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Changes in the Composition of Colostrums, Transition Milk and Milk of Crossbred Cows due to Transition Period Supplementation of Vitamin A, E and Zinc

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ABSTRACT

The present study was undertaken with the objective to study the effect of transition period supplementation of Vitamin A, E and Zinc on composition of colostrums, transition milk and milk of cross bred cows. Thirty-five cross-bred advanced pregnant cows and divided into 5 groups of 7 each at 30 days prior to calving were selected from the NDRI experimental herd. The cows were supplemented individually with Vitamin A (T1) (100000 IU/animal/day), Zinc (T2) (60 ppm/animal/day), Vitamin E (T3) (2500 IU/animal/day) and combination (T4) of all to study cumulative effect of all micronutrients. One group without supplementation acted as control (T0). Colostrum (up to 4th day postpartum), transition milk (day 5 to day 7) and milk (day 8 to day 30) were collected and estimated for milk composition and SCC of the experimental crossbred cows. Total solid percentage in colostrums was significantly ($p<0.05$) higher in all supplemented groups compared to control . Significantly higher ($p<0.05$) colostrum fat percentage in T3 and T4 compared to T0, T1 and T2. Significantly higher ($p<0.05$) colostral protein percentage in T4 group from day 1 to day 4 compared to T0, T1, T2 and T3. Significantly higher transition milk and milk total solid percentage, fat percentage and protein percentage value ($p<0.05$) in T4 group when compared with T0, T1, T2 and T3 groups. It can be concluded that Vitamin A, Vitamin E and Zinc when supplemented to peripartum crossbred cows during the transition period improves the nutritional quality of colostrums and milk.

HIGHLIGHTS

- Supplementation of micronutrients showed significant increase in colostrums total solid percentage, fat percentage and protein percentage.
- Supplementation of micronutrients showed significant increase in transition milk and milk total solid percentage, fat percentage and protein percentage.
- Vitamin A, vitamin E and zinc when supplemented to peripartum crossbred cows during the transition period improves the nutritional quality of colostrums and milk.

Keywords: colostrums, crossbred cows, transition period, micronutrients, milk

Transition period, the most critical time period of a dairy cow during its life time in which a pregnant, non-lactating dairy cow transit to a non-pregnant lactating stage accompanied by a drastic change in its physiological status and to enhance the performance of a dairy cow during this period micronutrient plays an important role (Singh *et al.,* 2020). The mammary gland, a modified sweat gland found in female mammals is underdeveloped at birth in both males and females and develops as a secondary sex

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characteristic in females on attaining puberty. It reaches full size and begins producing milk with the birth of the first child (Alhussien and Dang, 2018). A thick, creamy-yellow, sticky milk produced by cows immediately after calving and up to 3-4 days (Pandey *et al.,* 2021) is colostrum. In this study colostrum had been considered for 4 days.

Various environmental and genetic factors influencing quality and composition of colostrums are, animal factors (breed, Individuality, parity, illnesses, dry period length of cows, postpartum time) and environment factors (milking time, prepartum nutrition, season) (Arslan *et al.,* 2021). Colostrum contains more fat, protein, ash, vitamins and minerals, growth factors cytokines, which gradually decrease in the first three days of calving while low in lactose which is high in milk (Vipin *et al.,* 2020; O'Callaghan *et al.,* 2020; Arslan *et al.,* 2021; Pandey *et al.,* 2021). Colostrum is distinguished by its very high immunoglobulin G (IgG) concentration, which is important for conferring passive immunity to calves as immunoglobulin concentration and the intestinal permeability decrease rapidly over the first 24 h after parturition and thus adequate supply of colostrum is very important for calves (Alhussien *et al.,* 2021).

