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Evaluation of metabolic status and milk compositions of indigenous cattle with subclinical mastitis and its amelioration by nutritional supplementations

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ABSTRACT

Indigenous cattle that were in early lactation and positive for subclinical mastitis were allocated into 2 groups; one group was administered with nutrional supplements (50 g mixture of vitamins A, D, E and thiamine, riboflavin, pantothenic acid, biotin, niacin, trisodium citrate dihydrate, methionine, manganese, copper, zinc, cobalt, selenium and live yeasts orally daily for 7 days), while other was kept as negative control. Milk composition of mastitic milk and metabolic status of affected cows were evaluated at day 0 and day 7 post-therapy. On day 0, remarkable alteration in milk composition as well as in metabolic status of affected animals was recorded in comparison to the healthy control. However, the altered nutrional panels as well as milk compositions were ameliorated toward normalcy at day 7 post-therapy in mastitic cows administered with nutrional supplements. At day 7 post-therapy, remarkable improvements in somatic cell count was also recorded in these cows when compared with day 0 values within the group, but the values were still significantly higher than the healthy control. Thus, subclinical mastitis in indigenous cattle could bestow remarkable alterations in milk compositions and metabolic status. The altered metabolic panels and milk compositions can be ameliorated toward normalcy by administering nutritional supplements.

Key words: Metabolic profile, Milk composition, Nutritional supplementation, Subclinical mastitis

India has attained the first position among the milk producing countries in the world, but still per animal milk productivity is substantially low. One of the most important causes of low milk production could be subclinical mastitis. These subclinical infections are a serious concern for dairy farmers because of decreased milk production, reduced milk quality, and increased transmission of pathogens (Mweu *et al*. 2012). Mastitis causes injury to milk secretory cells in the mammary gland, which interferes with the synthesis of lactose, fat and protein and decreases the food value of milk (Forsback *et al.* 2010). The causes of mastitis involve a complex relationship of 3 major factors, that is, host resistance, bacterial agents and the environmental factors (Mweu *et al*. 2012, Dimri *et al.* 2013). The increased susceptibility of the mammary gland to intramammary infections during the transition period has been linked to a compromised state of the innate defense system as well as negative energy balance (Esposito *et al.* 2014). Altered metabolic status of the lactating cows bestows favorable conditions for intramammary infections. Globally, in the recent years, researchers are investigating the role of non-conventional treatment for prevention and control of bovine mastitis. The disease remains the most frequent cause of antibacterial use on dairy farms, and contributes to a substantial portion of total drug and veterinary costs incurred by the dairy industry (Wellnitz and Bruckmaier 2012). Thus, in the present study, we indented to evaluate the metabolic status and milk composition of indigenous cattle with subclinical mastitis and its amelioration by nutritional supplementations.

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MATERIALS AND METHODS

Indigenous cattle that were in early lactation (within 1 month of parturition) and detected positive for subclinical mastitis by California mastitis test (CMT) and high somatic cell count (>5 lakh cells/ ml) were allocated into group 1 and 2 with 32 cows in each. For healthy controls (group 3) 29 indigenous cows, free from mastitis and in early lactation, were used. In group 1, each cow with subclinical mastitis was administered with a nutritional commercial preparation @ 50 g PO daily for 7 days. Each 50 g of nutritional commercial preparation contained, vitamin A (70,000 IU),

D (20,000 IU), E (1,000 IU), trisodium citrate dihydrate (25 g), niacin (2,000 mg), thiamine hydrochloride (200 mg), riboflavin (200 mg), pantothenic acid (50 mg), biotin (20 mg), methionine (10 g), manganese sulphate (4 g), copper sulphate (1200 mg), zinc sulphate (500 mg), cobalt sulphate (50 mg), selenium (4 mg) and live yeast (50,000 million CFU). In group 2, nutrional supplements were not administered and were kept as standard negative control. Blood and milk samples were obtained from each cow at day 0 and day seventh post-therapy.

