Indian Journal of Animal Sciences **85** (2): 178–182, February 2015/Article

Effect of supplementing certain vitamins and chelated trace minerals on reducing incidence of sub-clinical mastitis in crossbred cows

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Received: 9 July 2014; Accepted: 13 October 2014

ABSTRACT

Crossbred cows (93) having a history of sub-clinical mastitis (SCM) and clinical mastitis (CM) in previous lactation were selected from 57 farms in Sabarkantha district of Gujarat. Twenty crossbred cows from 12 farms served as control, and the remaining (n=73) were fed daily 10 g supplement per animal containing coated vitamins E and A, chelated copper (Cu), zinc (Zn), chromium (Cr), along with iodine(I), for 4 weeks prior to calving. Milk samples were collected post calving on day 10, 30, 60 and 90 for analysis of somatic cell counts (SCC), sodium (Na), potassium (K), chloride (Cl) content, pH and electrical conductivity (EC). Blood samples were also collected for neutrophil count, measurement of immunoglobulin and ferric reduction anti-oxidant power (FRAP). The milk producers were provided with Mastect strip for weekly checkup of SCM. Out of 73 supplemented cows, only 15 (21%) showed signs of SCM and CM with Mastect strip and California mastitis test, which was confirmed by SCC in milk (average 7.26×10^5 cells/ml milk). However, 16 animals (80%) out of 20 in control group were affected by SCM as indicated by the SCC (average 10.11×10^5 cells/ml milk), which was later aggravated to CM. Milk pH, EC, Na and Cl content in milk were higher in animals affected by SCM than the normal animals. In supplemental group, SCC in 58 (79%) animals was within the normal range ($1.22–2.36\times10^5$ cells/ml milk) and no signs of SCM or CM were observed. Milk lactose, protein, solids-not-fat (SNF) content and FRAP were higher in unaffected as compared to mastitis affected animals. On feeding the supplement, blood neutrophil count decreased, whereas, immunoglobulin and FRAP activity increased significantly ($P<0.05$). The inference could be drawn that feeding a vitamins and chelated minerals based supplement 4 weeks prior to calving could significantly help in reducing the incidence of SCM in crossbred cows.

Key words: Crossbred cows, Minerals, Somatic cell counts, Sub-clinical mastitis, Vitamin E

Field surveys of major livestock diseases in India have ranked mastitis as number one disease of dairy animals. Sub-clinical mastitis (SCM) is 30–40 times more prevalent than clinical mastitis and causes heavy economic losses, especially in crossbred cows. Additionally, the disease is important from consumers' and processors' point of view (Wheelock *et al.* 1996). The milk from an affected animal may harbour the organisms potentially pathogenic for humans (Sharma *et al.* 2006). Amongst others, proper feeding of advanced pregnant cows with appropriate vitamins and minerals can significantly improve immune function, as their deficiencies can result in immune-depression (Suttle and Jones 1989, Weiss and Spears 2006).

Ration fed to dairy animals in India, is often deficient in vitamins E and A, zinc, copper (Garg *et al.* 2014), chromium that are primary vitamins and minerals affecting immune function and susceptibility to SCM and CM (Drake *et al.* 1992, Torre *et al.* 1996, Harmon and Torre 1997, Bhanderi and Garg 2012). The dietary level of certain vitamins and minerals required for optimal immune function is higher than that required for growth and reproduction (NRC 2001). Therefore, it becomes paramount important to supplement the ration with requisite vitamins and minerals, especially prior to calving to improve immune status of animals. In view of this, the present study was undertaken to investigate the efficacy of feeding coated vitamins and chelated minerals based supplement for reducing the incidence of SCM and CM in crossbred cows, maintained under field conditions in Sabarkantha district of Gujarat.

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MATERIALS AND METHODS

Experimental design: A primary survey was conducted in Sabarkantha district of Gujarat, to identify advanced pregnant crossbred cows having a history of sub-clinical and clinical mastitis in previous lactation. Based on the survey out of 185, animals (93) from well managed 57 progressive dairy farms were identified; 75 animals at 45 farms were taken under the supplemental group and fed daily one sachet of 10 g supplement per animal for 4 weeks prior to calving. The supplement contained coated vitamins

E and A, chelated trace minerals (Cu, Zn, Cr) along with iodine. The levels of supplemental Cu and Zn were considered in view of deficiency of these minerals in the total ration, considering total dietary mineral intake and the requirement. Vitamins E and A were supplemented as per NRC (2001), considering the requirement during advanced stage of pregnancy. At 12 farms, 20 animals were maintained without supplement and served as control.