Micronutrient Supplementation to dairy cows during transition period was found to be the promising management practice to improve the quality of colostrum and milk which when fed to new born calves improves their survivability (Alhussien *et al.,* 2021). It could be supplemented either orally or parentral, most commonly it is supplemented orally (Dang *et al.,* 2013; Prince *et al.,* 2017; Singh *et al.,* 2020). However, parenteral supplementation was also found to be efficient (Mattioli *et al.,* 2019; De *et al.,* 2015). Mattioli *et al.,* 2019 reported that parenteral supplementation of copper, zinc, selenium, manganese and vitamins A and E had positive effects on body weight. Vitamins A and E, and the trace elements such as zinc are critical for optimum health and production of dairy cows and calves (Dang *et al.,* 2013; Prince *et al.,* 2017; Singh *et al.,* 2020) which is a potent antioxidant to prevent cellular oxidative damage in cell membranes (vitamins) as well as in the cytoplasm (trace elements). Moreover, these micronutrients strengthen the immune system and reduce the level of stress in animals around parturition and improves the production and quality of colostrums and milk (Singh *et al.,* 2020, Alhussien *et al.,* 2021). There is an enhanced immune responses and antioxidant status in perinatal calves due to micronutrient supplementation (Ottaviano *et al.,* 2019). Micronutrient interventions during the prepartum period improve the health status of dairy calves and subsequently the well-being of their calves (Alhussien *et al.,* 2021). Vitamin E consists of eight fat-soluble compounds, tocopherols, and tocotrienols, of which α-tocopherol has the highest activity and is the major tocopherol present in the milk of cows. Supplementation of vitamin E during the dry period has been found to maximize colostral α-tocopherol, minimize the incidence of postpartum metabolic diseases (Dang *et al.,* 2013; Weiss *et al.,* 1990; Smith *et al.,* 1997). Vitamin E Supplementation to the periparturient cows improves the nutritional quality of colostrums as it can cross mammary gland barriers and comes in milk (Prince *et al.,* 2017). Zinc is an important component of many enzymes that are involved in various immunological, endocrine, and reproductive processes (Nayeri *et al.,* 2014, Allahyari *et al.,* 2019). β-carotene, the main dietary precursor of vitamin A, is an antioxidant and has immune regulatory properties (Alhussien *et al.,* 2021).

Therefore, the present study was undertaken to see the alteration in the composition of colostrum due to micronutrient supplementation of dams during the transition period and milk.

MATERIALS AND METHODS

The present study was carried out at Livestock Research Centre (LRC), National Dairy Research Institute (NDRI), Karnal-132001. The geographical location of NDRI, Karnal is at coordinates 29°42'13"N latitude and 76°58'44"E longitude situated at an altitude of 250 meters above the mean sea level. The place comes under semi-arid region where the climatic condition is hot during summer (45°C) and cold (4°C) during winter season. The annual rainfall is about 70 cms and relative humidity varies from 41 to 85 %.

Thirty-five crossbred cows in the advanced stage of pregnancy (≈-30 days) were selected and grouped into five equal groups based on their most probable producing ability (MPPA) for milk (average 3849.22 kg), parity (average 2.5) and body weight (average 488.268 kg). Seven crossbred cows were assigned to control, while seven in each of the 4 treatment groups. Only animals

which would be expected to calve from September, 2019 to April, 2020 had been considered. All the cows were apparently free from any physiological, anatomical and infectious disorders. Based on their milk yield and no. of parity, the animals within the same range of yield and at the same parity had been blocked and randomly allocated to one of the five different treatments; each group compromising seven animals (Table 2). T0 without any supplementation acted as control. The experimental cows had been supplemented individually with Vitamin A (T1), Zinc (T2), Vitamin E (T3), and also with a combination of Vitamin E, Vitamin A and Zinc (T4), to study the cumulative effect of all micronutrients.

All experimental cows were maintained under loose housing management system. 30 days before the expected date of calving, the pregnant cows were shifted to pregnant animal shed consisting of an open paddock and individual calving pen provided with ample space, proper ventilation and drainage and feeding and watering facilities. After 5 days post calving, cows were again shifted to milking shed, which was large, open and brick paved and was adjacent to milking byre.

All the cows were medium milk yielding (>10-15 kg/day) and machined milked thrice daily i.e., in morning (5.00 to 6.00 am), noon (12.00 to 1.00 pm) and evening (5.00 to 6.00 pm). The pulsators were adjusted to give a pulsation rate of around 50 pulsations per minute with uniform vacuum level of 400 mm Hg.

All the cows were fed as per NDRI standard practices (ICAR, 2013) adopted during prepartum period. Cows were fed adlib available green succulent roughages (November-December: mustard and jowar; Jan-Mar: berseem and oats) and dry fodders and silage with limited concentrates based on the animal's body weight and the nutritional composition of the fodders offered.

