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Evaluation of dietary betaineaine supplementation on milk yield and milk composition in Deoni cows

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Abstract
A study was conducted to assess effects of dietary betaine supplementation on milk yield and composition, SCC in lactating Deoni cows. Twelve lactating cows were randomly divided into control and treatment group with six in each group. Control group was fed basal diet alone whereas treatment group was supplemented with anhydrous betaine hydrochloride @ 15 g/ day in the diet during the study period of eight weeks in addition to basal diet. The average daily milk yield improved significantly ($p<0.05$) in treatment group from 5th week; milk fat and solids not fat from 3rd week of after supplementing betaine. There was no significant difference in milk lactose level between two groups during the study period. Somatic cell count in the milk declined significantly ($p<0.05$) in betaine supplemented Deoni cows from 4th week of study. It can be concluded that dietary betaine supplementation to lactating Deoni cows enhances milk yield and improves milk composition and udder health.

Keywords: Betaine, lactating, milk yield, milk fat and somatic cells

1. Introduction
Trimethylglycine (TMG), commonly referred to as betaine, is a derivative of the amino acid glycine and is a molecular chaperone (Sharma *et al*., 2009) [20] which is found in living organisms. It has three reactive methyl groups and a dipole structure with an equal number of oppositely charged ions functional groups hence is a Zwitterion. Betaine can be a nutrient (Craig, 2004) [2], endogenously synthesized through the metabolism of choline, or exogenously consumed through dietary intake. Although betaine concentrations in foods vary depending on cooking and preparation methods, grain products and vegetables such as wheat bran (1340 mg·100 g^{-1}), wheat germ (1240 mg·100 g^{-1}), spinach (600–645 mg·100 g^{-1}), and beets (114–297 mg·100 g^{-1}) are the best sources of dietary betaine (Sakamoto *et al*., 2002) [16]. Betaine increases the ability of cells to retain water and lowers body temperature by lowering the activity of ion pumps required for osmoregulation, which frees up more energy for growth. Betaine is transported across cell membranes utilizing a Na (+)-coupled betaine-specific transporter of the betaine-choline-carnitine transporter family involved in the response to hyperosmotic stress (Perez *et al*., 2011) [11]. Under thermoneutral (TN) conditions, the benefits of adding betaine to lactating dairy cow diets are enhanced VFA production, higher FCM yield, and increased milk production (Wang *et al*., 2010) [23]. Betaine availability to rumen epithelial or other nondigestive tract cell types would be constrained by rumen bacteria use and degradation (Nakanishi *et al*., 1990) [10]. The duodenal digesta contains fed betaine, implying that some betaine escapes the rumen (Nakai *et al*., 2013) [9]. Betaine serves as an excellent natural feed additive in animal nutrition (Rashmi *et al*., 2023) [15].
In order to better understand how dietary betaine supplementation affects lactating Deoni cow’s lactation efficiency. The present study was set out to investigate the effect of dietary betaine supplementation on milk composition, milk yield and somatic cell count in Deoni cows.

2. Material and Methods
2.1 Experimental Design
The current study was carried out in twelve apparently healthy, lactating Deoni cows with age group of 3-8 years and body weight of around 325-350 kgs were selected from Livestock Research and Information Centre (Deoni), Halliked of KVAFSU, Bidar. The study was carried out from October to December 2020 for a period of 2 months.

The average day time temperature ranged from 25 ºC to 27 ºC during the study period. Analysis of milk samples was carried out in Department of Livestock Products Technology of Veterinary College, Bidar. Selected animals were divided into two groups of 6 animals each based on parity and body weight. a. Group I (Control) with Basal diet and b. Group II (Treatment) with Basal diet + Betaine @ 15 g/ day. During the experimental period all the animals were provided with same feeding and managemental inputs as per the standard conditions.

2.2 Estimation of Milk yield and Chemical Composition of Milk

Milking was done twice (5.30 AM and 4.30 PM) a day in all the animals throughout the experiment. Milk yield of individual cows of both morning and evening milking was recorded by using electronic weighing machine every day in the course of experimental period. The milk yield of both morning and evening milking of a day was added to get the daily milk yield. 4% FC M was calculated by using formula (Bouraoui *et al*., 2002) [1].

