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Raw Milk Quality and Udder Health Status of Lactating Crossbred Sahiwal Cows Supplemented with β-carotene Enriched Mineral-Vitamin Premix

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ABSTRACT

The aim of this study was to assess the effect of supplemented β-carotene enriched mineral-vitamin premix on lactating crossbred Sahiwal cows and analyzing the raw milk quality and udder health of the animals. Twenty-four disease-free lactating crossbred Sahiwal cows with a close date of calving were randomly divided into two equal groups as Control group (CONT; n = 12) and Beta-carotene group: 500 mg/cow/d (BETA; n = 12). These were animals from 7 days post-partum (dpp) until 105 dpp, and thereafter the raw milk quality and udder health status of these animals were determined. Statistical analysis of the data regarding post-partum udder health status fortnightly from 45 to 105 days of the experimental period was observed ($P<0.001$). Lower somatic cell counts in raw milk of 12.50 to 10.02 $Log10^4$ cells/ml were observed in BETA-group cows when compared with CONT-group cows with 12.69 to 13.17 $Log10^4$ cells/ml. The Modified California Mastitis tests from 45 to 105 dpp were lower in the BETA-group cows (2.27 to 1.35) than in the CONT-group cows (3.59 to 3.96). The post-partum methylene blue dye reduction test from 45 to 105 dpp indicated ($P<0.001$) that raw milk quality was significantly higher in BETA-group cows (107.96 to 139.89 in minutes) than in CONT-group cows (90.81 to 80.78 in minutes) throughout the post-partum period. This study revealed that orally supplementing crossbred Sahiwal cows with β-carotene improved their raw milk quality and udder health.

HIGHLIGHTS

- β-carotene supplemented cows exhibited lower raw milk somatic cell counts.
- Subclinical mastitis was less common in β-carotene supplemented cows.
- β-carotene supplemented cows showed superior raw milk quality.

Keywords: β-carotene, Lactating cows, MBRT, MCMT, SCC, Subclinical mastitis

Vulnerable udder health is most directly linked with mastitis, which ranks first among diseases that cause a significant economic loss to dairy animal owners. Mastitis, more specifically in subclinical mastitis (SCM) form is the most cost-effectively significant animal illness affecting the dairy sector (Seegres *et al.,* 2003). Mastitis is the single most common reason for premature culling and the real cost of mastitis is often underestimated by farmers. The related monetary losses include drug costs, discarded milk that is not suitable for human consumption, veterinary advice, workload, lower milk production, premature culling, costs for replacement animals, feed costs for non-producing animals and decreased price per liter for lower-quality milk in connection with higher milk somatic cell counts (SCC). Clinical mastitis (CM) refers to an inflammatory response

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within the udder that is accompanied by a suite of visible signs whereas subclinical mastitis (SCM) is frequently asymptomatic and is characterized by an increase of somatic cell counts (SCC) in milk obtained from infected quarter of the udder. According to Sharma *et al.* (2012) the prevalence of clinical and subclinical mastitis in cows in India was between 1 and 10% two years ago, whereas recent studies have reported the incidence of SCM ranging from 19.20 to 83% in cows. Therefore, it is critical to understand the variables that determine its incidence, including management and feeding methods.

Milk somatic cell counts (SCC) include both milk-producing and immunological cells. They are discharged in raw milk during the regular milking process. The SCC index is used to determine the health status of the mammary glands and the quality of raw milk in dairy cows worldwide (Yang and Li, 2015). Among the numerous milk quality screening procedures, estimating milk SCs is the most efficient method for detecting subclinical mastitis. In all developed countries, milk somatic cell count is used to detect the presence of clinical and subclinical mastitis in dairy herds and to guarantee that milk has a longer shelf life (Sharma *et al.,* 2011; Petzer *et al.,* 2017). In underdeveloped countries like India, however, milk is still priced depending on its fat content.

In India the lower livestock productivity is mainly due to feed shortages and unbalanced feeding practices. To minimize animal feed costs, labor expenditures, and to increase animal productivity, it considers the time requirements that may be met by combining concentrates with minerals and vitamins in the formulation of a balanced diet. This will assist in providing the livestock with the correct nutritional requirements. The high-producing dairy cow requires minerals and vitamins to maintain a healthy immune system, a mammary gland capable of producing milk, milk components, milk quality, as well as optimal fertility and udder health. (Erickson and Kalscheur, 2020). The premix is a highly efficient mineral-vitamin additive that is used to enrich combined feeds and feed of all farm animals in order to increase their productivity. It is most effective when used regularly at the recommended doses. Beta-carotene ($C_{40}H_{56}$) is one additive that is gaining popularity at the moment.

