

## ASSESSMENT OF ALTERATION IN METABOLIC PROFILE AND MILK COMPOSITION OF BUFFALOES WITH SUBCLINICAL MASTITIS

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**ABSTRACT**

The present study aimed to assess the metabolic status and milk composition of buffaloes with subclinical mastitis. Forty buffaloes in early lactation from local buffalo dairy farms of Mathura district Uttar Pradesh (India) and instructional livestock farm complex of DUVASU, Mathura, found positive for subclinical mastitis at the quarter level by California mastitis test (CMT) (++) score and high somatic cell counts (SCC) (>5 lakh cells per ml) were included in the present study. Another 20 clinically healthy buffaloes in early lactation and free of mastitis were used as healthy control group. Blood samples (5 mL) were collected from the both healthy and subclinical mastitic buffaloes and were used for estimation of serum metabolites including glucose, total cholesterol, triglycerides, total protein, albumin, urea, calcium (Ca), magnesium (Mg) and phosphorus (P). Further, milk samples were obtained from each affected quarter of buffaloes having subclinical mastitis as well as from healthy group and used for assessment of milk composition. To evaluate the milk quality, milk lactose, protein, fat, solid-not-fat (SNF), salt, specific gravity and depression in freezing point were estimated. Statistical differences between groups were evaluated by using ANOVA

with general linear models. Significantly lower serum glucose ( $P \leq 0.01$ ), Ca ( $P \leq 0.05$ ) and P ( $P \leq 0.05$ ) levels were revealed by buffaloes with subclinical mastitis as compared to healthy control group. Whereas, serum urea level of buffaloes with subclinical mastitis was significantly higher ( $P \leq 0.01$ ) than healthy control group. However, significant alteration in serum total cholesterol, triglycerides, total protein and magnesium contents was not revealed by buffaloes with subclinical mastitis in comparison with the healthy controls.

Milk samples obtained from buffaloes with subclinical mastitis revealed significantly higher ( $P \leq 0.01$ ) total protein and salts contents as well as depression in freezing point as compared to that of the healthy control group. However, significantly lower lactose ( $P \leq 0.01$ ), fat ( $P \leq 0.01$ ), SNF ( $P \leq 0.01$ ) contents and specific gravity ( $P \leq 0.05$ ) was estimated in mastitic milk as compared to non-mastitic milk (healthy control group). Therefore, it can be concluded that remarkably altered metabolic status of buffaloes could be associated with subclinical mastitis. In tandem, subclinical mastitis could result in marked alteration in the milk composition conferring the poor milk quality of buffaloes.

**Keywords:** buffaloes, *Bubalus Bubalis*, metabolic

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status, milk composition, subclinical mastitis

## INTRODUCTION

The dairy sector in the India has shown remarkable development in the past decade and the country become the largest producer of milk and value-added milk products among the milk producing countries. This was mainly achieved by momentous improvement in the productivity of bovine population, particularly of buffaloes, during the past three decades. Population of buffaloes has increased at a faster rate than cattle in India, confirming their pivotal role in the agricultural economy of the sub-continent. Albeit, India has attained the first position in the milk production but still per animal milk productivity is substantially not enough. One of the most important causes of low milk production could be mastitis. Mastitis can be defined as an inflammatory disease of the mammary gland parenchyma; characterized by a range of physical and chemical changes of milk and pathological changes in the udder tissues (Contreras and Rodriguez, 2011). 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 (Ozawa *et al.*, 2011; Wellnitz and Bruckmaier, 2012). It adversely affects animal health, quality of milk and economics of milk production globally and entails huge financial losses (Tesfaye *et al.*, 2010; Plozza *et al.*, 2011). It may be in the form of clinical or subclinical. Subclinical cases show no visible changes in the appearance of the milk or the udder, but milk production decreases, composition is altered and bacteria are present in the secretion (Mansor *et al.*, 2013). Presently, subclinical mastitis is considered

to be the main form of mastitis in modern dairy herds (Tesfaye *et al.*, 2010; Mweu *et al.*, 2012).

The occurrence of disease is an outcome of interplay between the infectious agents and management practices stressing the defense of udder (De Vlieghe *et al.*, 2012; Dimri *et al.*, 2013). Inflammation in mastitis may damage the mammary secretary epithelium and decreases the food value of milk by interfering with the synthesis of lactose, fat and protein (Ullah *et al.*, 2005). Milch buffaloes were earlier thought to be less susceptible to mastitis compared to cows, but similar frequencies for both the species were reported in the recent past from different quarters (Moroni *et al.*, 2006). Identification of factors predisposing animals for subclinical mastitis is imperative for the development of control and prevention strategies. Metabolic demands associated with late pregnancy, parturition, and initiation of lactation would be expected to increase the production of reactive oxygen species (Sordillo, 2009). Additionally, increased susceptibility of the mammary gland to intramammary infections during transition period has been linked to a compromised state of the innate defense system (Sordillo, 2009) as well as negative energy balance (Esposito *et al.*, 2014). Altered metabolic status of the lactating buffaloes can bestow favorable condition for the intramammary infections. Thus, in the present study we indented to evaluate the metabolic status and milk composition of buffaloes with subclinical mastitis.

