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RESEARCH ARTICLE

Combined effect of trisodium citrate and vitamin E supplementation during the transition period on body weight and other production parameters in Sahiwal cows

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Abstract: The present study was carried out to investigate the combined effect of supplementation of trisodium citrate (25 gm) and vitamin E (1000 IU)/ animal/ day during the transition period in Sahiwal cows. A total of 30 healthy multiparous cows were selected and randomly divided into two groups, i.e. control and treatment. Body weight(BW), body condition score (BCS), dry matter intake (DMI) and DMI/100 kg body weight of cows were estimated starting from three weeks before to three weeks after calving. Milk samples were collected at day 1, 3, 7, and then at weekly interval up to day 56 postpartum for assessment of various milk parameters. The BW, BCS, DMI, and DMI/ 100 kg body weight of cows were significantly ($P < 0.05$) higher in treatment as compared to control group. The DMI and DMI/ 100 kg body weight were minimum at the time of calving and increased around 3rd week postpartum. Milk somatic cell counts (SCC), pH and electrical conductivity (EC) were lower in the treatment as compared to the control group. However, milk yield, fat, lactose and solid not fat (SNF) were higher in the supplemented cows as compared to the control. Milk SCC, pH, EC, fat, protein, and SNF levels were maximum during the colostrum phase in both groups and declined to normal levels afterwards. However, milk lactose levels followed an inverse pattern to that of fat and protein. Our findings reveal that the supplementation of trisodium citrate and vitamin E during the transition period can improve body condition score and productivity of indigenous dairy cows.

Keywords: Calving, Colostrum, Early lactation, Body condition score, Somatic cell counts, Milk composition

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Introduction

The udder health, milk yield and body condition of dairy animals determine the profitability of herd, and thus it needs dynamic, and time-dependent management approaches, especially feeding management during the transition period (Esposito *et al.,* 2014). The main problem after calving in dairy cattle is the instability between the body reserves and nutrients requirements for milk production (Kuhla *et al*., 2011). Major metabolic changes around this period are also associated with the initiation and rapid increase of milk production (Dobbelaar *et al.,* 2010). The abruptly increased metabolic rate associated with late pregnancy, parturition, and initiation of lactation results into a greater stress exposure which contribute to periparturient disorders in the dairy cows (Sordillo and Aitken, 2009). There is a decrease in dry matter intake (DMI) about 1 to 2 weeks before calving which cannot simultaneously meet the nutrient demand of the growing fetus and lactogenesis (Grummer, 1995). Therefore, a gap in nutrient demand occurs which has adverse effects on the nutrient metabolism causing different metabolic disorders and decreased productivity. Provision of adequate mineral and vitamin supplementation during the peripartum period can be used as a strategy to enhance the milk quality and quantity as well as at the same time maintaining DMI and body condition score (BCS) of transition animals (Weiss and Wyatt, 2002; Cortinhas *et al.,* 2010).

Trisodium citrate has buffering and antimicrobial properties which can maintain pH of milk (~6.6) and prevent the formation of calcium ion crystals and thus cure mastitis and improve milk quality (Shennan and Peaker, 2000; Yousaf *et al.,* 2010). Vitamin E is a universally accepted potent fat soluble antioxidant which has a positive impact on health, production and growth performance of dairy animals (Dang *et al.,* 2013; Chandra *et al*., 2013*).* Many workers have studied either the effect of trisodium citrate (Stumpf *et al.,* 2013; Prakash *et al*., 2013; Mbonwanayo *et al*., 2016) or vitamin E (Politis *et al*., 2004; Chandra *et al*., 2013) supplementation on production performance of dairy animals. However, the combined effect of these supplements has not been studied in indigenous dairy cow around the transition period. Therefore, the current study was undertaken to see the effect of combined supplementation of trisodium citrate (25gm) and vitamin

E (1000 IU)/ animal/ day during the transition period on body conditions as well as milk yield and quality of indigenous Sahiwal cows. The doses of vitamin E and trisodium citrate fed to the cows in this study were fixed based on earlier studies (Chandra *et al*., 2013; Dhillon and Singh, 2013).

Materials and Methods

Ethical permission

The guidelines for animal experiments outlined by the Institutional Animal Ethics Committee which approved this study and the ethical guidelines of the National Dairy Research Institute (NDRI), Karnal were followed during all the experiment.