A member of the carotenoids family of phytochemical pigments, Beta-carotene (BETA) can be found in a variety of foods including fruits and vegetables, plants, phytoplankton, and photosynthetic microbes (Eggersdorfer and Wyss, 2018). BETA is a precursor of retinol (vitamin A), a fat-soluble vitamin required for cell division and differentiation, bone development, and reproduction (Lopez-Flores *et al.,* 2020). Due to the inability of animals to synthesize vitamin A (VA), the primary natural precursor of vitamin A in cattle is beta-carotene (BETA). This is primarily obtained through green forages. Distinct response of 500 mg/cow/day beta-carotene supplementation has significantly decreased the clinical mastitis in Holstein Friesian cows (Bian *et al.,* 2007). Chew *et al.* (1982) also found that cows with lower plasma beta-carotene and vitamin A concentrations had the higher score in the California mastitis test. Wang *et al.* (1988) examined that additional dietary beta-carotene had a beneficial impact on reducing the risk of mastitis. The animals with lower plasma beta-carotene and vitamin A levels had a higher index of the Modified California mastitis test (MCMT) (Chew *et al.,* 1982). The positive influence of beta-carotene as a supplement was found in the SCC of milk (Halik *et al.,* 2016; Kadyan *et al.,* 2020). In contrast, SCC in cows that were supplemented with beta-carotene was lower than those cow's which were un-supplemented (Rakes *et al.,* 1985). Milk SCC is affected by cow age, breeds, milk yield, health, number of lactation, teat and udder shape (Ahlawat *et al.,* 2008; Alhussien *et al.,* 2016; Alhussien and Dang, 2017).

There are very few studies on the effect of beta-carotene supplementation on raw milk quality and udder health status in indigenous cows, notably in India. Thus, the purpose of this study was to determine the effect of supplemented β-carotene enriched mineral-vitamin premix on lactating crossbred Sahiwal cows raw milk quality and udder health status.

MATERIALS AND METHODS

The experiment of this study has been 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. 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; parity = 4.41±0.60, milk yield = 8.53±0.12 kg) and (ii). Beta-carotene (BETA; n = 12; live weight = 419.98±14.69 kg; parity = 4.83±0.72 kg, milk yield = 8.62±0.13 kg). β-carotene (500 mg/cow/d) was given orally to the cows in BETA group for the duration of the 105 days trial (Fig. 1). Wheat straw *(Triticum aestivum) ad lib* and green lucerne *(Medicago sativa)* as green fodder in a mixed-ration were fed to the experimental groups twice daily. For lactating cows, the nutritional needs of the NRC (2001) were taken into consideration while designing their diets. Table 1 depicts the composition of the concentrate combinations that were fed to animals during the experiment.

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

Ingredients (kg/100 kg)	Concentrate mixture	
	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

Fig. 1: Supplemented β-carotene enriched mineral-vitamin premix used for experimental cows

Collection of raw milk samples to perform milk tests

The individual raw milk samples were taken from each lactating cow in every 15 days interval, starting from 7 days after calving until 105 days post-partum. At 15 days intervals, approximately 100 ml of raw milk was collected from 2 sequential milking, morning and evening (4.00 A.M. and again at 4.00 P.M.). Prior to sampling of raw milk, the teats were carefully disinfected, dried, and the first 2-3 strips of fore milk were discarded. Animal tag numbers were recorded on the sample collection bottles of all raw milk samples collected aseptically in clean and sterilized plastic bottles. Immediately after raw milk collection, slides were prepared for somatic cell counts (SCC) and raw milk sample was tested for Modified California mastitis test (MCMT) and methylene blue dye reduction test (MBRT). The raw milk samples were collected in sterile sampling bottles immediately after milking and transported to the laboratory for methylene blue dye reduction test (MBRT) and somatic cell counts (SCC) within one hour of collection. MCMT was tested on the dairy farm right after milking itself.

Statistical analysis

Fortnightly data related to raw milk tests for SCC, MCMT and MBRT were considered for statistical analysis. Prior to analysis, the SCC was converted into a log scale to reduce variance of heterogeneity. The student's t-test approach was used to compare data with the assistance of the IBM SPSS statistical software package (2012). The standard error of the mean (SEM) is used to express the variability in data. A significant level was determined using the probability value $P<0.001$.

RESULTS AND DISCUSSION

Somatic cell counts ($Log10^4$ cells/ml of raw milk)

Data related to post-partum fortnightly means of raw milk somatic cell counts (SCC) $Log10^4$ SCC (cells/ml of raw milk) of both CONT-group and BETA-group lactating crossbred Sahiwal cows are shown in ((Table 2). Statistically analyzed data revealed that SCC $Log10^4$ (cells/ml of raw milk) were found almost similar during day 0 to 30 dpp lactation in cows both CONT-group (12.59 to 12.63 $Log10^4$ cells/ml of raw milk) and BETA-group

(12.57 to 12.51 Log10^4 cells/ml of raw milk). However, after 30 dpp experiment period cows in BETA-group, the fortnightly raw milk SCC significantly decreased (P<0.001) from 12.50 to 10.02 Log10^4 cells/ml of raw milk. Whereas in the case of cows in the CONT-group it increased from 12.69 to 13.17 Log10^4 cells/ml of raw milk over 105 dpp lactation period (Fig. 2).