## MATERIALS AND METHODS

Lactating Indian water buffaloes from local buffalo dairy farms of Mathura district Uttar Pradesh (India) and instructional livestock farm complex of DUVASU, Mathura were screened for

subclinical mastitis at the quarter level by using California Mastitis Test (CMT) and Somatic Cell Counts (SCC). Forty buffaloes in early lactation (within one month of parturition) and found positive for subclinical mastitis by CMT (++) score) and high SCC (>5 lakh cells per ml) were used for the study. Simultaneously, 20 buffaloes in early lactation and free of mastitis were used as healthy control group for comparing the estimated panels of subclinical mastitic buffaloes.

#### **Assessment of metabolic status**

5 mL blood sample each was collected from the both healthy and subclinical mastitic buffaloes by jugular venipuncture into a tube containing clot activators and was used for serum harvesting. To evaluate the metabolic status, serum metabolites including glucose, total cholesterol, triglycerides, total protein, albumin, urea, calcium (Ca), magnesium (Mg) and phosphorus (P) were determined by using automated biochemistry analyzer (BS-120 Chemistry Analyzer; Shenzhen Mindray Biochemical Electronics Co. Ltd.) using the biochemistry estimation kits from RFCL Ltd. and Dialab.

#### **Assessment of milk composition**

20 mL milk samples were also obtained from each affected quarter of buffaloes having subclinical mastitis and used for assessment of milk composition. Moreover, 20 mL milk samples were also obtained from healthy controls and used as standard for milk composition comparison. To evaluate the milk quality, milk quality assay panels including lactose, protein, fat, solid-not-fat (SNF), salt, specific gravity and depression in freezing point were estimated by using milk analyzer Lactoscan of Milkotronics Ltd.

#### **Statistical analysis**

Statistical differences between groups were evaluated by using using ANOVA with general linear models in SPSS 16. Data were presented as mean  $\pm$  standard error ( $\pm$ SE) and the significance level was set as  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

Serum metabolic panels; glucose, total protein, triglyceride, cholesterol, blood urea, calcium (Ca), magnesium (Mg) and phosphorus (P) were estimated to assess the metabolic status of buffaloes suffering from subclinical mastitis and compared with the healthy control (Table 1). Serum glucose level of buffaloes with subclinical mastitis was significantly lower ( $P \leq 0.01$ ), when compared with the healthy control. Additionally, significantly lower levels of serum calcium ( $P \leq 0.05$ ) and phosphorus ( $P \leq 0.05$ ) were also recorded in buffaloes with subclinical mastitis. Whereas, serum urea level of buffaloes with subclinical mastitis was significantly higher ( $P \leq 0.01$ ) than healthy control group. However, significant alteration in serum total cholesterol, triglycerides, protein and magnesium contents was not revealed buffaloes with subclinical mastitis in comparison with the healthy control.

Remarkably decreased serum calcium, phosphorus, glucose levels of buffaloes with subclinical mastitis indicate that the buffaloes with altered nutritional status 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 animals experience a substantial periparturient immunosuppression (Esposito *et al.*, 2014).

Immunosuppression together with marked changes in endocrinological, nutritional and metabolic status cause increased concentrations of circulating cortisol around parturition. These dynamic changes seem to be central to the metabolic disturbances which favors the establishment of infection in the postpartum dairy animals (Goff, 2006; Spears and Weiss, 2008). The severity of this immunosuppression is exacerbated by factors such as negative energy balance (Ohtsuka *et al.*, 2006), hypocalcaemia (Ducusin *et al.*, 2003) and increased circulating levels of cortisol for prolonged periods around calving (Burton *et al.*, 2005). Moreover, hypocalcemia reduces feed intake so that greater body fat mobilization occurs in early lactation. It also reduces all muscle contraction including the teat sphincter muscle responsible for closure of the teat orifice after milking, thus increasing the risk of mastitis.