Location and climatic conditions of the study area

This study was conducted at Livestock Research Centre (LRC) of NDRI, Karnal, Haryana, India. This institute is located at 290 43' N latitude and 760 58' E longitudes at an altitude of 245 meters above the mean sea level in the bed of Indo-Gangetic alluvial plain. The experiment was conducted from September-2016 to February-2017. The meteorological variables data during the study period were collected from Central Soil Salinity Research Institute and have been presented in Table 1. All the climatic variables were recorded twice daily at 7.30 A.M. and 2.30 P.M; however, we are presenting the average value of each climatic variable during each month.

Selection of animals, health, housing and feeding management

For the present study, thirty peripartum healthy Sahiwal cows were randomly selected from LRC of NDRI, Karnal and divided into two groups of fifteen animals each. All of the selected cows were multiparous (2–4) parity. During the experimental period, routine health management of the farm was followed and animals were having any health disorder were excluded from the study. The cows were kept under asbestos roofing in a well-ventilated stall. They were shifted from dry pregnant cow paddocks (brick on edge flooring) to the calving pen just 1 week before the expected date of calving and retained till 4–5 days after calving. The control cows were fed as per standard practices followed at LRC, NDRI, Karnal for transition animals. The ingredients of total mixed ration fed to the animals were as following berseem fodder (20 %), ground yellow maize (28.7 %), corn silage (14.5 %), de-oiled mustard cake (4.9 %), groundnut cake (16 %), rice bran (8.4 %), wheat bran (6.5 %), dicalcium phosphate (0.7 %), and salt (0.3 %). However, the treatment group has been offered a similar diet of that of control group along with a mixture of trisodium citrate (25gm) and vitamin E (1000 IU)/ animal/ day, during the transition period, 3 weeks before calving to 3 weeks after calving at the morning time.

Milk collection and analysis

Composite milk samples representing all the quarters of mammary gland were collected into sterile milk bottles (100 ml/cow). Both hand and machine milking were used for the collection of milk samples from the experimental cows. Hand milking was performed during the colostrum phase; however, machine milking was performed during the rest of the experiment. Milk samples were checked for somatic cell counts (SCC), pH, electrical conductivity (EC), and composition, i.e. fat, protein, lactose and solid not fat (SNF) percentage by using Lactoscan automatic analyzer (Bulgaria). All these parameters were estimated at day 1, 3, 7 and then at weekly interval up to day 56 postpartum. The milk samples were maintained at 28-32^{0}C at the time of analysis, which is the calibration temperature of the analyzer. The milk yield was immediately recorded during milking using a sensitive balance.

Body weight and BCS measurement of Sahiwal cows

The fortnight body measurements of each animal were recorded early in the morning between 7:30 to 8:30 am consecutively for two days on a platform of electrical balance with high accuracy before providing the animal with any feeding stuff and water. Thus each observation on body weight was based on an average of two observations. The BCS of the animals was estimated on weekly interval following the BCS card of lactating cows which was developed by Aggarwal *et al.* (2010).

Dry matter intake **(**DMI**)** and DMI/ 100 kg body weight

The DMI of each group was recorded at weekly interval on two consecutive days during which weighed amount of concentrate mixture, green fodder and roughages were offered daily, and residue weighed, and DM determined. The DM content of fresh as well as leftover of concentrate mixture and green fodder was estimated by drying the sample in the electric oven at 100±10°C for 24 hours. DM of each group was calculated using the following formula.

DMI = DM of the feed offered - DM of residual feed

DMI/ 100 kg body weight = DMI / body weight of animal (kg) ×100

Statistical analysis

All the data has been presented as mean ± SEM. Statistical analysis was done by one-way ANOVA test for between days within the same group and repeated measures two-way ANOVA (mixed model) for between groups analysis. This followed by Duncan multiple range test (DMRT) comparison test using SAS software, version 9.1 of SAS system for window, copyright © (2011), SAS Institute Inc., CARY, NC, USA. The difference at P *d"* 0.05 was considered to be statistically significant.

Results and Discussion

The results related to the changes observed in the body weight, BCS, DMI, and DMI/ 100 kg body weight during the transition period of Sahiwal cows have been presented in Table 2. Body weight of dam displayed a gradual increment till the day of calving as most of the fetus's growth occurs during the last trimester of the gestation period. After that, it reduced dramatically after calving due to the expulsion of the fetus, fetal fluids and fetal membrane and stress experienced during calving which is similar to the findings reported by Brar and Nanda (2007). We also noticed that the body weight of treatment cows was numerically high as compared to control cows but there was no significant difference. However, Khan *et al.* (2015) reported that vitamin E supplemented to Murrah buffaloes during the transition period had a higher body weight gain compared to control group.