During postpartum period, cows were fed adlib available fodders and concentrates based on their milk production level. The proximate average composition of the concentrate offered to the experimental animals is presented in table 1.

All the cows were medium milk yielding (>10-15 kg/day) and hand milked twice daily i.e., in morning (6.00 to 7.00 am), noon (3.00 to 4.00 pm) for colostrums during 4 days.

Table 1: Composition of the concentrate mixture (Cows)

Ingredients	Parts (%)
Maize (cracked)	33
Mustard cake (oiled)	12
GNC (oiled)	21
Wheat bran	20
Deoiled rice bran	11
Mineral mixture	2
Common salt	1

The cows were supplemented individually with vitamin A, E, Zinc and their combination from 30 days before the expected date of calving till calving to study the effect of micronutrient supplementation. One group without any supplementation were acted as control. Doses of Vitamin A, E and Zinc supplemented daily have been depicted in Table 2. Individual fresh colostrum samples pooled from all the four quarters from all the cows were collected daily up to 7 days, 300 ml of colostrum was collected aseptically in clean milk bottles. Individual fresh milk samples pooled from all the four quarters from all the cows were collected at weekly intervals up to 30 days post calving. About 100 ml of milk was collected aseptically in clean milk bottles.

Table 2: Supplemented doses of micronutrients to crossbred cows

Groups	No. of cows	Dose/Animal/day
Control (T0)	7	Nil
Vitamin A (VA) (T1)	7	100000 IU
Zinc (T2)	7	60 ppm
Vitamin E (VE) (T3)	7	2500 IU
Vitamin E + Zn + Vitamin A (T4)	7	Combination of the above

The samples were brought to the laboratory immediately after collection for SCC, Colosrum composition and whey separation. Colostrum and milk composition i.e., fat, protein, lactose, total solids and ash were estimated by lactoscan milk analyzer. Colostrum and milk SCC were estimated by Lactoscan SCC kit X 4 (manufactured by Milkotronic Ltd.) consisting of: Lactochip, Sofia green lypholized dye, Automatic pipette tip. 100 µL of milk sample is mixed with a dye, kept for 2 min and 8 µL of dyed sample was used for charging lactochip and somatic cell counting is done by the machine.

RESULTS AND DISCUSSION

The present study has been done to study the effect of VA, VE, Zinc and their combination supplementation on colostrum, transition milk and milk composition.

Colostrum composition

The colostrum composition (Means±SE) i.e., total solids, fat, protein, lactose and ash percentage on day 1 to day 4 in supplemented and control cross bred cows have been presented in table 3. Total Solids, fat, protein and ash percentage increases from day 1 to day 4 in all groups and highest value was reached on day 4th of colostrum. Lactose percentage was lowest on day 1 and reached higher on day 4 in all groups (Table 3).

Colostrums total solid percentage was significantly ($p<0.01$) higher in all supplemented groups (T1, T2, T3 and T4) compared to control (T0) on 1st and 2nd day. On the 3rd and 4th day combination supplemented group (T4) showed significantly ($p<0.01$) higher value compared to control. However individual supplementation (T1, T2 and T3) showed numerically higher values in solid percentage on all 4 days compared to control (T0).

Similarly, supplementation of micronutrients showed significant increase in *colostrums fat percentage* in T3 and T4 from day 1 to day 4 compared to control and T1 but no such significant changes was seen among the treatment groups. Whereas fat percentage in T1 and T2 was non significant ($p>0.05$) with that of control, but was numerically higher and on day 3 it was significantly higher ($p<0.05$) in T2 group also.

