

## Original Research Article

### Effect of $\beta$ -Carotene on Somatic Cells Count in Cow Milk

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#### ABSTRACT

Milk somatic cells (SCs) are a mixture of milk-producing cells and immune cells. These cells are secreted in milk during the normal course of milking and are used as an index for estimating mammary health and milk quality of dairy animals worldwide. Milk with low SC means better milk products with a longer shelf life. In present study 12 crossbred cows selected and divided in 2 groups. Cows of groups 1 and 2 were supplemented with 0, 500 mg beta carotene (BC) respectively in the lactating period which was continued 30 days of lactation. The average somatic cell counts (SCC) was recorded higher in group-1 (control) ( $0.87 \times 10^5$ / ml) as compared to group-2 (treatment) ( $0.84 \times 10^5$ / ml). The difference was found significant during 4<sup>th</sup> week from supplementation.

#### Keywords

Somatic cells,  
Crossbred cows,  
Beta carotene

#### Introduction

Somatic cells are mostly milk secreting epithelial cells and white blood cells (WBCs). Prescott and Breed (1910) recommended the use of the term 'body cells' because research at that time had recommended that the cells in milk were separate epithelial cells. By the late 1960's, the term 'somatic' (meaning body) cell count became common. Since number of cells in milk is strictly related with inflammation and udder health, these somatic cell counts (SCC) are accepted as the international standard measurement of milk quality [National Mastitis Council (NMC)

Annual Meeting Proceedings, 2001]. There is a straight line relationship between milk loss and SCC (Sharma *et al.*, 2011). High SCC levels in the milk causes deterioration of the milk quality. For instance, use of such poor quality milk in the production of cheese leads to reduced curd firmness, reduced cheese yield, increased fat and casein loss in whey and compromised sensory quality (Ma *et al.*, 2000). These effects would badly affect the yield of milk protein concentrate (MPC), which consist of casein type and whey proteins. Moreover, an elevated SCC score may also lead to shorter (or minimized) shelf life and undesirable milk flavors. Such

changes in milk quality have significant impact on export of milk and milk products and may cause heavy economic loss.

Bindas *et al.*, (1984) conducted a study, in which seventy-eight Holstein cows alternately were assigned at calving to receive 600 mg beta-carotene supplementation and determine effects on cow and found not significantly to improving milk yield & milk fat & SCC in milk and effect of beta carotene not significantly to reduce mastitis. Somatic cells were not different between supplemented and control cows. Wang *et al.*, (1988) conducted a study which comprises fifty-four lactating Holstein cows were assigned supplemented cow with 600 mg beta carotene per day and found not significantly to improving milk yield and milk fat % & SCC in milk.

Oliveira *et al.*, (2015) applied the prepartum supplementation of dairy cows with  $\beta$ -carotene (1.2 g/cow per d). No evidence was observed for positive responses to  $\beta$ -carotene supplementation in colostrums density, milk yield, and milk fat concentration.

However,  $\beta$ - carotene supplementation tended to increase milk protein concentration. A trend was observed for a decreased proportion of primiparous cows with a milk fat to protein ratio greater than 1.5 when  $\beta$ -carotene was supplemented. A weak trend was observed for a reduction in the incidence of SCC greater than 200,000 cells/mL in multiparous cows supplemented with  $\beta$ -carotene.

## **Materials and Methods**

### **Place of work and animals**

The study was carried out on normal cows of Dairy farm, Department of Animal Husbandry & Dairying, Institute of

Agricultural Sciences, Banaras Hindu University, Varanasi (Uttar Pradesh) during lactation period. The animals were kept under normal routine management practices followed at the Institute's dairy farm.

### **Selection of cattle**

Crossbred cows in lactation (before drying) were selected from Institute dairy farm herd. All the cows were free from physiological, anatomical and infectious disorders. Age of all cattle was around 7-8 years.

### **Feeding**

The nutrient requirements of all experimental animals were mostly met with *ad libitum* green fodder and measured amount of concentrate. The green fodders, grown in the Institute farm, were supplied according to the seasonal availability. During summer and rainy seasons predominantly maize and sorghum were fed whereas in winter, fodders like berseem, oat, were fed. Feedings were spread in 3 to 4 feeding during day and night. The concentrate was fed @ 1.5kg/day/ animal for body maintenance in general, for Milking cows were given additional concentrate @ 1.0 kg for every 2.5 kg milk production, above 5.0 kg milk yield. The concentrate to the milking animals was fed in divided allowances during two times milking.

Concentrate mixture had 20% CP and 70% TDN consisted of 33% maize, 21% ground nut cake (oiled), 12% mustered cake (oiled) 20% wheat bran, 11% de-oiled rice bran, 2% mineral mixture and 1% common salt.

### **Treatment details**

Cows of groups I and II were supplemented with 0, 500 mg beta carotene (BC) respectively in the lactating period which was continued 30 days of lactation.