It has been demonstrated that hypocalcemia directly impairs immune cell response to an activating stimulus (Kimura *et al.*, 2006). In agreement to the results of the present study, an association between altered metabolic status of parturient cattle and occurrence of various diseases including mastitis has recently been addressed by different scientific workers (Goff, 2008; Sordillo and Raphael, 2013; Esposito *et al.*, 2014; Sharma *et al.*, 2014 ). An elevated serum urea level of buffaloes with subclinical mastitis could be resultant of stress induced protein catabolism of diseased buffaloes. Therefore, altered metabolic status of buffaloes with early lactation could be accountable as a predisposing cause of subclinical mastitis.

For analysis of milk quality of buffaloes with subclinical mastitis, milk quality panels including lactose, protein, fat, SNF, salt, specific gravity and depression in freezing point were

assessed and compared with the healthy control group (Table 2). Total protein and salts contents as well as depression in freezing point of milk samples obtained from buffaloes with subclinical mastitis were significantly higher ( $P \leq 0.01$ ) in comparison with the milk quality panels of healthy control group. However, lactose, fat and SNF contents of milk samples obtained from buffaloes with subclinical mastitis were significantly lower ( $P \leq 0.01$ ) in comparison with the healthy control group. Moreover, milk specific gravity of these buffaloes was also significantly lower ( $P \leq 0.05$ ) in comparison with the healthy control.

Mastitis and the elevation of SCC are commonly accomplished with evident changes in milk composition (Forsback *et al.*, 2010; Leitner *et al.*, 2011). Both, clinical and subclinical mastitis may alter milk composition by altering protein composition, concentration of salt and lactose (Pyörälä, 2003). During mastitis, the net synthesis of casein, lactose and fat in the mammary gland is generally decreased (Forsback *et al.*, 2010). The result of the present study clearly indicates remarkably increased total protein and salts levels as well as remarkable reduction lactose, fat, SNF contents of milk from affected quarters compared to the healthy quarters. Remarkable reduction in specific gravity of milk from affected quarters was also recorded when compared with the specific gravity of milk from 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 damaged epithelial cells. The osmotic pressure of milk is maintained by the balance of concentration of lactose and of soluble minerals. Therefore, changes in the sodium and potassium content of mastitic milk could results in reduced lactose synthesis and thus milk production.

Table 1. Comparison of metabolic profile of subclinical mastitic and healthy buffaloes.

<b>Panels</b>	<b>Healthy control (n=20)</b>	<b>Subclinical mastitic buffaloes (n=40)</b>
Glucose (mg/dl)	48.6±2.34	38.07±0.90 <sup>a</sup>
Triglyceride (mg/dl)	11.29±0.89	11.26±0.09
Cholesterol (mg/dl)	102.85±4.2	101.92±1.5
Total Protein(g/dl)	6.46±0.12	6.43±0.04
Urea (mg/dl)	24.29±1.9	31.84±0.86 <sup>a</sup>
Calcium (mg/dl)	8.82±0.15	7.66±0.05 <sup>b</sup>
Magnesium (mg/dl)	2.39±0.12	2.41±0.01
Phosphorus (mg/dl)	4.60±0.09	4.31±0.04 <sup>b</sup>

<sup>a</sup>statistically significant difference ( $P \leq 0.01$ ), when compared with the healthy control.

<sup>b</sup>statistically significant difference ( $P \leq 0.05$ ), when compared with the healthy control.

Table 2. Comparison of milk quality assay panels of subclinical mastitic and healthy buffaloes.

<b>Panels</b>	<b>Healthy control milk (n=20)</b>	<b>Subclinical mastitic milk (n=40)</b>
Fat (%)	8.03±0.23	4.61±0.26 <sup>a</sup>
SNF (%)	9.60±0.02	8.33±0.09 <sup>a</sup>
Protein (%)	3.87±0.01	4.32±0.08 <sup>a</sup>
Lactose (%)	5.23±0.01	4.23±0.06 <sup>a</sup>
Salt (%)	0.687±0.032	0.707±0.004 <sup>a</sup>
Specific gravity	1.0297±0.0005	1.0187±0.0039 <sup>b</sup>
Depression in freezing point	0.546±0.002	0.491±0.005 <sup>a</sup>

<sup>a</sup>statistically significant difference ( $P \leq 0.01$ ), when compared with the healthy control.

<sup>b</sup>statistically significant difference ( $P \leq 0.05$ ), when compared with the healthy control.

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 of cattle and buffaloes (Forsback *et al.*, 2010; Hussain *et al.*, 2012; Malek dos Reis *et al.*, 2013; Sharma *et al.*, 2014)

An increased permeability of the blood-milk barrier could also result 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 estimated. The result 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 outcome 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).

## CONCLUSION

Thus, it can be concluded that remarkably lowered serum metabolites including, glucose, calcium and phosphorus could be associated with subclinical mastitis of buffaloes. In tandem, subclinical mastitis could confer remarkable alteration in milk quality of the affected quarters in buffaloes.

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