Milk lactose, protein, fat, solid-not-fat (SNF), salt contents, specific gravity and depression in freezing point were assessed using milk analyzer. Serum glucose, total protein, triglyceride, cholesterol, urea, calcium (Ca), magnesium (Mg) and phosphorus (P) levels were estimated to evaluate the nutritional status of the affected and treated cows by using chemistry analyzer using kits. All data were expressed as mean±S.E. The SCC values of each cow were transformed to $\log_{10}$ to obtain a normal distribution. Statistical analysis was conducted to determine the difference among the groups at the same sampling time by using MANOVA, post-hoc Tukeys test with general linear models in SPSS 16. The comparison between pre- and post-treatment values within the group was analyzed by the paired t-test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

On day 0, significant increase in protein content ($P \leq 0.0001$) and depression in freezing point (P= 0.019) of milk from affected cows ware recorded when compared with the healthy control (Table 1). However, lactose, fat and SNF contents and specific gravity were significantly lower ($P \leq 0.0001$) in comparison to the healthy control (Table 1). While, no significant alteration was recorded in salt content. At day 7 post-therapy, significant ameliorations in protein content and depression in freezing point of milk samples obtained from the affected cows administered with nutritional supplements ware recorded (Pd"0.0001) when compared with the day 0 values within the group (Table 1). However, milk samples of cows without nutrional supplementations revealed significantly higher contents of protein ($P \leq 0.0001$) and depression in freezing point (P=0.001) in comparison to the healthy control. Moreover, significant improvements in lactose ($P \leq 0.0001$), fat (P=0.027), SNF (P= 0.023) contents and specific gravity ($P \leq 0.0001$) were recorded in affected cows administered with nutritional supplements when compared with day 0 values within the group. But, milk specific gravity of these cows was still significantly lower (P=0.002) in comparison to the healthy control. However, lactose, fat and SNF contents of the affected cows without nutrional supplementations ware still significantly lower ($P \leq 0.0001$) in comparison to the healthy control and day 0 values within the group. Moreover, significant deterioration (P=0.038) in milk specific gravity of these cows was recoded. Additionally, the values of specific gravity of these cows were also significantly lower ($P \leq 0.0001$) in comparison to the healthy control. On day 0, $\log_{10}$ values for SCC of cows with SCM ware significantly higher ($P \leq 0.0001$) in comparison to the healthy control (Table 1). At day 7 post-therapy, the affected cows administered with nutrional supplements revealed significant reduction in (P=0.003) SCC values in comparison to their day 0 values (Table 1). However, significant reduction in SCC values of untreated affected cows was not recorded when compared with their day 0 values. Moreover, SCC values of the both groups (with and without nutritional supplementations) were still significantly higher ($P \leq 0.0001$) in comparison to the healthy control.

The results of the present study indicate higher contents of total protein and lower contents of lactose, fat and SNF in milk from affected quarters compared to the healthy quarters. It is well known that lactose content decreases during mastitis as a consequence of the lower blood-milk barrier caused by increased tight junction permeability and

Table 1. Comparison of milk compositions and SCC of subclinical mastitic and healthy cows at day 0 and day 7 post-therapy (mean±SE)

Panels	Subclinical mastitis (without nutritional supplements) (n=32)		Subclinical mastitis (with nutritional supplements) (n=32)		Healthy controls (n=29)
	Day '0'	Day '7'	Day '0'	Day '7'	-
$\log_{10}$ SCC	5.89±0.03^{A}	5.98±0.02^{A}	5.874±0.03^{A}	5.74±0.03^{A,I}	5.53±0.01
Fat (%)	3.70±0.07^{A}	3.69±0.07^{A}	3.80±0.08^{A}	4.09±0.08^{E}	4.27±0.11
SNF (%)	8.67±0.11^{A}	8.58±0.11^{A}	8.67±0.01^{A}	9.08±0.08^{F}	9.15±0.05
Sp. Gra.	1.017±0.001^{A}	1.014±0.001^{A,D}	1.018±0.00^{A}	1.025±0.001^{C,G}	1.028±0.00
Protein (%)	4.009±0.04^{A}	4.04±0.04^{A}	4.0006±0.06^{A}	3.6±0.04^{G}	3.48±0.04
Lactose (%)	3.94±0.11^{A}	3.817±0.10^{A}	3.946±0.09^{A}	4.79±0.06^{G}	4.948±0.02
F.P (Depreciation)	0.553±0.001^{H}	0.554±0.001^{B}	0.554±0.002^{H}	0.549±0.001	0.55±0.001
Salt (%)	0.688±0.004	0.689±0.004	0.689±0.003	0.686±0.003	0.685±0.005

$^{A, B \text{ and } C}$ statistically significant difference at ($P \leq 0.0001$), (P=0.001) and (P=0.002) respectively when compared with healthy controls. $^{D, E, F, G, H \text{ and } I}$ statistically significant difference at (P=0.038), (P=0.027), (P=0.023), (Pd+0.0001), (P=0.019) and (P=0.003) respectively when compared with day 0 values of the same group.

damaged epithelial cells. Reduction in lactose content of mastitic milk could also be due to its losses into the circulation through damaged epithelial cells and leaky tight junctions. Moreover, lowered SNF contents of milk from affected quarters might be the effect of decreased lactose content. Since lactose and protein are the major components of SNF, it appeared that drop in SNF was mainly due to decreased lactose content in mastitic milk. Our findings are in agreement with the recent scientific reports demonstrating remarkably reduced content of lactose in subclinical mastitic milk (Forsback *et al.* 2010, Hussain *et al.* 2012, Malek dos Reis *et al.* 2013).