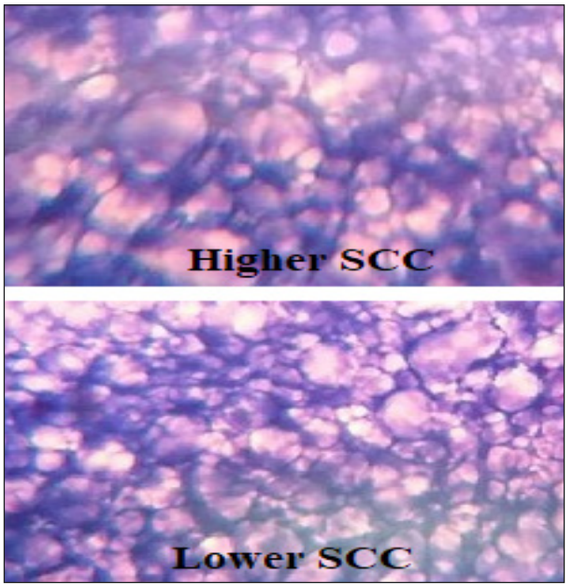

Fig. 2: Determination of SCC (higher SCC in CONT-group and lower SCC in BETA-group) in raw milk using microscopic method

Raw milk samples of cows in both groups showed similar SCC in early stage 0 to 30 dpp experiment period, which is normally required for the udder's self-defense mechanism. After 30 dpp SCC in BETA-cows positively decreased and cows in CONT-group showed regular increase in SCC over 105 dpp. This was a visible sign for the incidence of SCM in cow's udder along with lower milk yield. Our findings are similarly supported by Halik *et al.* (2016) on impact of supplementing cows with synthetic β-carotene (400 mg/cow/d) where the overall milk somatic cell counts recorded in the eight weeks of lactation period was significantly higher in the non-supplemented group in comparison with β-carotene supplemented group. On the other hand, Kadyan *et al.* (2020) found that when buffaloes were supplemented with β-carotene (300 mg/buffalo/d), the total milk SCC was considerably low in the β-carotene supplemented group than in the non-supplemented group.

Table 2: Effect of β-carotene supplementation on fortnightly somatic cell counts (SCC) in lactating crossbred Sahiwal cows

Days	Treatment groups		SEM	P-value
	CONT	BETA		
	Log10^4 SCC (cells/ml)			
0	12.59	12.57	0.02	0.765
15	12.61	12.54	0.02	0.232
30	12.63	12.51	0.02	0.38
45	12.69^a	12.50^b	0.03	<0.001
60	12.72^a	12.43^b	0.04	<0.001
75	12.80^a	12.35^b	0.07	<0.001
90	12.85^a	11.54^b	0.20	<0.001
105	13.17^a	10.02^b	0.50	<0.001

^{a,b}Mean values for each experiment within a row with different superscript letters differ significantly (P<0.001); CONT = Control group; BETA = Beta carotene group; SEM = Standard error the mean; SCC = Somatic cell counts.

Subclinical mastitis (SCM)/Modified California mastitis test (MCMT)

The present study presents the results from Modified California mastitis test (MCMT) that have been statistically examined (Table 3). The occurrence of subclinical mastitis (SCM) may have a negative effect on udder health status and eventually resulting in decreased milk production. Analyzed data of fortnightly MCMT grade revealed that grades were similar during day 0 to 30 dpp lactation in cows of both groups in CONT-cows (3.39 to 3.46 in grades) and BETA-cows (3.37 to 3.36 in grades). After that 30 dpp there was significant (P<0.001) decrease in the milk MCMT grades of BETA-group cows (from 2.27 to 1.35 in grades) compared to case of CONT-group cows where it increased (from 3.59 to 3.96 in grades) over 105 dpp experimental period. A comparison among fortnightly days of lactation revealed that similar raw milk SCC and MCMT grades are found in cows of both groups during early lactation stages (0 to 30 dpp). However, after 30 dpp in BETA-group cows had a lowering trend, but an increasing trend was observed in CONT-group cows over 105 dpp lactation period. This may be related to a higher incidence of SCC, which causes milk to become more alkaline. The presence of more DNA due to higher somatic reactions with MCMT reagents resulted in higher MCMT grades in the CONT-group than in the BETA-group. The current research findings confirm

those reported by Singh *et al.* (2020), that treatment group cows had significantly lower (P<0.01) milk SCC and MCMT grades than in control group cows. Bian *et al.* (2007) reported a significant decrease in clinical mastitis in cows supplemented with beta-carotene (300 and 500 mg/cow/day) when compared to the non-supplemented group. Whereas, in another study it was observed that supplementing Sahiwal cows with vitamin E lowered the somatic cell counts (SCC) and percent of subclinical mastitis (SCM) (P<0.05) in supplemental group than un-supplemented groups. (Chandra *et al.,* 2015).

