Research Article

A Study on Reducing the Incidence of Sub-Clinical and Clinical Mastitis in Dairy Cows by Feeding a Vitamins and Minerals based Strategic Feed Supplement

B. M. Bhanderi[1*] and M. R. Garg[2]

National Dairy Development Board, Anand 388 001 (Gujarat)

One hundred twelve high yielding (>20kg/animal/day) crossbred cows having a history of clinical and sub-clinical mastitis in previous lactation were selected from 24 farms in Mohali and Ropar districts of Punjab. Thirty-three cows from eight farms served as control, and remaining were fed daily 10 g supplement per animal, containing chelated copper, zinc, chromium, vitamins E and A along with iodine for four weeks before calving. After calving, milk samples were collected on 10th and 40th day for analysis of somatic cell counts (SCC), sodium content, pH and electrical conductivity. The farm owners were provided with Mastect strips for routine check up for sub-clinical mastitis. Out of 79 experimental cows, only 17 showed positive signs for sub-clinical and clinical mastitis with Mastect strip test, which was confirmed by SCC in milk (2.97×10^5 cells/ml milk). However, out of thirty three, twenty nine animals in control group were affected by sub-clinical mastitis as indicated by SCC (3.19×10^5 cells/ml milk), which was later aggravated to clinical mastitis. The pH, electrical conductivity, SCC and sodium content in milk were higher ($P<0.01$) in animals affected by sub-clinical mastitis than the normal animals. In experimental group, SCC in 62 animals was within the normal range ($0.75\text{-}2.50 \times 10^5$ cells/ml milk) and no signs of sub-clinical or clinical mastitis were observed. The inference could be drawn that feeding a vitamins and chelated minerals based strategic feed supplement for four weeks prior to calving can reduce the incidence of sub-clinical and clinical mastitis significantly in dairy cows under farm conditions.

***Keywords:* Sub-clinical mastitis, minerals, vitamin E, somatic cell count, crossbred cows**

INTRODUCTION

Mastitis is a serious infectious disease of dairy animals causing great economic losses due to reduction in milk yield, as well as lowering its quality and nutritive value. Sub-clinical mastitis (SCM) is 30-40 times more prevalent than clinical mastitis. Additionally, the disease is important from consumers' and processors' point of view (Wheelock *et al.*, 1996). The milk from an affected animal may harbor the organisms potentially pathogenic for humans (Sharma *et al.*, 2006). Mastitis affects the milk quality in terms of decrease in milk protein, fat, lactose contents and increase in somatic cell counts. Dairy animals are most susceptible to mastitis during two weeks prior to calving and two weeks post-calving. Amongst others, proper feeding of dry 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). Rations fed to high yielding cows in India are often deficient in zinc, copper (Garg *et al.*, 2008), chromium and vitamins A and E that are primary minerals and vitamins affecting immune function and susceptibility to sub-clinical and clinical mastitis (Drake et al., 1992; Torre *et al.*, 1996; Harmon and Torre, 1997). It has been reported that 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 utmost desirable to supplement the ration with requisite vitamins and minerals, especially prior to calving to improve immune function of animals.

*1 *Corresponding Author: Scientist-II, Animal Nutrition Group, 2nd Floor, CALF Building, National Dairy Development Board, Anand 388 001 (Gujarat), India, Phone: +91-2692-226270, E mail: bhanderi@nddb.coop*

2 *General Manager, Animal Nutrition Group, 2nd Floor, CALF Building, National Dairy Development Board, Anand 388 001 (Gujarat), India*

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In view of this, the present study was undertaken to investigate the efficacy of feeding a coated vitamins and chelated minerals based strategic feed supplement for reducing the incidence of sub-clinical and clinical mastitis in dairy cows, maintained under farm conditions.

MATERIALS AND METHODS

A primary survey was conducted in Mohali and Ropar districts of Punjab, to identify advanced pregnant crossbred cows having history of mastitis in previous lactation and one hundred twelve animals from well managed 24 progressive dairy farms were identified. Seventy nine animals at 16 farms were taken under the experimental group and fed daily one sachet of 10 g supplement per animal for four weeks prior to calving. The supplement contained chelated trace minerals (Cu, Zn, Cr), vitamins A and E 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 A and E were supplemented as per NRC (2001), considering the requirement during advanced stage of pregnancy. Thirty-three animals at 8 farms were maintained without supplement and served as control. On an average, animals were fed on maize fodder (12-15 kg), berseem (15-20 kg) and ad lib wheat straw. Each cow was fed homemade concentrate mixture or compound cattle feed, based on the level of milk production at the time of milking. The average milk production of crossbred cows identified for the study was 22.9 kg/animal/day with 3.2 per cent fat in milk while in production. All the animals under control and experimental groups were checked weekly for sub-clinical mastitis, post calving for a period of 120 days, using Mastect strip test, developed by Indian Immunologicals Ltd., Hyderabad. Milk samples were collected from