[(0.4 x kg milk) + (0.15 x kg milk) x fat %)]

At weekly intervals, 50mL milk samples of both morning and evening milking were taken in sterile plastic cups from all the animals for the estimation of milk chemical composition viz., fat, solids not fat (SNF), protein and lactose was estimated by electronic milk analyser. Milk sample was taken in the sample cup and stirred it by using the ultrasonic stirrer. Stirred milk sample was fed to the electronic milk analyser and within 30sec, the printed report of milk component was analysed.

2.3 Determination of Somatic Cell Counts

About 5ml of milk samples were collected in a sterile plastic vial during the middle of the milking for the estimation of somatic cell count. The somatic cell counts of milk samples were determined microscopically by the method as described by Singh and Ludri (2001) [21]. At weekly intervals in the milk obtained from both morning and evening milking. The collected samples were immersed in crushed ice during transport until conducting of necessary laboratory tests (National Mastitis Council, 1990) [10].

2.4 Preparation of Milk Smears

Initially on clean glass slide, 1 cm^2 area was drawn by using diamond marker pen and marked glass slides were washed using warm water and air-dried. Then collected individual milk samples were mixed by vortexing for 10 sec. By using micropipette, about 10 µl of agitated milk sample was taken and spread on marked clean glass slide, smear was prepared and air-dried for 10 min. After air-drying, the smear was stained using Newman Lampert Stain solution (0.5 g methylene blue, 40 ml xylene, 56ml 95% ethyl alcohol and 4ml glacial acetic acid) for 60–90 sec. After staining slides were washed with distilled water and air dried, somatic cell counts were determined under oil immersion objective.

2.5 Counting of somatic cells

Examination of milk smear was done at random. One square cm area of smear was divided into four equal parts by dividing it at the right angle. Cells were counted in five fields from each divided area. Thus, the cells were counted in total 20 fields. The average number of cells per sq cm area was calculated. For counting of cells per ml of milk, the average number of cells per field was multiplied by multiplication factor.

Derivation of common microscopic factor, volume factor and multiplication factor Microscopic factor: Common microscopic factor was determined as per Prescott and Breed (1910) [13] as under:

Diameter of an oil immersion microscopic field of Olympus microscope used in study

D= 0.1 cm.

Radius of oil immersion microscopic factor = 0.01 cm.

Area of oil immersion microscopic field = πr^2

= 22/7 × (0.01)2 cm^2

= 31.42 × 10^{-5} cm^2

No. of fields in 1 cm2 area=1cm/Area of an oil immersion microscopic field = 1/31.42× 10^{-5} = 3182.68

Volume Factor: 10µl of milk is used for the preparation of smear whereas SCC is expressed as number of cells present in 1ml (1000 µl) of milk. Therefore, the correction factor for volume is 100

Multiplication Factor:

Multiplication factor = Microscopic factor X Volume factor

= 3182.68 X 100

= 318268

Calculation of Somatic Cell Count

If, number of cells counted in 20 fields = X

Then total numbers of cells in 1 cm^2 area are = X/20 x 318268

Here =X/20 = Average No. of cells in 20 field of 1 cm^2area

Total number of cells in 1 ml of milk = Avg. No. of cells in 20 fields of 1 cm^2 area×318268

3. Results and Discussion

3.1 Milk yield and Fat Corrected Milk (4% FCM)

In the present study, the average daily milk yield and Fat Corrected Milk (4% FCM) in the treatment group were significantly (p<0.05) higher as compared to that of control group at 5th, 6th, 7th and 8th week of study period. Along the study period, in treatment group, the average daily milk yields from 5th week to 8th week and 4% FCM from 4th week to 8th week were significantly higher (p<0.05) compared to milk yield before the betaine supplementation. The results obtained in the present study are in agreement with the studies of Wang *et al*. (2010) [23], Peterson *et al*. (2012) [12], Monteiro *et al*. (2017) [7], Shankhpal *et al*. (2019) [19] and Shah *et al*. (2020) [17] in lactating cows, who reported dietary supplementation of betaine improved milk yield significantly compared to those of control cows. Fernández *et al*. (2004) [4] in Murciano-Granadina goats and Tsiplakou *et al*. (2017) [22] in lactating ewes who observed higher milk yield in groups fed with betaine than that of control animals. It is postulated that when betaine is supplemented, rumen fermentation decreases betaine concentrations, with an increase in acetate concentrations (Mitchell *et al*., 1979) [6]. An increase in acetate was shown to increase the rate at which milk is synthesized in some studies (Purdie *et al*., 2008) [14] However, Wang *et al*. (2019) [24] and Fernández *et al*. (2009) [5] were of the opinion that betaine supplementation in cows and goats did not have any detectable effect on milk yield among treatments than that of control groups.