### **Collections of milk samples and recording milk yield**

The milk of cows for analysis was collected during milking and was further analyzed in laboratory. The milk samples were collected from the experimental cows on day 1, 8, 15, 22, and 29.

### **Milk samples**

All Cow milk samples were collected from dairy farm, Institute Of Agriculture Science, BHU Varanasi (UP). Raw milk samples were collected at milking time in a clean and dry container. The samples were kept in chilled condition and transported to the lab, where they are stored. The analysis of milk samples was carried out at Animal Husbandry & Dairying Department, Institute Of Agriculture Science, BHU Varanasi (UP).

### **Preparation of samples for analysis**

Cow milk samples for chemical analysis were prepared as per the method described in BIS Handbook (SP 18: 1981).

### **Chemical and glassware**

During the entire study, Chemicals used for chemical analysis for various properties were of analytical reagent grade and branded glassware's were used after washing them with diluted nitric acid followed by distilled water and drying in an oven.

### **Estimation of somatic cell counts in the milk preparation of milk smears for SCC count**

After collection of milk samples, 5 $\mu$ l of milk was spread on a 1cm<sup>2</sup> Chemicals used (1x1cm) area of a degreased microscopic slide and dried in a horizontal position. The films were air dried and then smears were

defatted with xylene (10 min) and rinsed smoothly with 96% Methanol, air dry and successively dyed with Leishman strain solution for 10 min. The slides were rinsed with water and air dried.

### **Chemicals used**

Xylene

Methanol

Leishman Solution

### **Steps**

Milk samples smeared on a glass slide

Smear dried and dipped in xylene for 10 minutes and dried

Smear was fixed in Methanol for 10 seconds and then dried in air

Dipped in Leishman strain solution for 15 minutes

Washed and air dried

### **Direct microscopic counts**

Somatic cells in the milk smear were counted using a microscope with a magnification of 40X in 10 fields and average number of cells per field was multiplied by the microscopic factor. The microscopic factor was determined by using ocular and stage micrometer. The number of somatic cells in one ml of milk was calculated using the following formula.

SCC / ml of milk (lakh) = Average cells count in on field x microscopic factor.

### **Estimation of microscopic factor**

Magnifications under 40 X

Diameter using ocular micrometer =  $54 \times 0.01 \text{ mm} = 0.54 \text{ mm}$

So, the radius =  $0.54/2 = 0.27 \text{ mm}$ .

Area of one microscopic field =  $\pi r^2$   
=  $\pi \times (0.27)^2 \text{ mm}^2$   
=  $0.2289 \text{ mm}^2$   
=  $0.002289 \text{ cm}^2$

$5 \mu\text{l}$  milk is spread over an area of  $1 \text{ cm}^2$   
So,  $0.002289 \text{ cm}^2 = 1 \text{ field}$   
 $1 \text{ cm}^2 = 1/0.002289 \text{ field}$   
=  $436.8719 \text{ fields}$   
 $5 \mu\text{l}$  milk remains in  $436.8719 \text{ fields}$

$1000 \mu\text{l}$  milk remains in  $436.8719 \times 1000/5$   
fields  
=  $87374.38 \text{ fields}$   
=  $87374.38 \text{ fields}$   
So, microscopic factor =  $0.87374 \text{ lakh}$

### Statistical Analysis

The data obtained during investigation were subjected to statistical analysis using t test to compare difference among group's means for different parameters by using SPSS 16.0 software.

### Result and Discussion

The study was conducted in a commercial dairy herd in dairy farm to evaluate the effect of supplementation of  $\beta$ - carotene on milk yield, milk composition and somatic cell counts (SCC). Experimental procedures were approved by the Department of Animal Husbandry & Dairying, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (Uttar Pradesh). An attempt has been made to summarize the

experimental findings of present study in this chapter under following sub-headings.

### Somatic cell count ( $10^5/\text{ml}$ )

Effect of supplementation of  $\beta$ - carotene somatic cell counts (SCC) is presented in Table 1 and depicted in Fig. 1. It is clearly indicated by the presented data that somatic cell counts (SCC) differed non-significantly by using supplementation of  $\beta$ - carotene. The average somatic cell counts (SCC) was recorded higher in group-1 (control) ( $0.87 \times 10^5/\text{ml}$ ) as compared to group-2 (treatment) ( $0.84 \times 10^5/\text{ml}$ ). The difference was found significant during 4<sup>th</sup> week from supplementation. It is clearly indicated by the present findings that there is no effect of supplementation of  $\beta$ - carotene on somatic cell counts (SCC) of milk. Similar findings were obtained by earlier workers Oldham *et al.*, (1991) and Rakes *et al.*, (1985) who observed that there is no effect on somatic cell counts (SCC) with supplementation of  $300 \text{ mg/d}$  concentration of  $\beta$ - carotene.