Table 1: Locations and farm size in Mohali and Ropar districts of Punjab for identification of trial animals

Location	Farm size (n)	No. of dry/ advanced pregnant animals	No. of animals having history of clinical/ sub-clinical mastitis in previous lactation
Control group			
Farm-1(Kheni, Mohali)	145	70	8
Farm-2 (Alipur, Mohali)	55	30	3
Farm-3 ((Peer Suhana, Mohali)	20	7	2
Farm-4 (Sahpur, Mohali)	140	60	7
Farm-5 (Chkalan, Ropar)	85	27	4
Farm-6 (Khurad, Ropar)	30	12	2
Farm-7 (Manouli Kalan, Ropar)	46	26	3
Farm-8 (Kalara, Ropar)	56	32	4
Total	577	264	33
Experimental group			
Farm-1 (Tanouri, Ropar)	66	16	4
Farm-2(Kotla, Ropar)	40	20	6
Farm-3 (Khurad, Ropar)	34	14	3
Farm-4 (Saheni, Ropar)	69	34	8
Farm-5 (Chouta Kalan, Mohali)	80	35	9
Farm-6 (Manouli Kalan, Ropar)	38	18	5
Farm-7 (Saheni, Ropar)	71	26	2
Farm-8 (Kalara, Ropar)	83	33	4
Farm-9 (Alipur, Mohali)	46	16	4
Farm-10 (Manouli Kalan, Ropar)	35	10	2
Farm-11 (Manouli Kalan, Ropar)	85	25	4
Farm-12 (Manouli Kalan, Ropar)	45	15	3
Farm-13 (Gupalpur, Ropar)	95	35	5
Farm-14 (Manouli Kalan, Ropar)	70	34	5
Farm-15 (Balogi, Ropar)	125	60	2
Farm-16 (Ganga, Ropar)	122	72	13
Total	1104	463	79

all four quarters of an animal in sterile polyethylene screw capped, wide mouth vials after squirting few streams, on 10th and 40th day after calving, from all the animals under control and experimental groups for estimation of sodium content (Brooks *et al.*, 1996) and somatic cell counts (IDF, 1984). The milk samples were mixed thoroughly before a final amount of 0.01 ml of milk was pipetted and spread evenly on the entire area of the special slide (special circular slide with an area of 1 cm^2 circle). After drying, the slide was stained with the prepared stain (New Man stain) for two minutes and then the cells were counted under oil immersion. The pH and electrical conductivity in milk samples were also measured on the spot with the help of portable digital pH/conductivity meter (Model Eutech, PCSTEST35-01X441506). The data on milk pH, electric conductivity, somatic cell counts and sodium content 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).

RESULTS AND DISCUSSION

Farm Size and Animal Identification

Farm size and number of animals having history of clinical and sub-clinical mastitis in previous lactation are presented in Table 1. Average number of cows per farm was 70. A total of 727 animals in advanced stage of pregnancy were screened for the study. Out of 727, 112 animals were identified for the study, having history of clinical and sub-clinical mastitis in the previous lactation. Mean pH, electrical conductivity, somatic cell counts and sodium content in milk for the control and experimental groups post calving are shown in Table 2.

Effect of Feeding Supplement on pH and Electrical Conductivity of Milk

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 sub-clinical mastitis was 6.60 and 6.63 on day 10 and 40, respectively, which was significantly higher ($P<0.01$) than that of recorded in the normal animals. In normal animals (n=62), pH was 6.42 and 6.48 on 10th and 40th day, respectively. In control group, out of 33, 29 animals were affected by sub-clinical mastitis 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, electrical conductivity in normal animals was 6.42 and 6.48 mS/cm on day 10 and 40, which increased ($P<0.01$) to 6.60 and 6.63 mS/cm in infected animals. Similar trend was observed in control group animals affected with mastitis (Table 2). The electrical conductivity 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. Electrical conductivity 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 and Sodium Content in Milk