The average value of BCS at the day -21 before calving was almost similar in both control and treatment groups. It increased linearly over the time till the date of calving then gradually decreased with the progress of lactation. Vitamin E has an antioxidant characteristic for preventing of free radicals action in tissue which improves health status, mitigates stress effects and ultimately increases feed intake and productivity of cows (Chauhan *et al*., 2016). Moreover, declining DMI around parturition can be compensated by increasing dietary mineral concentration such as sodium, calcium and selenium which improve animal appetite and feed intake during this critical period (Tahmasbi *et al*., 2012). In agreement with the above cited literature, we have also observed a higher scale of BCS in the treatment group as compared to control group as vitamin E and trisodium citrate were helpful in enhancing the DMI and ultimately BCS of supplemented cows. Similar findings have been reported by Chandra *et al*. (2013) in Sahiwal cows supplemented with vitamin E and zinc during the transition period. However, Stumpf *et al.* (2013) reported no difference between BCS of sodium citrate supplemented Jersey cows as compared to the control group.

The DMI depression 2-3 weeks before parturition is well documented (Marquardt *et al*., 1977). Hayirli *et al.* (2003), reported that DMI usually decreases with the progress of gestation till the day of calving and then it gradually increases during lactation, which is similar to our findings. Our results are also in close agreement with Chandra *et al*. (2013), who has also supplemented vitamin E in Sahiwal cows while in contrary with Panda and Kaur (2008) who has supplemented vitamin E in Murrah buffaloes. We observed higher DMI and DMI/100 kg body weight in the treatment group as compared to the control group which might

Table 1 Meteorological variables data recorded from September- 2016 to February- 2017

		Temperature (°C)				
Year	Month	Max	Min	Dry bulb	Wet bulb	Relative humidity (%)
2016	September	33.5 ± 1.90^{c}	22.7 ± 0.91^{e}	31.5 ± 1.95^{d}	26.2 ± 0.90^{d}	63.0 ± 3.39^{d}
	October	31.0 ± 1.73^{c}	17.6 ± 0.86^{d}	31.0 ± 1.80^{d}	21.6 ± 0.85^{c}	46.0 ± 2.66^{b}
	November	25.1 ± 1.83^{b}	11.4 ± 0.74^{c}	27.0 ± 1.66^{c}	17.2 ± 0.63^{b}	39.0 ± 2.24^{a}
	December	18.2 ± 1.34^{ab}	06.6 ± 0.55^{a}	19.7 ± 1.33^{b}	14.8 ± 0.41^{a}	54.0 ± 2.98^{c}
2017	January	16.3 ± 1.44^{a}	06.0 ± 0.50^{a}	15.7 ± 1.38^{a}	13.7 ± 0.92^{a}	68.0 ± 3.64^{d}
	February	21.4 ± 1.58^{b}	08.3 ± 0.61^{b}	22.0 ± 1.46^{b}	15.6 ± 0.75^{a}	51.0 ± 2.45^{bc}

Values are expressed as mean ± SE. Values within a column with different superscript letters (a, b, c, d, e) differ significantly at $P < 0.05$. They are arranged alphabetically from the smallest value to the biggest value

Table 2. Body weight (BW), body condition score (BCS), dry matter intake (DMI), and DMI/ 100 kg BW of two different groups of Sahiwal cows, i.e. not supplemented (control) and supplemented (treatment) with mixture of trisodium citrate (25gm) and vitamin E (1000 IU)/ day during transition period

Parameters	Groups/ Days	-21	-14	-7	0	+7	+14	+21
BW (kg)	Control	417.97 ± 10.12—		420.80 ± 10.34––		398.76 ± 8.90 ––		390.66 ± 12.24*
	Treatment	419.68 ± 8.87 ––		423.72 ± 8.26 ––		400.32 ± 9.73 ––		392.62 ± 10.75*
BCS	Control	3.2 ± 0.16	3.22 ± 0.29	3.25 ± 0.39	3.34 ± 0.16	3.12 ± 0.35	$2.94 \pm 0.13^{*}$	$2.87 \pm 0.23^{*}$
	Treatment	3.15 ± 0.23	3.36 ± 0.28	3.42 ± 0.278	$3.51 \pm 0.12^{*}$	$3.42 \pm 0.20^{*}$	$3.3 \pm 0.10^{\#}$	$3.0 \pm 0.18^{\#}$
DMI	Control	9.39 ± 0.28	9.17 ± 0.29	8.70 ± 0.38*	8.13 ± 0.12*	8.90 ± 0.11	10.94 ± 0.13*	11.51 ± 0.57*
	Treatment	9.44 ± 0.31	9.21 ± 0.31	9.09 ± 0.11	$8.61 \pm 0.16^{*\#}$	9.3 ± 0.61	$12.19 \pm 0.27^{*\#}$	$13.19 \pm 0.19^{*\#}$
DMI/100 kg BW	Control	2.24 ± 0.19	2.18 ± 0.12	2.06 ±0.25	$1.98 \pm 0.17^{*}$	2.23 ± 0.31	$2.77 \pm 0.11^{*}$	$2.94 \pm 0.22^{*}$
	Treatment	2.24 ± 0.20	2.18 ± 0.10	2.14 ± 0.21	2.08 ± 0.28	2.32 ± 0.28	$3.07 \pm 0.13^{*\#}$	$3.35 \pm 0.11^{*\#}$