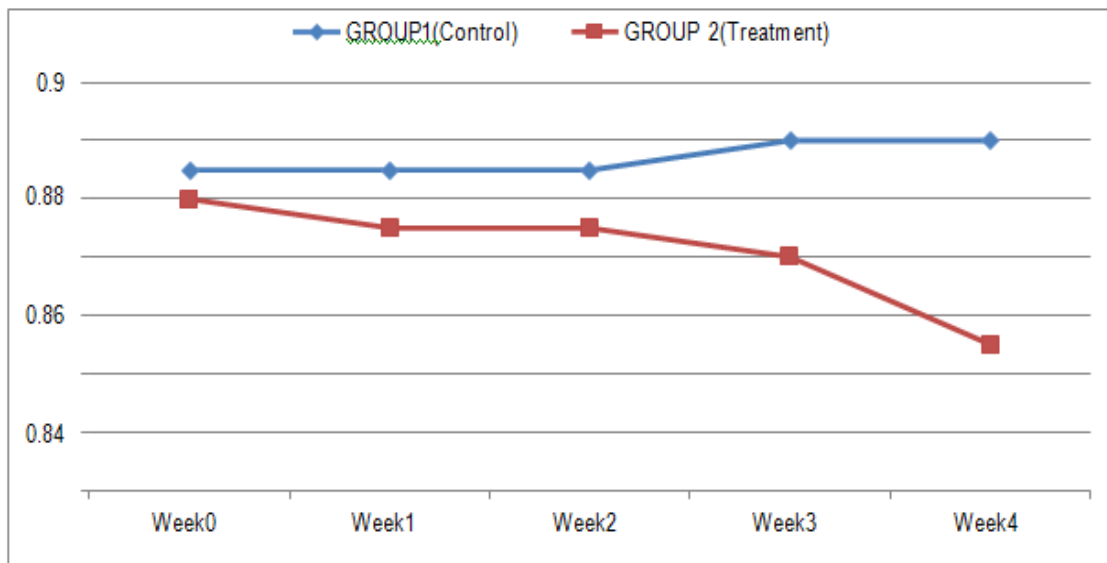
Similarly, Bindas *et al.*, (1984) also observed non-significant effect on somatic cell counts (SCC) when cattle were supplemented with  $600 \text{ mg/d}$  dose of  $\beta$ - carotene. Marcek *et al.*, (1985) conducted a study to determine the effect of feeding  $300 \text{ mg}$  supplemental beta-carotene from 30 days before expected parturition to 98 days postpartum on periparturient. Supplementation had no effect on the somatic cell counts. Oliveira *et al.*, (2015) observed reduction in the incidence of SCC greater than  $200,000 \text{ cells/ml}$  in multiparous cows supplemented with  $\beta$ - carotene. Bian *et al.*, (2007) and Wang *et al.*, (2013) also observed positive effects of  $\beta$ - carotene supplementation before calving on the incidence of mastitis in dairy cows.

**Table.1** Effect of beta carotene supplementation on somatic cell count (SCC) ( $10^5/\text{ml}$ ) in milk

WEEK	MEAN $\pm$ SD		SEM	P VALUE
	GROUP 1 (Control)	GROUP 2 (Treatment)		
0	0.87 $\pm$ 0.12	0.86 $\pm$ 0.09	0.04	0.843
1	0.87 $\pm$ 0.09	0.85 $\pm$ 0.11	0.04	0.696
2	0.87 $\pm$ 0.11	0.85 $\pm$ 0.10	0.04	0.438
3	0.88 $\pm$ 0.10	0.84 $\pm$ 0.08	0.03	0.168
4	0.88 $\pm$ 0.08	0.81 $\pm$ 0.10 <sup>a</sup>	0.03	0.040

Mean bearing different superscript in a column differ non-significantly ( $p < 0.05$ )

**Fig.1** Effect of beta carotene supplementation on somatic cell count (SCC) ( $10^5/\text{ml}$ ) in milk



Ondarza *et al.*, (2009) also reported that a supplement of  $\beta$ -carotene had no effect on SCC. Gonzalo *et al.*, (2009) treated ewes (half-udders) at 75 d postpartum an intramuscular injection of 200 mg  $\beta$ -carotene and they found that SCC significantly decreased during 60 days after  $\beta$ - carotene treatment (single dose).

### Somatic cell count ( $10^5/\text{ml}$ )

The presented data that somatic cell counts (SCC) differed non-significantly by using supplementation of  $\beta$ - carotene. The average somatic cell counts (SCC) was recorded

higher in group-1 (control) ( $0.87 \times 10^5/\text{ml}$ ) as compared to group-2 (treatment) ( $0.84 \times 10^5/\text{ml}$ ). The difference was found significant during 4<sup>th</sup> week from supplementation.

It is clearly indicated by the present findings that there is overall significant effect of supplementation of  $\beta$ - carotene on somatic cell counts (SCC) of milk. Keeping in view the research outcomes summarized above, it can be concluded that the supplementation of  $\beta$ - carotene in the ration of cattle herd resulted in improvement of milk quality in term of decreased in somatic cell counts.

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