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CORRELATIONS OF BLOOD SERUM AND MILK BIOCHEMICAL PROFILES WITH SUBCLINICAL MASTITIS IN CHOLISTANI CATTLE

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Mastitis is a complex and multifarious disease that adversely affects the physico-chemical properties, composition and yield of milk and risks of premature culling of affected animals. A total of 320 Cholistani cattle were screened for subclinical mastitis using California mastitis test in and around district Bahawalpur. The blood and milk samples were only collected from subclinical infected cattle. Milk samples obtained from cattle with subclinical infection revealed significantly ($P<0.05$) negative correlation of Na with K, Ca, Mg, P, Fe and Zn while significant ($P<0.05$) positive correlation with aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase. Serum samples from infected animals revealed significant ($P<0.05$) positive correlation of Ca with P and Zn while significant negative correlation of Zn and P with enzymes. Milk samples from non-infected cows showed a significant ($P<0.05$) negative correlation between Ca and K while positive correlations between Ca and P. Blood samples from non-infected animals revealed significant positive correlation between zinc, AST and ALP while significant ($P<0.05$) positive correlation between LDH, ALP and AST. From the present study, it was concluded that correlation of milk and blood electrolytes, trace minerals and enzymes are suitable diagnostic technique to detect sub-clinical mastitis.
Keywords: Cholistani cattle, subclinical mastitis, milk, blood, minerals, enzymes.

INTRODUCTION

In Pakistan, about 35 million people are involved with livestock which contribute nearly 11% to GDP (Rehman *et al.,* 2017). Hence the dairy animals play a pivotal role in the economy and socio-economic development of farmers in the country (Zaman *et al.,* 2017). The production potential of dairy animals is mainly influenced by different diseases and among those mastitis is the major threat for milk producing animals (Ali *et al.,* 2016; Qayyum *et al.,* 2016a; Aqib *et al.,* 2017).Mastitis significantly affects the quality and the overall yield of milk (Qayyum *et al.,* 2016b; Song *et al.,* 2017). Moreover, the disease adversely alters the physico-chemical properties and composition of milk (Qayyum *et al.,* 2016c; Risvanli*et al.,* 2017). This alteration in milk is due to changes in serum composition due to clinical or subclinical mastitis that contributes in impairment of immune response (Megalia*et al.,* 2001).

Trace minerals have critical significance in maintaining the udder health of lactating cows (O'Rourke, 2009). The investigation of different minerals in milk and udder secretions could be valuable, reliable and a useful tool to determine the efficiency of bovine mammary glands (Mohamed *et al.,* 1999). In this context, potassium (K) being the most important mineral in milk is considered as a highly sensitive indicator of clinical as well as subclinical mastitis in lactating animals (Khan and Khan, 2006). Several researchers aimed to develop various indirect tools to diagnose the sub-clinical intra mammary infections, however, none of them proved satisfactory (Guha *et al.,* 2012, 2013). Somatic cell count (SCC) is one of the indirect tests used for the diagnosis of sub clinical mastitis but it also has few limitations. Thus, there is a need for an accurate and reliable diagnostic method of sub-clinical mastitis that must accomplish two main criteria such as an accurate bacteriological examination and changes in biochemical parameters related with inflammatory conditions of udder.

But usually these are much time consuming procedures being used for the diagnosis of mastitis (Katsoulos*et al.,* 2010). The estimation of sodium (Na) and K in mastitis positive milk samples could be accurate biomarkers of mammary infections (Haron*et al.,* 2014). Previously, it is reported that mastitis causes significant increase in zinc, iron and cobalt in milk samples collected from infected animals (Gera *et al.*, 2011). Zinc affects the keratinization of teat canal and thus protects the mammary parenchyma against the bacterial penetration during post milking duration (Davidov*et al.*, 2013).