Table 3: Variation in colostrum composition due to supplementation of vitamin A, Zinc, vitamin E and its combination in crossbred cows

Postpartum days	Treatment groups*					P value
	T0	T1	T2	T3	T4	
	Colostrum Total solid%					
1	19.54^{a}±1.51	22.75^{b}±0.83	24.25^{b}±1.09	24.12^{b}±0.94	24.98^{b}±0.77	0.007
2	14.04^{a}±1.38	18.04^{bc}±0.94	16.14^{ab}±0.95	15.68^{ab}±1.38	20.89^{c}±0.85	0.002
3	11.51^{a}±1.44	14.53^{a}±1.24	13.86^{a}±1.27	12.00^{a}±1.32	19.68^{b}±0.77	0.001
4	10.19^{a}±1.13	13.61^{ab}±1.28	12.46^{ab}±1.18	12.03^{ab}±1.11	15.51^{b}±1.43	0.056
	Colostrum Fat%					
1	6.05^{a}±0.16	6.21^{a}±0.16	6.46^{ab}±0.3	6.78^{b}±0.06	6.99^{b}±0.19	0.007
2	5.08^{a}±0.17	5.53^{a}±0.18	5.69^{ab}±0.29	6.28^{b}±0.27	6.38^{b}±0.23	0.002
3	4.69^{a}±0.2	5.11^{ab}±0.2	5.68^{b}±0.33	5.88^{b}±0.37	5.92^{b}±0.27	0.014
4	4.54^{a}±0.21	4.61^{a}±0.24	5.21^{b}±0.21	5.33^{ab}±0.16	5.42^{b}±0.34	0.03
	Colostrum Protein%					
1	12.05^{a}±0.34	12.32^{a}±0.51	12.58^{a}±0.67	13.19^{a}±0.63	15.43^{b}±0.43	0.001
2	7.77^{a}±0.44	7.98^{a}±0.85	8.59^{a}±0.72	8.69^{a}±0.73	10.67^{b}±0.46	0.031
3	6.61^{a}±0.41	7.21^{ab}±0.68	7.12^{ab}±0.55	7.6^{ab}±0.62	9.06^{b}±0.55	0.048
4	5.07^{a}±0.62	5.76^{a}±0.54	6.33^{ab}±0.45	6.48^{ab}±0.47	7.52^{b}±0.51	0.03
	Colostrum Lactose%					
1	2.61±0.15	2.62±0.17	2.68±0.14	2.86±0.14	2.87±0.09	0.523
2	3.43±0.06	3.50±0.36	3.51±0.15	3.66±0.20	3.98±0.23	0.422
3	3.51±0.28	3.97±0.22	4.00±0.15	4.09±0.13	4.19±0.13	0.137
4	3.67^{a}±0.13	4.09^{ab}±0.15	4.28^{b}±0.15	4.30^{b}±0.15	4.48^{b}±0.18	0.009
	Colostrum Ash%					
1	1.09^{a}±0.09	1.22^{ab}±0.11	1.35^{ab}±0.1	1.49^{b}±0.11	1.55^{b}±0.14	0.033
2	0.89±0.06	0.95±0.05	0.96±0.03	1.06±0.08	1.13±0.13	0.185
3	0.74±0.04	0.81±0.05	0.82±0.06	0.82±0.07	0.89±0.06	0.493
4	0.71±0.02	0.72±0.05	0.73±0.03	0.74±0.03	0.82±0.05	0.347

^{abc}Means with different superscripts within a row differ significantly ($p<0.05$); *T0: Control; T1: Vitamin A supplemented; T2: Zinc supplemented; T3: Vitamin E supplemented; T4: Combination supplemented.

Micronutrient supplementation showed significant ($p<0.05$) increase in *colostrums protein percentage* in T4 group from day 1 to day 4 compared to control (T0) and other treatment groups (T1,T2, T3) but no such significant changes was seen among the treatment groups. Whereas numerically higher protein percentage was observed in T3 followed by T2, T1 and finally in T0, however it was non-significant ($p>0.05$).

No significant ($p>0.05$) (Means±SE) difference in *colostrums lactose percentage* from 1st to 3rd day between treatment and control groups. However, on the 4th day mean values of lactose was significantly ($p<0.05$) higher in T2, T3 and T4 group as compare to T0 and T1 (Table 3).

Higher *colostrums ash percentage* ($p<0.05$) in T3 and T4 group when compared with T0, T1 and T2 groups and no significant ($p>0.05$) (Means±SE) difference in colostrums ash% from 2nd to 4th day between treatment and control groups. Even though there was non-significant ($p>0.05$) changes but still supplemented groups was found to have numerically higher colostrums lactose and colostrums ash percentage than unsupplemented groups.