Feeding of animals: On an average, animals in Sabarkantha were fed local grasses/ hybrid napier (4–5 kg), sorghum/maize green (10–12 kg), sorghum straw (2–3 kg) and *ad lib.* wheat straw. Each cow was fed home-made concentrate mixture or compound cattle feed, based on the level of milk production, while in lactation. The average milk production of crossbred cows identified for the study was 21.8 kg/animal/day with 3.55% fat in milk while in production.

Laboratory analysis: All the animals under control and experimental groups were checked weekly for SCM, post calving for a period of 90 days, using Mastect strip and California Mastitis Test (CMT). Milk samples were collected from all 4 quarters of an animal in sterile polyethylene screw capped, wide mouth vials after squirting few streams, on day 10, 30, 60 and 90 after calving, from all the animals under control and experimental groups for estimation of Na, K and Cl content (Brooks *et al.* 1996) and SCC (IDF 1984), lactose, protein and SNF, using commercial kit. The pH and EC in milk samples were also measured on the spot with the help of portable digital pH/ conductivity meter. About 10 ml blood from jugular vein was collected from the animals before supplementation and on day 10, 30, 60 and 90 after calving. The blood samples were analysed for the IgG, IgM, IgA using a kit, FRAP activity (Benzie and Strain 1996) and neutrophils by using automatic haematology analyser. Feeds and fodder samples were analysed for mineral levels using spectrometer .

Statistical analysis: The data on pH, EC, SCC, Na, K, Cl, lactose, protein, SNF and FRAP in milk and neutrophil, immunoglobulin and FRAP activity in blood were subjected to variance for statistical significance as per Snedecor and Cochran (1986) with SPSS package programme, SPSS 9.00 software for Windows, SPSS Inc., Chicago, IL (SAS 2012).

Table 1. Advanced pregnant cows having a history of SCM and CM in their previous lactation

Particulars	Number of animals	Percentage
Mastitis acquired in early lactation (0–2 months after calving)	86	92
Mastitis acquired in late lactation (7 months after calving)	7	8
Animals having one or two teats dry	30	32
Animals having swelling on teat or flakes in milk or both	41	44
Animals recovered after treatment	11	12
Animals with severity 1 & 2 (less)	19	20
Animals with severity 3 (moderate)	41	44
Animals with severity 4 & 5 (severe)	34	36
Animals with one teat affected	60	64
Animals with two teats affected	7	8
Animals with four teats affected	26	28

RESULTS AND DISCUSSION

Animal identification: Number of animals having previous history of clinical and sub-clinical mastitis in earlier lactation is presented in Table 1. Out of 185 animals in advanced pregnancy screened for the study, 93 animals were identified having a history of SCM and CM in the previous lactation. Data showed that 92% animals acquired mastitis during early stage of lactation. Out of 93, one teat was affected with SCM or CM in 64% animals.

Minerals content in feeds and fodder: The feeds and fodder samples were analysed for calcium, phosphorus, magnesium, copper, zinc and manganese and values are depicted in Table 2. Feedstuffs commonly fed to the animals were found to be deficient in copper and zinc.

California mastitis test (CMT): The CMT is a simple, inexpensive, rapid screening test and is useful in determining which quarter of the cow is most affected. The test is useful in indicating sub-clinical and chronic mastitis. The CMT estimates the number somatic cells present in the milk. The CMT was conducted by mixing the test reagent with an equal quantity of milk. It reacted with materials from the nuclei of the somatic cells in the milk to form a gel. The reaction was then visually scored depending on the amount of gel formed. On the basis of result of Mastect strip and CMT, animals affected by SCM were 80 and 21% in control and supplemental groups, respectively (Table 3).