An increased permeability of the blood-milk barrier also results in an influx of serum proteins and enzymes (such as plasminogen) from the blood, which may lead to increased proteolysis. Plasmin and other proteolytic enzymes, such as cathepsin, elastase and collagenase, all contribute to the degradation of caseins I in milk (Kelly *et al.* 2006). In the present study we have not estimated the milk caseins content, but total milk protein content was evaluated. The results of the present study revealed an elevated protein content in milk from affected quarters; might be the outcome of damaged blood-milk barrier and thus an influx of serum proteins. The increased protein content could also be the out come of a much greater decreased milk volume after infection than in per day synthesis of this component. Using the total protein content as a milk quality marker is therefore questionable, whereas measuring the whey protein content, in addition to total protein, could be more correct validation for the protein quality of milk. Our findings are in accordance with the other scientific reports demonstrating higher total protein content of milk owing to the increased SCC and mastitis (Nielsen *et al.* 2005, Forsback *et al.* 2010).

Our results clearly indicated remarkable amelioration of SCC and altered milk composition towards normalcy by administration of nutritional supplements in cows with SCM. The administered nutritional supplements are balanced mixture of vitamin A, vitamin D, vitamin E, trisodium citrate dihydrate, niacin, thiamine hydrochloride, riboflavin, pentothenic acid, biotin, methionine, manganese sulphate, copper sulphate, zinc sulphate, cobalt sulphate, selenium and live yeast. These supplements might have holistically balanced the altered panels owing to their positive effects on the health status of suffering cows. It has been established that dairy cattle subjected to the demands of late pregnancy, parturition or peak lactation may be subjected to oxidative stress or the production of reactive oxygen metabolites (Spears and Weiss 2008). Immune cells are sensitive to oxidative stress as their membranes contain high concentrations of polyunsaturated fatty acids that are vulnerable to lipid peroxidation as well as they produce large quantities of reactive oxygen metabolites when stimulated (Spears and Weiss 2008). Several trace elements and vitamins are potentially useful in maintaining an appropriate balance of antioxidants in the dairy cows to cope with the increased production of reactive oxygen metabolites around parturition. The lists of potentially useful trace elements that functions as or are key components of antioxidants include copper (Cu), selenium (Se), zinc (Zn), vitamin-E and ß-carotene (Machado *et al.* 2013). Therefore, these components of administered nutritional supplements in the present study might have augmented the immune status of affected cows by ameliorating various conduits, if immunological deficit was encountered and thus bestowed recovery from subclinical mastitic condition. In agreement to our findings, healing potentials of various nutritional supplements on subclinical mastitis and improvement in milk yield and milk quality have been reported by various recent scientific workers (Dimri *et al*. 2013, Machado *et al.* 2013, Mir *et al.* 2014). It has been reported extensively that mastitic milk is significantly low in citrate and a certain minimum concentration of citrate is essential for the normal synthesis of milk in the alveoli in the udder. The deficiency of citrate in a particular quarter may be due to nutritional, metabolic or some other intrinsic unknown factors. Thus, supplementation of trisodium citrate dihydrate in affected cows might have helped in faster recovery from the

Table 2. Comparison of metabolic status of subclinical mastitic and healthy cows at day 0 and day 7 post-therapy (mean±SE)

Panels	Subclinical mastitis (without nutritional supplements) (n=32)		Subclinical mastitis (with nutritional supplements) (n=32)		Healthy controls (n=29)
	Day 0	Day 7	Day 0	Day 7	
GLU (mg/dL)	39.3±1.4^{A}	36.7±1.6^{B}	38.7±1.9^{A}	44.6±1.6	46.2±1.4
TG (mg/dL)	10.6±0.4	10.0±0.5	10.5±0.4	11.7±0.5	11.8±0.7
CHOL(mg/dL)	115±3.9	112±2.9	114±3.2	121±2.9	123±4.7
Ca(mg/dL)	7.98±0.1^{D}	7.84±0.2^{F}	8.10±0.1^{D}	8.50±0.1	8.60±0.2
TP(g/dL)	6.94±0.1	6.834±0.2	6.94±0.1	7.035±0.1	7.08±0.2
UREA(mg/dL)	34.0±0.6^{B}	35.2±0.5^{B}	34.1±0.8^{B}	30.5±0.9^{E}	29.0±1.2
P(mg/dL)	5.9±0.05^{C}	5.85±0.06^{G}	5.91±0.07^{C}	6.07±0.07	6.16±0.09
Mg(mg/dL)	1.96±0.04	1.94±0.04	1.96±0.03	2.01±0.03	2.04±0.04