Increased colostrums composition (total solid%, protein%, fat%, lactose% and ash%) in the supplemented groups highlighted the importance of zinc sulphate, and vitamins A and E in improving colostrums quality. In agreement with our findings, Vipin *et al.* (2020), observed that vitamin A (1,50,000 IU) and E (3000 IU) supplementation at a higher dose in murrah buffaloes during late pregnancy helped to increase ($p<0.05$) total solid percentage and protein percentage and colostrum lactose percent in groups supplemented with vitamin A and E @ 75,000 IU and 1,500 IU/day respectively only on the third day. Singh *et al.,* 2021, also reported that combined supplementation of antioxidant micronutrients (Zn, Cu, and vitamins A and E) to the periparturient Murrah buffaloes starting from 2 months prior to calving improved the quality of colostrums. Present findings correlates with that of Mutoni *et al.* (2012), in which they supplemented vitamin E in Sahiwal cows reported improvement of colostrum fat (4.88% vs. 5.05%, $p<0.05$) percentage, protein percentage (4.27% vs. 4.35%) and colostrum lactose (3.12 % vs. 4.37 %, $p<0.05$) as compared to the non-supplemented group. Nieto *et al.* (2015) observed in ewes receiving intramuscular injections of vitamin E (4 IU vitamin E kg^{-1} of live weight) in weekly intervals from 50 days before lambing until 60 days of lactation improved lactose content in colostrums (1.9% vs. 1.2%, $P < 0.001$) while non-significant change in colostrums protein and fat percentage. Colostrum contain higher mineral content than milk Puppel *et al.* (2019). Therefore, a better understanding of the association between antioxidant micronutrients and their effect on nutritional quality of colostrums composition may help to design effective nutritional management of transition cows and their calves, as colostrum ultimately have impact on the health status of newborn calves.

Effect of supplementation on Vitamin A, Zinc, Vitamin E and their combination on transition milk composition

The transition milk composition (Means±SE) i.e., total solids, fat, protein, lactose and ash percentage from day 5 to day 7 taken daily in supplemented and control cross bred cows have been presented in table 4.

Supplementation of micronutrient to transition cows revealed significantly higher value ($p<0.05$) in T4 group when compared with T0, T1, T2 and T3 groups in transition milk total solid percentage, fat percentage and protein percentage and no significant ($p>0.05$) (Means±SE) difference in transition milk lactose% and ash%. However, micronutrient supplemented group revealed numerically higher transition milk composition (total solid%, fat%, protein%, lactose % and ash%) as compare to unsupplemented group. Transition milk fat percentage was also significantly ($p<0.05$) higher in T2 and T3 groups on day 5 when compared with T0 group.

Gakhar *et al.* (2010), found significant increase in milk SNF in copper supplemented group and decrease in unsupplemented group at 3rd -7th days post calving. Not much authors have studied the composition of transition milk and changes in it due to micronutrient supplementation. However our research revealed that the transition milk total solids percentage, fat percentage and protein percentage was found to be lower than colostrums that is upto 4th day postpartum and higher than milk after 7th day postpartum. As lactation proceeds total solids percentage, fat percentage and protein percentage decreases while lactose percentage increases.

Table 4 : Variation in transition milk composition (postpartum day 5 to day 7) due to supplementation of Vitamin A, Zinc, Vitamin E and its combination in crossbred cows