Effect of feeding supplement on pH and electrical conductivity of milk: Mean pH, EC, SCC, FRAP, Na, Cl, K, protein, lactose and SNF content in milk for the control and experimental groups post calving are shown in

Table 2. Macro and micro mineral levels in feeds and fodder

Particular	Local grass	Hybrid napier	Maize green	Sorghum green	Sorghum straw	Wheat straw	Concentrate mixture
Ca (%)	0.76	0.50	0.60	0.58	0.57	0.45	0.79
P (%)	0.25	0.15	0.24	0.23	0.19	0.06	1.12
Mg (%)	0.59	0.24	0.59	0.50	0.49	0.21	0.66
Cu (ppm)	13.66	10.08	8.07	13.11	7.02	5.23	10.48
Zn (ppm)	41.79	32.39	51.05	63.36	28.31	19.71	79.09
Mn (ppm)	62.28	132.99	62.64	53.86	55.83	47.88	100.4

Table 3. Effect of feeding mastitis supplement on incidence of SCM and CM in crossbred cows

Days after calving	Control animals (n=20)		Supplemental animals (n=73)	
	Normal	Affected with SCM/CM*	Normal	Affected with SCM/CM*
Day 10	3 (15%)	17 (85%)	56 (77%)	17 (23%)
Day 30	3 (15%)	17 (85%)	58 (79%)	15 (21%)
Day 60	4 (20%)	16 (80%)	58 (79%)	15 (21%)
Day 90	4 (20%)	16 (80%)	58 (79%)	15 (21%)

*Note: Based on the Mastect strip and CMT.

Table 4. Milk pH can be considered as an economical easy and rapid method to detect the sub-clinical mastitis under field conditions. In supplemented group, pH of milk from animals affected by SCM was 6.86, which was significantly higher (P<0.05) than that of recorded in the normal animals. In normal animals (n=58), pH was 6.50. In control group, out of 20, 16 animals were affected by SCM and showed higher pH than the unaffected animals. In mastitis, increased permeability of the gland to blood sodium and chloride ions leads to increase of milk pH (Kellogg 1990).

In supplemented group, EC in normal animals was 4.03, which increased (P<0.01) to 6.24 mS/cm in affected animals. Similar trend was observed in control group animals affected by mastitis (Table 4). The EC of mastitis affected cows in experimental group was higher than those in control group. This might have resulted due to increase in chloride ions in the milk. EC of milk to detect mastitis is based on the ionic changes, which occur during inflammation, as a result of increased sodium and chloride concentrations in milk (Popovic 2004).

Somatic cell counts in milk as an indicator of SCM: Milk samples from all the animals in control and experimental groups were checked for SCM. Out of 93, 31 animals were found to be positive for SCM in both groups, which were subjected to SCC for confirmation. SCC is an indicator of both resistance and susceptibility of dairy cows to mastitis and can be used to monitor the level or occurrence of SCM in individual cow (Harmon 1994, Torre *et al.* 1996). Increase in SCC indicates inflammatory reaction of udder tissues. In supplemented group, cows detected negative with Mastect strip and CMT showed SCC in the range of 1.22 to 2.36 ($x10^5$/ml of milk). Cows affected by SCM showed SCC in the range of 6.39 to 8.71 ($x10^5$/ml milk), which was significantly (P<0.01) higher than the normal animals. Similarly, animals in control group affected by SCM showed significantly (P<0.01) higher SCC than the normal animals. This increase of SCC indicated inflammatory reaction and might be due to shift of leucocytes to the udder after entry of infection in the mammary gland and as a protective mechanism against infection (Kellogg *et al.* 2004, Spears and Weiss 2008). The high SCC causes a rise in whey protein and a decrease in casein resulting in a considerable lower cheese yields. A shorter shelf life and adverse milk flavours are other consequences of high SCC in milk.

Effect of feeding mastitis supplement on milk electrolytes: Major milk electrolytes are Na, Cl and K. Levels of Na and Cl increased during SCM, whereas, level of K decreased. In supplemented group, average Na and Cl content in normal animals were 57.18 and 122.85 mg/dl, respectively which increased significantly (P<0.05) to 118.6 and 163.5 mg/dl in animals affected with SCM. The level of K was significantly (P<0.05) lower in affected animals. These imbalances result into decrease in quality and taste of milk. Bacterial infection of the udder results into damage to the ductal and secretary epithelium, which leads to increase in permeability of the blood capillaries, thus Na^+ and Cl^- pour into the lumen of the alveolus and in order to maintain osmolarity, K^+ level decrease proportionately (Wheelock *et al.* 1996). The trace minerals and vitamins in the supplement might have helped in preventing damage to ductal and secretary epithelial due to low Na content in unaffected animals.