$^{A, B, C, D, F \text{ and } G}$statistically significant difference at (P=0.001), (P<70.0001), (P=0.005), (P=0.003), (P=0.006), and (P=0.011) respectively when compared with healthy controls. ^{E}statistically significant difference (P=0.004), when compared with day 0 values of the same group.

condition. At day 7 post-therapy, the SCC values were significantly higher even in the nutritional supplemented cows; indicates that the complete recovery from SCM requires supplementation of vitamins and trace minerals for a longer period (> 7 days).

The levels of studied metabolic panels; glucose, total protein, triglyceride, cholesterol, blood urea, calcium, magnesium and phosphorus at day 0 and day 7 post-therapy are depicted in Table 2. On day 0, significantly lower levels of glucose (P=0.001) calcium (P=0.003) and phosphorus (P=0.005) ware recorded in affected cows when compared with the healthy control. However, urea level of affected cows was significantly higher (P≤0.0001). Significant differences for triglycerides, cholesterol, total protein and magnesium contents in these cows ware not found. At day 7 post-therapy, remarkable improvements in glucose, calcium and phosphorus levels in cows administered with nutritional supplements ware recorded and the values were almost comparable to the values of the healthy control. Contrarily, urea level of these cows was significantly reduced (P=0.004). However, glucose, calcium and phosphorus and urea levels of the affected cows without nutritional supplementations were not ameliorated towards normalcy.

Remarkably lowered levels of serum calcium and glucose in the affected cows indicate that the cows with lower serum calcium level and in negative energy balance could be more prone to intramammary infections. One of the reasons, that infectious diseases such as mastitis may be associated with a poorly managed transition period, is that the dairy cow experiences a substantial periparturient immunosuppression (Esposito *et al*. 2014). Immunosuppression together with marked changes in endocrinological, nutritional and metabolic status cause remarkably increased concentrations of circulating cortisol around parturition. These dynamic changes seem to be central to the metabolic disturbances which favor the establishment of infections in the postpartum cow (Goff 2006, Spears and Weiss 2008). The severity of this immunosuppression is exacerbated by factors such as negative energy balance and hypocalcaemia (Ducusin *et al.* 2003). Hypocalcemia reduces feed intake so that greater body fat mobilization occurs in early lactation. Additionally, it reduces all muscle contraction including the teat sphincter muscle responsible for closure of the teat orifice after milking, thus increases the risk of mastitis (Goff 2008). More recently it has been demonstrated that hypocalcemia directly impairs immune cell response to an activating stimulus (Kimura *et al.* 2006). Blood urea level can be used as an indirect measure of rumen ammonia in ruminants with normal kidney function. Although no specific disease state is associated with abnormal herd urea levels, valuable information concerning dietary protein content and utilization can be detected from herd urea levels. High blood urea levels are consistent with excessive protein intake. Thus, the elevated level of urea in mastitic cow could be the effect of highly degradable protein diet and/or proteolysis in the body of affected cows. Moreover, alterations in metabolic status could be accountable as a predisposing cause of subclinical mastitis in cattle with early lactation. In agreement to the results of the current study, an association between altered metabolic status of parturient cattle and occurrence of various diseases including mastitis has been recently addressed by different scientific workers (Goff 2008, Sordillo and Raphael 2013, Esposito *et al.* 2014). The supplementation of vitamins, trace minerals and yeasts might have enhanced the feed intake as well as udder immunity and thus, curtailing the intramammary infections of affected cows. In agreement, amelioration of subclinical mastitis by various nutritional supplementations has been documented (De and Mukherjee 2013, Dimri *et al*. 2013, Trevisi *et al*. 2014). Thus, it can be concluded that remarkable alterations in milk compositions and metabolic status may be ensued in indigenous cattle suffering from subclinical mastitis. The altered metabolic panels and milk compositions can be ameliorated toward normalcy by administering of nutritional supplements. Further, studies are required to evaluate the time period required for the complete recovery of subclinical mastitis by these nutritional supplementations.

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