Apart from minerals, enzymes concentrations like lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in milk are also potential indicators in diagnosis of subclinical mastitis in dairy cows (Babaei*et al.*, 2007; Yang *et al.*, 2011). These enzymes are cytoplasmic in nature and originate on disintegration of the leukocytes, damaged epithelial cells and interstitial cells of the udder (Hamadani*et al.*, 2013). Higher levels of these enzymes in milk and serum have been reported in lactating dairy animals suffering from sub-clinical mastitis and thus are considered as the best biomarker of udder health (Akerstedt*et al.*, 2011; Hussain *et al.*, 2012; Kalantari *et al.*, 2013). However, there is no available report about the correlation of complete milk and blood biochemical profile in both healthy and mastitic cattle. In Pakistan, Cholistani cattle breed being an excellent heat tolerant animal with high milk potential is usually kept under desert conditions in pastoral system. It is the only vital source of livelihood and socio-economic uplifting of nomads in Cholistan desert. Thus, the current study was designed with an aim to set-up a correlation between minerals and enzymes in milk and blood of healthy and mastitis Cholistani cows.

MATERIALS AND METHODS

***Collection of milk and blood samples*:** In this study, lactating Cholistani cows (n=320) were screened for subclinical mastitis in and around district Bahawalpur. All the animals with blood-tinged milk, having pus flakes, watery secretions from udder and signs of clinical mastitis and systemic involvement were not included in the study. After gross examination, the udder of each animal was cleaned and washed with luke warm water. Milk samples from different lactating cattle were collected aseptically and tested by using California Mastitis Test for the detection of subclinical mastitis (Kivaria *et al.*, 2007; Hussain *et al.*, 2012). Blood sample (5 ml) was collected from jugular vein of each animal into EDTA free tubes. Milk samples were centrifuge for serum separation while all the blood samples were placed on ice for serum separation. The serum was stored at -20°C until further analysis.

***Determination of electrolytes and minerals*:** Milk electrolytes and trace minerals were determined after wet digestion of samples according to the method Na and K (mg/dl) were determined by flame photometer and total phosphorus (P; mg/dl) was determined by colorimetric method on UV spectrophotometer at 720nm wavelength against the standard and blank solutions. The calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) were determined by atomic absorption spectrophotometer (Ahmad *et al.*, 2007).

Estimation of enzyme profile in milk:For estimation of different enzymes all the collected milk samples were centrifuged at 5000rpm for 10 min to remove fat from milk (Kausar*et al.*, 2017). The fat free milk samples were used to determine enzymes (IU/L) including LDH, aspartate aminotransferase (AST) and ALP using commercially available kits. LDH (Cormay, Catalog # 1-239) was determined at wavelength 340 nm, ALP at 405 nm (Cormy, Catalog # 1-212) and AST at 340 nm (Analyticon, Catalog # 1178) (Babaei *et al.*, 2007; Ghaffar *et al.*, 2017a).

***Biochemical changes in blood*:**Ca and Zn (mg/dl) were determined by atomic absorption spectrophotometer (Ahmad *et al.*, 2007) while total P (mg/dl) was determined by colorimetric method on UV spectrophotometer at 720nm wavelength against standard and blank. The enzymes including AST, ALP and LDH were measured through spectrophotometer by using commercially available kits.

***Statistical analysis*:** The data were processed using the statistical program SPSS (Ver 15.0) (SPSS, 2006). The level of significance was set at $p < 0.05$.

RESULTS

***Correlations of minerals and enzymes in milk of cows with subclinical mastitis*:** Na showed significantly (P<0.05) negative correlations with all other minerals while significantly (P<0.05) positive correlations with AST, ALT and LDH. K, Ca, Mg, P, Fe and Zn showed significant (P<0.05) positive correlations with each other while all these minerals have significant (P<0.05) negative correlations with alkaline phosphatase, lactate dehydrogenase and aspartate aminotransferase. LDH showed a significant (P<0.05) positive correlation with other two enzymes while AST had a significant (P<0.05) positive correlation only with ALP (Table 1).

***Correlations of minerals and enzymes in serum of cows with subclinical mastitis*:** Ca showed significant (P<0.05) positive correlations with P and while Zn a significant (P<0.05) negative correlations with all three enzymes. Phosphorus showed significant (P<0.05) positive correlations with Zn and significant (P<0.05) negative correlations with enzymes. Results indicated that Zn showed significant (P<0.05) negative correlation with serum enzymes. LDH showed significant (P<0.05) positive correlations with ALP and AST ALP showed significant (P<0.05) positive correlations with AST (Table 2).