Postpartum day	Treatment groups*					P value
	T0	T1	T2	T3	T4	
			Total solid%			
5	8.64±1.08^{a}	10.36±0.93^{ab}	11.7±0.75^{ab}	11.51±0.91^{ab}	13.00±1.09^{b}	0.036
6	8.78±1.07^{a}	10.12±1.08^{ab}	11.09±0.84^{ab}	11.01±0.69^{ab}	13.19±0.95^{b}	0.035
7	8.29±1.15^{a}	10.02±1.15^{ab}	11.3±0.67^{ab}	10.97±0.48^{ab}	12.44±0.83^{b}	0.032
			Fat%			
5	4.16±0.18^{a}	4.41±0.19^{ab}	5.18±0.26^{b}	5.09±0.23^{b}	5.16±0.24^{b}	0.004
6	4.13±0.16^{a}	4.12±0.16^{a}	4.83±0.37^{ab}	4.7±0.15^{ab}	5.16±0.33^{b}	0.012
7	3.77±0.15^{a}	4.2±0.2^{ab}	4.6±0.37^{ab}	4.59±0.07^{ab}	4.83±0.32^{b}	0.042
			Protein%			
5	4.59±0.54^{a}	5.31±0.45^{a}	5.61±0.41^{ab}	5.82±0.54^{ab}	7.19±0.37^{b}	0.008
6	4.35±0.45^{a}	5.11±0.47^{ab}	5.33±0.47^{ab}	5.37±0.40^{ab}	6.77±0.44^{b}	0.011
7	4.02±0.46^{a}	4.99±0.54^{ab}	5.34±0.51^{ab}	5.37±0.43^{ab}	6.56±0.32^{b}	0.009
			Lactose%			
5	4.59±0.22	4.52±0.25	4.45±0.13	4.18±0.3	4.32±0.15	0.705
6	4.57±0.18	4.8±0.22	4.83±0.2	4.46±0.21	4.41±0.17	0.454
7	4.49±0.24	4.75±0.26	4.91±0.19	4.76±0.11	4.93±0.1	0.504
			Ash%			
5	0.62±0.01	0.71±0.04	0.68±0.02	0.68±0.03	0.76±0.06	0.107
6	0.61±0.02	0.67±0.02	0.67±0.04	0.68±0.02	0.73±0.04	0.176
7	0.58±0.02	0.66±0.03	0.66±0.03	0.65±0.04	0.68±0.04	0.256

^{abc}Means with different superscripts within a row differ significantly ($p<0.05$); *T0: Control; T1: Vitamin A supplemented; T2: Zinc supplemented; T3: Vitamin E supplemented; T4: Combination supplemented.

Effect of supplementation on Vitamin A, Zinc, Vitamin E and their combination on milk composition

The milk composition (Means±SE) i.e., total solids, fat, protein, lactose and ash percentage from 8th day to 30th day taken weekly in supplemented and control cross bred cows have been presented in table 5.

Supplementation of micronutrient to transition cows revealed significantly higher value ($p<0.05$) in T4 group when compared with T0, T1, T2 and T3 groups in milk total solid percentage, fat percentage and protein percentage and no significant ($p>0.05$) (Means±SE) difference in milk lactose% and ash%. However, micronutrient supplemented group revealed numerically higher milk composition (total solid%, fat%, protein%, lactose % and ash%) as compare to unsupplemented group. Total solid percentage was significantly ($p<0.05$) higher in T2 and T3 groups on day 8 and 30, milk fat percentage was significantly ($p<0.05$) higher in T1, T2 and T3 groups on 22nd and 30th day and on day 8 only in T1 and T2 groups, milk protein percentage was significantly ($p<0.05$) higher in T3 group on 30th day when compared with T0 group.

Kellogg *et al.* (2004) indicated that Zn methionine increased lactational performance by increasing milk yield ($p<0.01$), energy corrected milk and fat corrected milk, with no change in milk composition while no effect on milk composition was observed by (Cope *et al.,* 2009), due to dietary treatment of zinc. Our research revealed only significant change in milk total solid percentage, fat percentage and protein percentage. Uchida *et al.* (2001), also reported no effect on milk production, milk fat and protein content and linear SCC to early lactation holstein cows due to supplementation of combination of Zn amino acid (AA), Mn AA, and Cu AA complexes, and Co glucoheptonate.

Similarly, Oliveira *et al.,* (2015), found that supplementing beta carotene @ 1.2 gram/day/cow for 14 days pre-partum increased milk protein content from 2.90 to 2.96 %. Singh *et al.* (2020), also found that strategic antioxidant micronutrient supplementation (Zn, Cu, and vitamins A and E) in the ration of peri-parturient buffaloes increased milk fat and protein percentage. In agreement with our findings, Griffiths *et al.* (2007), reported increase in milk production (6.3%), milk energy (5.6%), fat yield (6.4%), protein yield (6.5%), milk solids and decrease in mastitis ($p<0.10$) due to micronutrient supplementation. In our study, the milk

Table 5: Variation in milk composition (postpartum day 8 to day 30) due to supplementation of vitamin A, zinc, vitamin E and its combination in crossbred cows