3.2 Somatic Cell Count

As per observations, the somatic cell count (x 105) in the treatment group were significantly lower compared to that of control at 5th, 6th, 7th and 8thweek of study period. Along the study period, in treatment group, the somatic cell count at 7th week and 8th week were significantly lower compared to levels at 0 day and 1st week of study period. The results obtained in the present study are in agreement with the studies of Shah *et al*. (2020) [17] in lactating cows, who reported dietary supplementation of betaine decreased milk SCC significantly compared to those of control cows. However, Peterson *et al*. (2012) [12] in cows and Fernández *et al*. (2004) [4] and Fernández *et al*. (2009) [5] in goats have reported that betaine supplementation did not have any detectable effect on milk SCC among treatments and that of control groups.

Table 1: Mean Milk yield, 4% Fat corrected milk, Somatic cell counts in control and dietary betaine supplemented lactating Deoni cows (n=6).

Parameter	Milk Yield (Kg/Day)		4% Fat corrected Milk (Kg/Day)		Somatic cell count (x 10^5/ml)	
Period /Group	Control	Treatment	Control	Treatment	Control	Treatment
0 day	2.57±0.11^{xa}	2.54±0.13^{xa}	2.33±0.10^{xa}	2.33±0.08^{xa}	1.80±0.08^{xa}	1.79±0.05^{xc}
1st week	2.51±0.16^{xa}	2.60±0.12^{xa}	2.28±0.13^{xa}	2.45±0.09^{xab}	1.83±0.05^{xa}	1.73±0.06^{xbc}
2nd week	2.49±0.17^{xa}	2.73±0.18^{xab}	2.28±0.14^{xa}	2.67±0.16^{xabc}	1.85±0.04^{xa}	1.70±0.05^{xbc}
3rd week	2.48±0.15^{xa}	2.79±0.13^{xab}	2.28±0.14^{xa}	2.76±0.13^{xbc}	1.81±0.07^{xa}	1.64±0.06^{xbc}
4th week	2.48±0.11^{xa}	2.91±0.16^{xab}	2.28±0.13^{xa}	2.89±0.13^{ycd}	1.85±0.06^{ya}	1.61±0.03^{xb}
5th week	2.51±0.10^{xa}	3.11±0.12^{ybc}	2.27±0.11^{xa}	3.09±0.12^{ycd}	1.84±0.04^{ya}	1.60±0.03^{xab}
6th week	2.58±0.11^{xa}	3.22±0.14^{ybc}	2.39±0.11^{xa}	3.25±0.15^{yde}	1.83±0.04^{ya}	1.56±0.05^{xab}
7th week	2.50±0.13^{xa}	3.22±0.14^{ybc}	2.28±0.12^{xa}	3.24±0.12^{yde}	1.85±0.04^{ya}	1.52±0.05^{xa}
8th week	2.54±0.14^{xa}	3.32±0.17^{yc}	2.35±0.13^{xa}	3.38±0.16^{ye}	1.86±0.09^{ya}	1.51±0.04^{xa}

*Means with different superscripts for a particular parameter within a row (x, y) and within a column (a, b, c, d, e) differ significantly ($p<0.05$).