Milk lactose, protein and SNF content and FRAP activity: Mastitis causes considerable changes in milk composition in dairy animals. The lactose content was significantly lower

Table 4. Effect of feeding mastitis supplement on different parameters in crossbred cows

Particulars	Control animals (n=20)		Experimental animals (n=73)	
	Normal (n=4)	Affected with SCM/CM (n=16)	Normal (n=58)	Affected with SCM/CM (n=15)
pH	$6.52^{a}\pm0.01$	$6.99^{b}\pm0.04$	$6.50^{a}\pm0.02$	$6.86^{b}\pm0.05$
EC (mS/cm)	$4.56^{c}\pm0.16$	$6.78^{d}\pm0.12$	$4.03^{c}\pm0.07$	$6.24^{d}\pm0.06$
SCC ($x10^5$/ml of milk)	$2.01^{c}\pm0.06$	$10.11^{d}\pm0.32$	$1.51^{c}\pm0.28$	$7.26^{d}\pm0.51$
FRAP (µM/l)	$1126^{a}\pm10.71$	$454^{b}\pm14.31$	$1329^{a}\pm35.88$	$451^{b}\pm17.42$
Na (mg/dl)	$57.68^{a}\pm1.63$	$126.90^{b}\pm2.48$	$57.18^{a}\pm0.82$	$118.66^{b}\pm1.66$
Cl (mg/dl)	$137.79^{a}\pm1.05$	$176.22^{b}\pm4.93$	$122.85^{a}\pm3.64$	$163.55^{b}\pm3.20$
K (mg/dl)	$137.06^{a}\pm1.46$	$117.50^{b}\pm0.84$	$136.03^{a}\pm0.75$	$122.04^{b}\pm2.39$
Protein (%)	3.71±0.03	3.60±0.03	3.70±0.01	3.63±0.05
Lactose (%)	$4.67^{a}\pm0.09$	$4.11^{b}\pm0.03$	$4.47^{a}\pm0.09$	$3.94^{b}\pm0.05$
SNF (%)	9.25±0.13	8.97±0.11	9.42±0.03	9.15±0.09

[a,b]Values with different superscript in a row within respective parameter differ at P < 0.05; [c, d]Values with different superscript in a row within respective parameter differ at P < 0.01.

Table 5. Effect of feeding supplement on different blood parameters in crossbred cows

Particulars		Control animals (n=20)		Experimental animals (n=73)	
		Normal (n=4)	Affected with SCM/CM (n=16)	Normal (n=58)	Affected with SCM/CM (n=15)
Neutrophil (%)	Before	54.65±2.35	59.89±3.48	57.65±3.54	62.51±3.07
	Day 10	50.34^{a}±2.88	60.95^{b}±3.76	34.75^{a}±2.46	60.23^{b}±2.38
	Day 30	48.76^{a}±3.77	58.78^{b}±3.68	31.85^{a}±3.88	58.26^{b}±1.99
	Day 60	43.23^{a}±2.68	61.75^{b}±2.73	31.81^{a}±4.12	60.03^{b}±3.25
	Day 90	40.29^{a}±1.08	60.56^{b}±1.98	30.56^{a}±3.23	59.44^{b}±2.12
IgG (mg/ml)	Before	22.68±1.19	23.34±0.89	23.93±0.98	24.43±1.15
	Day 10	23.69±1.09	20.18±1.13	31.87^{a}±1.02	23.90^{b}±1.04
	Day 30	27.89^{a}±1.27	21.34^{b}±1.29	35.60^{a}±1.13	23.09^{b}±0.87
	Day 60	29.66^{a}±1.23	22.31^{b}±1.31	36.98^{a}±0.78	24.14^{b}±0.98
	Day 90	29.66^{a}±1.23	21.66^{b}±1.07	37.77^{a}±0.68	24.30^{b}±1.11
IgM (mg/ml)	Before	2.74±0.07	2.68±0.08	2.71±0.06	2.71±0.06
	Day 10	2.89±0.06	2.62±0.06	3.05^{a}±0.4	2.66^{b}±0.09
	Day 30	3.01^{a}±0.09	2.55^{b}±0.08	3.04^{a}±0.07	2.68^{b}±0.07
	Day 60	2.98^{a}±0.04	2.53^{b}±0.07	3.06^{a}±0.05	2.48^{b}±0.04
	Day 90	3.00±0.07	2.66±0.05	3.10^{a}±0.06	2.64^{b}±0.05
IgA (mg/ml)	Before	0.65±0.02	0.62±0.04	0.68±0.02	0.65±0.02
	Day 10	0.78±0.03	0.67±0.03	0.92^{a}±0.03	0.64^{b}±0.03
	Day 30	0.86^{a}±0.03	0.57^{b}±0.04	0.95^{a}±0.03	0.64^{b}±0.04
	Day 60	0.88±0.04	0.65±0.05	0.97^{a}±0.02	0.66^{b}±0.03
	Day 90	0.88±0.03	0.66±0.04	1.04^{a}±0.03	0.67^{b}±0.02
FRAP (µM/l)	Before	688.95±34.67	668.65±45.13	691.97±44.67	698.92±52.45
	Day 10	912.12^{a}±45.39	698.95^{b}±40.17	1170.06^{a}±34.67	689.10^{b}±47.88
	Day 30	999.45^{a}±50.36	590.11^{b}±38.18	1314.61^{a}±33.89	703.25^{b}±43.76
	Day 60	1009.48^{a}±30.37	645.16^{b}±40.87	1367.99^{a}±42.89	677.13^{b}±35.67
	Day 90	1029.66^{a}±41.23	629.66^{b}±56.33	1366.66^{a}±54.77	686.20^{b}±23.44

