

Table 2: Effect of beta-carotene supplementation on plasma beta-carotene content in lactating crossbred Sahiwal cows.

Days	Treatment		SEM[4]	P-value	Min BC[5]	Max BC[6]	Beta-carotene status[7]
	CONT[2]	BETA[3]					
	Mean BC mg/L[1]						
0	0.91	1.32	0.08	0.006	0.55	1.51	D
45	1.78	2.87	0.21	<0.001	1.40	3.52	M
85	2.64	3.89	0.23	<0.001	1.69	4.00	O

[1]Mean plasma beta-carotene concentration for group differ significantly (p<0.001) from each other row wise, [2]CONT = Control group, [3]BETA = Beta-carotene group [4]SEM = Standard error the mean of plasma beta-carotene within group, [5]Minimum beta-carotene value within group, [6]Maximum beta-carotene value within group, [7] Beta-carotene status of group; D = Deficient: <1.5, M = Marginal: ≥1.5, <3.5, O = Optimal: ≥3.5

Table 3: Effect of beta-carotene supplementation on plasma protein and energy parameters in lactating crossbred Sahiwal cows.

Days	Treatment		SEM	P-value
	CONT	BETA		
Protein parameters				
	Total protein (mg/dL)			
0	7.58	7.53	0.02	0.227
45	7.51	7.39	0.02	0.17
85	7.45	7.36	0.03	0.144
	Albumin (mg/dL)			
0	3.02	3.07	0.02	0.361
45	3.04	3.13	0.02	0.105
85	3.03	3.08	0.04	0.561
	Urea (mg/dL)			
0	15.34	15.29	0.29	0.930
45	15.10	12.46	0.44	<0.001
85	14.72	12.19	0.43	<0.001
Energy parameters				
	Glucose (mg/dL)			
0	4.58	4.65	0.03	0.372
45	4.30	4.38	0.09	0.668
85	3.92	4.12	0.09	0.315
	Total cholesterol (mg/dL)			
0	131.28	126.14	1.98	0.201
45	124.66	117.49	1.79	0.043
85	122.58	113.91	1.91	0.020

Reproductive performance

In the current investigation the signs of first post partum estrus interval from calving to first AI were noted in both group cows but the observable signs *i.e.* ropy clear discharge and standing while mounting by other animals *etc*. were more pronounced in BETA-group cows. Supplementation of beta-carotene to diet decreased the first post partum estrus interval from calving to first AI about 6 days was noted in BETA-group. Variation in the interval of calving to first AI from 74 to 81 dpp was less ($p<0.001$) for cows in the BETA-group than that for cows in the CONT-group during the experimental period (Fig 2). The overall conception rate was found to be 48.83% at first AI and 20.83% at second AI in all cows of CONT and BETA-groups put together (Table 4). In this study, the overall conception rate was higher ($p<0.001$) in BETA-group (83.33%) compared to the cows in CONT-group (50.00%). These results are in agreement with those reported by Bhatnagar *et al*. (2020) where in the impact of beta-carotene on the pregnancy diagnosis revealed highest conception rate in treated group over the dairy cows of control group. Supplemental effect of beta-carotene incorporated vitamin-premix showed estrus symptoms in 50% of crossbred Sahiwal infertile cows against the animals under control group upto 16.66% as reported by Kumar *et al*. (2019). Similarly, conception rates were also significantly higher in the beta-carotene supplemented cows (Bian *et al*., 2007; Trojacance *et al*., 2012; Colli *et al*., 2017; De Gouvea *et al*., 2018).

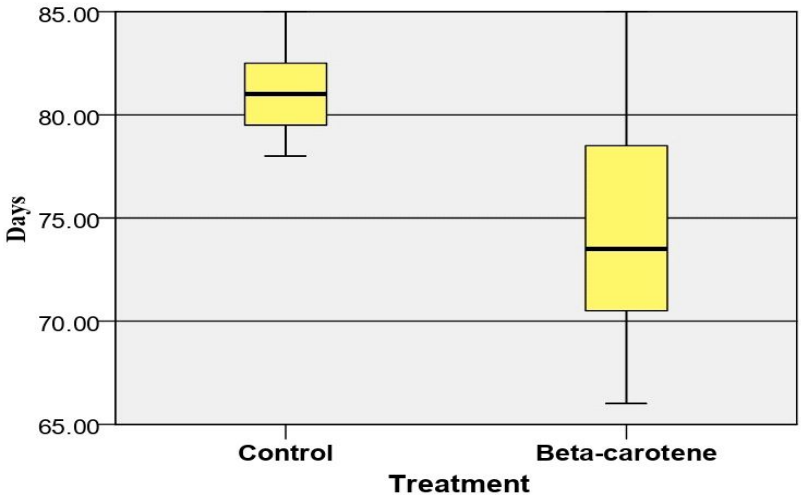

Fig 2: Effect of beta-carotene supplementation on post partum estrus in lactating crossbred Sahiwal cows.

Table 4: Effect of beta-carotene supplementation on reproductive performance in lactating crossbred Sahiwal cows.

Treatment	No of animals treated	Conception rate		
		At first AI	At second AI	Overall
		% of cows pregnant		
CONT	12	(4/12) 33.33	(2/12) 16.67	(6/12) 50.00
BETA (500 mg/cow/d)	12	(7/12) 58.33	(3/12) 25.00	(10/12) 83.33*
Overall	24	(11/24) 45.83	(5/24) 20.83	(16/24) 66.66

*p<0.001.

CONCLUSION

In conclusion, lactating crossbred Sahiwal cows with greater beta-carotene content in plasma decreased the first post partum estrus interval and increased the conception rate and also significantly decrease the blood plasma urea concentration during experimental period. Further studies are warranted on the possible association of content of beta-carotene in plasma at timed AI with improvement in fertility of dairy cows.

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