Values are expressed as mean ± SE. * indicates that the values are significantly ($P < 0.05$) different within the same group compared to day -21 and # indicates that the values are significantly ($P < 0.05$) different between the groups, i.e. control and treatment within the same day.

Table 3 Milk yield (kg/day/animal), somatic cell counts (SCC) ($\times 10^5$ cells/ml), pH, and electrical conductivity (EC) of two different groups of Sahiwal cows, i.e. not supplemented (control) and supplemented (treatment) with mixture of trisodium citrate (25 gm) and vitamin E (1000 IU)/ day during the transition period

Parameters	Groups/ Days	1	3	7	14	21	28	35	42	49	56
Milk yield	Control	––	––	6.34 ±0.30	6.41±0.28	6.60±0.37	6.70±0.22	6.86±0.36	7.12±0.27*	7.38±0.16*	7.89±0.26*
	Treatment	––	––	6.50±0.35	6.54±0.37	6.75±0.23	6.82±0.26	7.25±0.45	7. 50±0.20*	7.68±0.48*	8.56±022*#
SCC	Control	5.08±0.12	5.04±0.23	2.93±0.12*	2.43±0.34*	2.39±0.29*	2.36±0.18*	2.4±0.20*	2.45±0.24*	2.59±0.17*	2.65±0.15*
	Treatment	4.56±0.14#	4.48±0.24#	2.31±0.21*#	2.28 ±0.11*	2.22±0.15*	2.00±0.12*	1.87±0.21*#	1.89±0.11*#	2.02±0.12*#	2.23±0.15*#
pH	Control	6.98±0.02	6.96±0.03	6.78±0.02*	6.72 ±0.08*	6.69±0.01*	6.68±0.02*	6.7±0.05*	6.82±0.03*	6.85±0.06*	6.87±0.09
	Treatment	6.90 ±0.01#	6.87±0.02#	6.70±0.04*	6.67±0.03*	6.66 ±0.04*	6.65±0.07*	6.67±0.04*	6.68±0.03*#	6.69±0.04*#	6.67±0.05#
EC	Control	6.35±0.10	6.2±0.12	5.89±0.13	5.78±0.09*	5.77±0.12*	5.65±0.12*	5.59±0.12*	5.69±0.10*	5.8±0.14*	5.82±0.13*
	Treatment	6.10±0.10#	5.86±0.11#	5.80±0.14	5.75±0.08*	5.72±0.13*	5.61±0.13*	5.54±0.14*	5.57±0.07*#	5.61±0.03*#	5.60±0.07*#

Values are expressed as mean ± SE. * indicates that the values are significantly ($P < 0.05$) different within the same group compared to day 7 for milk yield and compared to day 1 for other parameters. # indicates that the values are significantly ($P < 0.05$) different between the groups, i.e. control and treatment within the same day

Table 4. Fat, protein, lactose and solid not fat (SNF) percentage in colostrum and milk samples of two different groups of Sahiwal cows, i.e. not supplemented (control) and supplemented (treatment) with the mixture of trisodium citrate (25 gm) and vitamin E (1000 IU)/ day during transition period