3.3 Milk Components

The betainine supplemented group showed significantly ($p<0.05$) higher milk fat (%), Solids-Not-Fat (%),Milk Protein (%) and Milk Lactose (%) levels when compared to that of control at 5th, 6th, 7th and 8th week of study period. Along the study period, in treatment group, the milk fat (%) and Solids-Not-Fat (%) level from 2nd week to 8th week were significantly ($p<0.05$) higher as compared to levels at 0 day, whereas no significant differences were found in control group along the study period. The results obtained in the present study are in agreement with the studies of Wang *et al*. (2010) [23], Monteiro *et al*. (2017) [8], Shankhpal *et al*. (2019) [19] and Shah *et al*. (2020) [17], who reported dietary supplementation of betaine in lactating cows improved milk fat, milk protein and SNF (Shankhpal *et al*. 2019) [19] but did not show any significant effect on milk lactose levels significantly compared to those of control cows. Similar results were noted by Fernández *et al*. (2004) [4] in Murciano-Granadina goats and Tsiplakou *et al*. (2017) [22] in lactating ewes who also reported higher milk fat in groups fed with betaine than that of control animals. However, Davidson *et al*. (2008) [3], Peterson *et al*. (2012) [12] and Zhang *et al*. (2014) [25] were of the opinion that milk fat percentage did not differ among treatments by betaine supplementation and that of control groups. Monteiro *et al*. (2017) [7] were of the opinion that milk lactose percentage tended to be decreased by betaine supplementation.

Table 2: Mean Milk fat, Milk solids not fat, Milk Protein and Milk lactose levels in control and dietary betaine supplemented lactating Deoni cows (n=6).

Parameter	Milk Fat (%)		Milk Solids not Fat (%)		Milk Proteins (%)		Milk Lactose (%)	
Period /Group	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
0 day	3.37±0.08^{xa}	3.47±0.10^{xa}	8.62±0.03^{xa}	8.63±0.04^{xa}	3.20±0.03^{x}	3.27±0.03^{x}	4.85±0.02	4.87±0.05
1st week	3.40±0.10^{xa}	3.62±0.09^{xab}	8.60±0.03^{xa}	8.78±0.05^{xab}	3.18±0.03^{x}	3.27±0.04^{x}	4.88±0.03	4.93±0.07
2nd week	3.45±0.09^{xa}	3.87±0.14^{xbc}	8.65±0.06^{xa}	8.85±0.08^{xb}	3.22±0.05^{x}	3.28±0.03^{x}	4.65±0.09	4.93±0.20
3rd week	3.45±0.06^{xa}	3.92±0.16^{ybc}	8.67±0.06^{xa}	8.95±0.05^{yb}	3.23±0.02^{x}	3.38±0.03^{x}	4.92±0.02	4.98±0.07
4th week	3.43±0.10^{xa}	3.98±0.20^{ybc}	8.68±0.07^{xa}	8.95±0.02^{yb}	3.13±0.04^{x}	3.32±0.07^{x}	4.77±0.07	5.02±0.19
5th week	3.36±0.06^{xa}	3.96±0.18^{ybc}	8.62±0.04^{xa}	8.94±0.09^{yb}	3.21±0.03^{x}	3.37±0.04^{y}	4.87±0.09	5.06±0.13
6th week	3.52±0.09^{xa}	4.07±0.11^{yc}	8.60±0.03^{xa}	8.95±0.04^{yb}	3.20±0.04^{x}	3.38±0.05^{y}	4.90±0.07	5.10±0.11
7th week	3.42±0.07^{xa}	4.05±0.13^{yc}	8.62±0.03^{xa}	8.91±0.07^{yb}	3.20±0.04^{x}	3.37±0.04^{y}	4.92±0.05	4.90±0.07
8th week	3.50±0.07^{xa}	3.47±0.10^{yc}	8.60±0.03^{xa}	8.95±0.09^{yb}	3.18±0.02^{x}	3.38±0.03^{y}	4.97±0.03	4.87±0.10

*Means with different superscripts for a particular parameter within a row(x, y) and within a column (a, b, c) differ significantly ($p<0.05$).

4. Conclusion

Dietary betaine supplementation @ 15 g/day/animal significantly ($p<0.05$) enhances the average daily milk yield in lactating Deoni cows. The betaine supplementation alters the milk composition by increasing the levels of fat, SNF and protein levels without affecting the lactose percentage in milk. Betaine supplementation improved the udder health in lactating Deoni cows as indicated by reduced the SCC in milk.

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