Postpartum day	Treatment groups*					P value
	T0	T1	T2	T3	T4	
			Total solid%			
8	8.01±0.44^{a}	9.08±0.43^{ab}	9.48±0.18^{b}	9.42±0.26^{b}	9.49±0.28^{b}	0.016
15	7.99±0.52^{a}	8.93±0.64^{ab}	9.42±0.32^{ab}	9.23±0.28^{ab}	9.73±0.18^{b}	0.006
22	7.98±0.40^{a}	8.82±0.63^{ab}	9.38±0.23^{ab}	9.34±0.19^{ab}	9.99±0.16^{b}	0.007
30	7.98±0.39^{a}	8.83±0.62^{ab}	9.44±0.20^{b}	9.33±0.15^{b}	9.82±0.17^{b}	0.009
			Fat%			
8	3.16±0.15^{a}	4.26±0.30^{b}	4.05±0.20^{b}	3.82±0.26^{ab}	4.35±0.10^{b}	0.003
15	3.21±0.15^{a}	4.2±0.22^{b}	4.19±0.13^{b}	3.75±0.24^{ab}	4.32±0.11^{b}	0.001
22	3.18±0.16^{a}	3.95±0.18^{b}	4.18±0.14^{b}	3.88±0.27^{b}	4.31±0.11^{b}	0.001
30	3.24±0.15^{a}	3.97±0.18^{b}	4.25±0.08^{b}	4±0.23^{b}	4.3±0.14^{b}	0.001
			Protein%			
8	3.7±0.16^{a}	3.84±0.18^{a}	3.97±0.23^{a}	4.13±0.20^{a}	5.32±0.13^{b}	0.001
15	3.68±0.14^{a}	3.91±0.17^{a}	4.00±0.22^{a}	4.21±0.20^{a}	5.34±0.15^{b}	0.001
22	3.59±0.11^{a}	3.97±0.13^{a}	4.09±0.21 ^{a}	4.08±0.23^{a}	5.29±0.17^{b}	0.001
30	3.55±0.13^{a}	3.86±0.26^{ab}	4.05±0.22^{ab}	4.25±0.23^{a}	5.32±0.16^{c}	0.001
			Lactose%			
8	4.77±0.24	4.75±0.17	5.05±0.09	5.18±0.05	5.18±0.11	0.107
15	4.88±0.22	5.11±0.21	5.12±0.08	5.17±0.09	5.18±0.14	0.682
22	4.95±0.25	5.01±0.26	5.03±0.09	5.03±0.14	5.20±0.14	0.914
30	4.97±0.15	4.98±0.19	4.95±0.25	5.07±0.14	5.11±0.11	0.959
			Ash%			
8	0.64±0.02	0.65±0.03	0.67±0.02	0.67±0.01	0.65±0.02	0.718
15	0.66±0.02	0.67±0.02	0.68±0.02	0.66±0.01	0.65±0.02	0.892
22	0.66±0.02	0.67±0.02	0.65±0.01	0.65±0.02	0.65±0.02	0.81
30	0.65±0.02	0.65±0.02	0.67±0.01	0.66±0.01	0.65±0.02	0.935

^{abc}Means with different superscripts within a row differ significantly ($p<0.05$); *T0: Control; T1: Vitamin A supplemented; T2: Zinc supplemented; T3: Vitamin E supplemented; T4: Combination supplemented.

composition that is milk total solid percentage, milk fat percentage and milk protein percentage was found to be maximum in the milk of the crossbred cows that belongs to the group supplemented with a combination of vitamins A, E, and zinc sulphate. Increased milk composition in the supplemented groups highlighted the importance of zinc sulphate, and vitamins A and E in improving milk quality.

Effect of supplementation of Vitamin A, Zinc, Vitamin E and their combination on colostrums, transition milk and milk somatic cell counts

The results (Means± SE) of colostrums, transition milk and milk SCC (X 10^3 cells/ml) during different days of supplemented and control crossbred cows have been presented in Fig. 1.