Parameters	Groups / Days	1	3	7	14	21	28	35	42	49	56
Fat	Control	4.90±0.16	4.73±0.14	4.0±0.12*	4.12±0.13*	4.2±0.13*	4.35±0.18*	4.40±0.18*	4.5±0.16*	4.50±0.17*	4.54±0.11*
	Treatment	5.23±0.15#	5.11±0.11#	4.23±0.18*	4.32±0.18*	4.40±0.13*	4.60±0.15*#	4.65±0.09*#	4.70±0.06*#	4.78±0.03*#	4.80±0.09*#
Protein	Control	15.35±0.74	14.35±0.10	3.85±0.27*	3.82±0.19*	3.40±0.13*	3.56±0.26*	3.82±0.15*	3.90±0.22*	3.95±0.12*	3.96±0.11*
	Treatment	15.78±0.13	15.23±0.12	3.45±0.15*	3.36±0.18*	3.20±0.16*	3.36±0.17*	3.49±0.16*	3.65±0.17*	3.68±0.26*	3.78±0.23*
Lactose	Control	2.50±0.23	2.72±0.40	3.35±0.37*	4.25±0.13*	4.35±0.13*	4.54±0.15*	4.61±0.28*	4.66±0.18*	4.65±0.18*	4.68±0.32*
	Treatment	2.55±0.30	2.84±0.26	3.50±0.10*	4.45±0.02*#	4.60±0.10*#	4.66±0.24*	4.68±0.34*	4.70±0.21*	4.75±0.21*	4.72±0.31*
SNF	Control	16.09±0.84	15.42±0.54	7.5±0.26*	7.56±0.42*	7.76±0.36*	7.82±0.33*	7.87±0.51*	7.96±0.29*	8.26±0.30*	8.45±0.47*
	Treatment	16.55±0.13	16.23±0.10	8.34±0.43*	8.52±0.40*	8.49±0.23*	8.54±0.29*	8.59±0.47*	8.62±0.31*	8.67±0.21*	8.68±0.35*

Values are expressed as mean ± SE. * indicates that the values are significantly ($P < 0.05$) different within the same group compared to day 1 and # indicates that the values are significantly ($P < 0.05$) different between the groups, i.e. control and treatment within the same day

be due to the lower metabolic stress and improved health status of vitamin E supplemented cows.

The results related to the weekly changes in milk yield, milk SCC, pH, and EC during the early lactation stage have been presented in Table 3. The milk yield of the treatment group was always higher than that of the control group, but significant ($P<0.05$) difference was observed only at day 56 postpartum. We observed the lowest milk yield at the first week of lactation in both groups and then increased gradually with the progress of lactation and attained maximum milk yield at day 42 onward which is in close agreement with Mukherjee *et al*. (2017). Moreover, the higher milk yield of treated cows as compared to the control group could be due to good udder health, higher DMI and higher BCS in the treatment group.

The average milk SCC, pH and EC were maximum in colostrum samples in both groups. However, significant ($P<0.05$) decline was noticed at day 7 and onwards in both groups and remained almost constant with the progress of lactation which is in agreement with many workers (Dang *et al.,* 2008; Hagnestam-Nielsen *et al.,* 2009; Alhussien *et al.,* 2016b). Any increase in the number of SCC above 2.5 lacks indicates high levels of mammary stress and big chances of mammary infection in indigenous Tharparkar and Sahiwal cows (Alhussien *et al*., 2016a; Alhussien and Dang, 2017). The higher level of milk SCC, pH, and EC in colostrum as compared to milk might be due to the sudden transition of the mammary gland from non-lactating to the lactating stage (Lee *et al.,* 1980). In our experiment, the treatment group has shown lower milk SCC, pH, and EC levels as compared to the control group. This is because citrate plays a buffering role inside the mammary glands and it regulates the homeostasis between Ca2+ and H+ ions and supports the fluidity of milk through its effect on casein micelles (Shennan and Peaker, 2000).

The results of the average fat, protein, lactose, and SNF percentage in colostrum and milk have been presented in Table 4. The average fat, protein, and SNF percentage were highest during the colostrum phase in both groups and then declined significantly ($P<0.05$) and reached normal levels in milk at day 7 postpartum. Afterward, fat percentage again increased gradually up to day 56 in milk while the percentage of protein and SNF remained constant. On the contrary to this lactose percentage was lowest during the colostrum phase, increased gradually till day 14 and remained constant up to day 56 postpartum. Fat percentage was higher in the treatment group of cows while statistically significant ($P<0.05$) difference was observed from day 28 to day 56 in milk, which is in close agreement with Sharma *et al*. (2014) who has supplemented trisodium citrate in dairy animals. This is because citrate is an essential for the tricarboxylic acid cycle and indirectly contributes to the synthesis of fat by providing reducing equivalents in the form of nicotinamide adenine dinucleotide phosphate (Garnsworthy *et al.,* 2006). The higher levels of lactose and SNF and the lower level of protein content in the milk of the treated cows might be because that the control cows had higher SCC as compared to the supplemented cows.

Conclusions

Our findings indicate that the combined supplementation of trisodium citrate and vitamin E can mitigate the stress of the transition period by increasing DMI which ultimately improves BCS and increase body reserve needed to meet the energy demand at the time of calving. Moreover, the supplementation helps in improving milk quality and udder health which is reflected by higher lactose, fat and SNF percentage and lower SCC, pH and EC levels in the milk samples of supplemented cows.

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