$^{a, b}$ Means with different superscript in a row differ significantly (P<0.05).

(P<0.05) in animals affected with SCM (Table 4). The decline in milk lactose in affected quarters probably is in part due to the damage to the alveolar epithelial cells and a consequent reduction in the synthetic and secretory capacity of the gland as a whole. There was reduction in protein and SNF content in milk in animals affected with SCM/CM. Casein, the major milk protein of high nutritional quality, declines and lower quality of whey proteins increase which adversely affects the quality of milk products such as cheese. Ferric reduction anti-oxidant power in milk reduced significantly in animals affected with SCM.

Effect of mastitis supplement on blood neutrophil count, immunoglobulin and FRAP activity: Neutrophils are the predominant cells found in the mammary tissue and mammary secretions during early stage of mastitis and constitutes more than 90% of the total leukocytes (Sordillo *et al*. 1987). In supplemented animals, neutrophil count before supplementation was 57.65%, which significantly decreased to 30.56% on day 90 after calving. The neutrophil count was in the range of 58–62% in mastitis affected cows in control and supplemental groups, which was significantly (P<0.05) higher as compared to unaffected animals (Table 5).

The levels of immunoglobulin and FRAP activity were significantly higher in unaffected animals than the animals affected with SCM. The neutrophils exert their bactericidal effect through a respiratory burst and produce hydroxyl and oxygen radicals that kill the bacteria. Antioxidants and trace minerals play important roles in immune function, which in turn can influence health of mammary gland in transition dairy cows (Politis *et al.* 1995). The killing ability of immune cells is shown to be increased by nutritional supplementation with vitamin E and Cr, which have consistently been shown to improve neutrophil function in dairy cows (Persson 1992, Politis *et al.* 1996). Iodine play key role in prevention of *Staphylococcus aureus* infections responsible mastitis in dairy cows (Borucki Castro *et al.* 2012). Zinc and vitamin A have a critical role in maintaining the health and integrity of skin due to their role in cellular repair and replacement, key to the natural defence mechanism of the mammary gland (Smith *et al.* 1984, Sordillo *et al.* 1997, Garg *et al*. 2008). Evidence clearly shows that vitamin E and trace minerals influence phagocytic cell function and cows fed diets deficient in either component are at greater risk of environmental streptococcal mastitis (Smith *et al.* 1984). In addition, it has been reported that Zn supplementation reduces SCC due to its role in keratin formation. Zinc, Cu and Cr play an important role in removing superoxide radicals (free radicals) from the body. These radicals can disrupt cellular membranes and cause cellular damage leaving the mammary gland more susceptible to infection, scarring, and

loss in milk production (Xin *et al.* 1991, Sharma 2007). Supplement containing vitamin E at higher level in the present study might have played a key role in protecting animals from SCM and CM.

It is evident from the present study that incidence of SCM and CM could be reduced significantly by supplementing the ration of pre-parturient cows with vitamins E and A along with specific trace minerals in the form of chelates.

ACKNOWLEDGEMENT

Financial assistance and necessary facilities provided by the management of National Dairy Development Board, Anand, for undertaking this study, are gratefully acknowledged. Necessary assistance provided by the veterinary officers of Sabarkantha District Cooperative Milk Producers' Union Ltd., Himmatnagar, Sabarkantha in identification of advanced pregnant animals, is gratefully acknowledged.

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