Supplementation of micronutrient to transition cows revealed significantly lower value ($p<0.05$) in T4 group when compared with T0, T1, T2 and T3 groups in colostrums, transition milk and milk SCC. On 4th, 6th and 15th day T2 and T3 groups also revealed significantly lower ($p<0.05$) SCC when compared with T0 and T1. On 2nd day T1 also revealed significantly lower ($p<0.05$) SCC when compared with T0 group. However, micronutrient supplemented group revealed numerically lower somatic cell count in colostrums, transition milk and milk as compare to unsupplemented group.

Various authors have studied decrease in somatic cell count due to micronutrient supplementation. β-carotene, the main dietary precursor of vitamin A, is an antioxidant and when supplemented (400 mg/day/cow) elevated antioxidant status in the mammary gland and hence extra supplementation may lead to decrease SCC (Arechiga *et al.,* 1998). Singh *et al.* (2020), also found that strategic antioxidant micronutrient supplementation (Zn, Cu, and vitamins A and E) in the ration of peri-parturient buffaloes improved the udder health by reducing the occurrence of mastitis. Combined supplementation of micronutrients

decreased milk SCC (3.05 ± 0.11 vs. 2.12 ± 0.10 × 105 cells/mL) (Alhussien *et al.,* 2021).

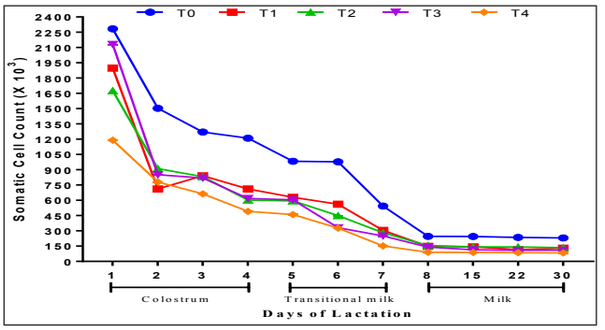

Fig. 1: Variation in Somatic Cell Count (X 10^3) in colostrum, transition milk and milk due to supplementation of Vitamin A (T1), zinc (T2), vitamin E (T3) and its combination (T4) in crossbred cows and control (T0) without supplementation

Reduction in SCC supplemented with Cu, Zn, vitamin E, and vitamin A have been seen by various researchers (Chatterjee *et al.,* 2005; Pechova *et al.,* 2005; Anwar *et al.,* 2014; Maurya *et al.,* 2014; Yang and Li, 2015; Singh *et al.,* 2020). Micronutrient Supplementation improve immune function by activating cell-mediated immune responsiveness (Chawla and Kaur, 2005) and thus there is reduced SCC. Zinc plays an important role in keratin formation, the keratin lining of the teat canal entraps bacteria and prevents their upward movement into the mammary gland which plays potential role in the reduction of SCC (Cope *et al.,* 2009). Our result showed decrease in somatic cell count in combination group that means eventhough when individually Vit A, Vit E and Zinc supplemented to crossbred cows reduction in SCC was not significantly lower but numerically it was lower, and for mastitis all micronutrients all equally important. The reduction in SCC may be partially attributed to the role of vitamin E that enhance the immune functions of mammary cells (Alhussien *et al.,* 2021), vitamin A maintains integrity and stability of epithelial tissue and mucosal surface of mammary tissue (Sordillo, 2016) and Zinc contributes to the keratin formation in the lining of the teat canal and these all prevents bacteria from gaining entry to the mammary gland (Hill *et al.,* 2019). However, Zinc and Vit A are found to be positively correlated as Zinc is required for metabolism (absorption, transport and assimilation) of vitamin A and hence deficiency in zinc limits the body's ability to move Vitamin A stores from the liver to body tissues (Hill *et al.,* 2019). And so in our findings maximum SCC reduction are found in groups which was supplementd with all three micronutrients.

CONCLUSION

Colostrum total solids% ($p<0.01$), fat% ($p<0.05$), protein% ($p<0.05$) and ash% ($p<0.05$) were found to be significantly higher in treatment group as compared to control and it was highest in T3 and T4 group. Milk protein% and lactose% was found to be significantly ($p<0.01$) higher in T4 group. The colostrum and milk SCC were found to be significantly ($p<0.01$) lower in micronutrient supplemented group as compared to control.

From this study, we can conclude that Vitamin E, Vitamin A and Zinc supplementation to peripartum crossbred cows during transition period may improve the nutritional status of colostrums and milk.

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