Table 3: Effect of β-carotene supplementation on fortnightly subclinical mastitis (SCM)/Modified California mastitis test (MCMT) in lactating crossbred Sahiwal cows

Days	Treatment groups		SEM	P-value
	CONT	BETA		
	MCMT (grades)			
0	3.39	3.37	0.02	0.710
15	3.43	3.38	0.01	0.14
30	3.46	3.36	0.01	0.004
45	3.59[a]	2.27[b]	0.13	<0.001
60	3.72[a]	1.98[b]	0.18	<0.001
75	3.83[a]	1.83[b]	0.20	<0.001
90	3.89[a]	1.64[b]	0.23	<0.001
105	3.96[a]	1.35[b]	0.27	<0.001

[a,b]Mean values for each experiment within a row with different superscript letters differ significantly (P<0.001); CONT = Control group; BETA = Beta carotene group; SEM = Standard error the mean; MCMT = Modified California mastitis test

Raw milk quality/methylene blue dye reduction test (MBRT)

Statistically analyzed fortnightly data of raw milk MBRT (in minute) is presented in (Table 4). Analyzed MBRT data of the cows in both groups from 0 to 30 dpp fortnightly were similar. Good raw milk quality was recorded in CONT-group cows (97.75 to 91.77 minutes) and BETA-group cows (98.21 to 93.33 minutes). Conversely, after 30 dpp experiment period from 45 to 105 dpp the fortnightly MBRT was considerably (P<0.001) higher in BETA-group cows (107.96 to 139.89 minutes) with the quality of raw milk found very good instead of as in case of CONT-group cows (90.81 to 80.78 minute) with quality of raw milk found fair. The time required for degradation of the blue color in milk provided by methylene blue (MB) indicate the quality of milk, i.e., low color reduction time indicate that the milk is of good quality and vice versa (Bongard *et al.,* 1995; Merker *et al.,* 1997; Impert *et al.,* 2002). Imran and Bassette, (2010) reported that an excellent quality milk sample would take 8-10 hours to the change of the color of MB into colorless. The MBRT is reliant on the capacity of bacteria found in milk to proliferate and absorb dissolved oxygen, hence lowering the medium's oxidation-reduction potentials (Srujana *et al.,* 2011). Bacteria found in milk samples were the agents responsible for oxygen consumption, which occurred more often in raw milk samples from CONT-group cows than in raw milk samples from BETA-group cows. Thus, the time required to reduce the dye (in minutes) as a reference for the number of microorganisms in raw milk concurred with Singh *et al.* (2020), who demonstrated that cows in the treatment groups had substantially (P<0.01) higher raw milk quality or MBRT than cows in the control group. According to De Frain *et al.* (2009), trace minerals also improved the cow milk quality.

Table 4: Effect of β-carotene supplementation on fortnightly methylene blue dye reduction test (MBRT) for milk quality test in lactating crossbred Sahiwal cows

Days	Treatment groups		SEM	P-value
	CONT	BETA		
	MBRT (minutes)			
0	97.75	98.21	0.19	0.241
15	94.78	95.85	0.35	0.140
30	91.77	93.33	0.32	0.12
45	90.81[a]	107.96[b]	1.78	<0.001
60	92.84[a]	110.88[b]	1.88	<0.001
75	86.80[a]	120.88[b]	3.55	<0.001
90	81.87[a]	127.89[b]	4.79	<0.001
105	80.78[a]	139.89[b]	6.16	<0.001

[a,b]Mean values for each experiment within a row with different superscript letters differ significantly (P<0.001); CONT = Control group; BETA = Beta carotene group; SEM = Standard error the mean; MBRT = Methylene blue dye reduction test

CONCLUSION

In conclusion, lactating crossbred Sahiwal cows

supplemented with β-carotene during post-partum fortnightly from 45 to 105 dpp showed significantly lower somatic cell counts (SCC) in raw milk of BETA-group cows when compared with CONT-group cows. Similarly in Modified California mastitis tests from 45 to 105 dpp, BETA-group cows had lower MCMT grades than cows in the CONT-group, which had higher MCMT grades. During the post-partum experimental period, the raw milk quality as evaluated by the methylene blue dye reduction test (MBRT) from 45 to 105 dpp was considerably higher in BETA-group cows with very good raw milk quality than in CONT-group cows with fair raw milk quality.

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