Milk samples from all the animals in control and experimental groups were checked for sub-clinical mastitis (SCM). Out of 112, 46 animals were found to be positive for SCM, which were subjected to somatic cell counts (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 sub-clinical mastitis 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 test showed SCC in the range of 0.9 to 2.5 ($x10^5$/ml of milk) and 0.73 to 2.75 ($x10^5$/ml milk) on 10th and 40th day, respectively. Cows affected by SCM showed SCC in the range of 1.56 to 4.90 ($x10^5$/ml milk) and 1.39 to 4.11 ($x10^5$/ml of milk), on 10th and 40th day, respectively which was significantly higher than the normal animals (Table 2). Similarly, animals in control group affected by SCM showed higher SCC than the normal animals. On the basis of results of Mastect strip test and SCC in milk, animals affected by mastitis were 88 and 21 per cent in control and experimental groups, respectively. 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).

Table 2: Effect of feeding chelated minerals and vitamins based supplement on different parameters in high yielding crossbred cows

Particular	Milk pH		Electrical conductivity (mS/cm)		Mastect strip test		Somatic cell count (x10^5/ml of milk)		Sodium content (mg/dl)	
	Day 10	Day 40	Day 10	Day 40	Day 10	Day 40	Day 10	Day 40	Day 10	Day 40
Control group (n=33)										
Normal animals (n=4)	6.44^{a} ±0.07 (6.30-6.60)	6.34 ±0.10 (6.12-6.54)	4.37^{c} ±0.10 (4.14-6.65)	4.57^{a} ±0.11 (4.34-4.88)	N	N	2.05^{a} ±0.36 (1.24-2.98)	2.37^{a} ±0.39 (1.27-3.10)	54.0^{c} ±7.13 (38-67)	68.0^{c} ±8.87 (45.5-88)
Animals affected with sub-clinical/clinical mastitis (n=29)	6.64^{b} ±0.03 (6.38-6.87)	6.59 ±0.05 (6.23-7.85)	5.19^{d} ±0.06 (4.59-5.83)	4.97^{b} ±0.07 (4.49-5.80)	S	S	3.02^{b} ±0.12 (1.65-4.11)	3.36^{b} ±0.15 (2.39-5.0)	146.0^{d} ±13.1 (52.8-345)	133.9^{d} ±11.5 (55.4-234)
Experimental group (n=79)										
Normal animals (n=62)	6.42^{c} ±0.02 (6.30-6.74)	6.48^{c} ±0.02 (6.26-6.69)	4.48^{c} ±0.04 (4.03-5.15)	4.52^{c} ±0.04 (4.24-5.40)	N	N	1.40^{c} ±0.06 (0.9-2.50)	1.46^{c} ±0.06 (0.73-2.75)	80.0^{c} ±4.22 (34-234)	67.53^{c} ±3.52 (51.0-171)
Animals affected with sub-clinical/clinical mastitis (n=17)	6.60^{d} ±0.04 (6.42-6.85)	6.63^{d} ±0.04 (6.31-6.90)	5.12^{d} ±0.06 (4.78-5.93)	5.23^{d} ±0.06 (4.86-5.84)	S	S	3.07^{d} ±0.22 (1.56-4.90)	2.87^{d} ±0.19 (1.39-4.11)	136.2^{d} ±14.8 (57.9-234)	128.1^{d} ±12.3 (61.5-212.5)

$^{a,\ b}$ Means with different superscript in a column differ significantly ($P<0.05$)
$^{c,\ d}$ Means with different superscript in a column differ significantly ($P<0.01$)
N= Normal animals; S= Suspected with sub-clinical mastitis.

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 *Staphyllococcus 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). In addition, it has been reported that Zn supplementation reduces SCC due to its role in keratin formation. Zinc and Cu 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 sub-clinical and clinical mastitis.

Major milk electrolytes are sodium, potassium and chlorides. Levels of sodium and chloride increase during clinical or sub-clinical mastitis, whereas, potassium decreases. These imbalances result into decrease in quality and taste of milk. In supplemented group, sodium content in normal animals was 80 and 67.53 mg/dl, on 10th and 40th day, respectively which increased significantly ($P<0.01$) to 136.2 and 128.1 mg/dl in SCM affected animals (Table 2). 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 sodium content in unaffected animals.

CONCLUSIONS

It is evident from the present study that incidences of sub-clinical and clinical mastitis could be reduced by supplementing the ration of dairy animals with vitamins A and E along with specific trace minerals in the form of chelates, for about four weeks prior to calving.

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