Table 1. Correlations between minerals and enzymes in milk of cows having sub-clinical mastitis

Correlation	Na	K	Ca	Mg	P	Fe	Zn	Cu	LDH	AST	ALP
Na	-	-0.96**	-0.94**	-0.77**	-0.92**	-0.87**	-0.52**	-0.08	0.91**	0.93**	0.98**
K	-	-	0.96**	0.77**	0.94**	0.87**	0.51**	0.14	-0.93**	-0.92**	-0.97**
Ca	-	-	-	0.74**	0.93**	0.84**	0.49**	0.23	-0.92**	-0.87**	-0.96**
Mg	-	-	-	-	0.82**	0.78**	0.35**	0.12	-0.79**	-0.72**	-0.76**
P	-	-	-	-	-	.86**	0.49**	0.12	-0.93**	-0.87**	-0.93**
Fe	-	-	-	-	-	-	0.51**	0.09	-0.84**	-0.85**	-0.88**
Zn	-	-	-	-	-	-	-	0.13	-0.54**	-0.48**	-0.52**
Cu	-	-	-	-	-	-	-	-	-0.21	0.02	-0.08
LDH	-	-	-	-	-	-	-	-	-	0.84**	0.92**
AST	-	-	-	-	-	-	-	-	-	-	0.93**
ALP	-	-	-	-	-	-	-	-	-	-	-

** Correlation is significant at the 0.05 level (2-tailed).

Table 2. Correlation between minerals and enzymes in serum of cows having sub-clinical mastitis

Correlation	Ca	P	Zn	LDH	ALP	AST
Ca	-	0.83**	0.88**	-0.85**	-0.91**	-0.92**
P	-	-	0.85**	-0.95**	-0.81**	-0.92**
Zn	-	-	-	-0.88**	-0.94**	-0.92**
LDH	-	-	-	-	0.85**	0.93**
ALP	-	-	-	-	-	0.92**
AST	-	-	-	-	-	-

** Correlation is significant at 0.05 levels (2-tailed).

Table 3. Correlations between minerals and enzymes of milk of non mastitic cows.

Correlation	Na	K	Ca	Mg	P	Fe	Zn	Cu	LDH	AST	ALP
Na	-	-0.11	-0.09	0.04	0.04	0.21	-0.08	0.30	-0.03	0.14	-0.26
K	-	-	-0.48*	-0.31	0.24	-0.07	-0.23	0.04	-0.28	-0.12	0.11
Ca	-	-	-	0.08	0.46*	-0.08	-0.04	0.01	0.36	0.34	-0.07
Mg	-	-	-	-	-0.14	-0.06	-0.42	-0.39	0.37	0.28	-0.21
P	-	-	-	-	-	0.06	-0.13	0.06	0.13	0.41	-0.29
Fe	-	-	-	-	-	-	-0.06	0.14	0.03	0.50*	-0.18
Zn	-	-	-	-	-	-	-	0.14	-0.16	-0.27	0.04
Cu	-	-	-	-	-	-	-	-	-0.21	0.17	0.46*
LDH	-	-	-	-	-	-	-	-	-	-0.05	-0.19
AST	-	-	-	-	-	-	-	-	-	-	-0.02
ALP	-	-	-	-	-	-	-	-	-	-	-

* Correlation is significant at 0.05 level (2-tailed).

Table 4. Correlations between minerals and enzymes in serum of non mastitic cows.

Correlation	Ca	P	Zn	LDH	ALP	AST
Ca	-	-0.01	-0.08	-0.18	-0.02	0.17
P	-	-	0.41	-0.06	-0.11	-0.25
Zn	-	-	-	-0.17	-0.46*	-0.52*
LDH	-	-	-	-	0.42	0.51*
ALP	-	-	-	-	-	0.47*
AST	-	-	-	-	-	-

* Correlation is significant at the 0.05 level (2-tailed).

***Correlations of minerals and enzymes of milk in healthy animals*:** K showed a significant ($P<0.05$) negative correlations with Ca while Ca showed significant ($P<0.05$) positive correlations with P. Iron showed significant positive correlation with AST. Copper showed significant ($P<0.05$) positive correlations with ALP (Table 3).

***Correlations of minerals and enzymes in blood in healthy animals*:**Zinc showed a significant ($P<0.05$) negative correlations with AST and ALP. However, LDH and ALP showed significant ($P<0.05$) positive correlation with AST (Table 4).

DISCUSSION

The current study was planned to estimate the micro and macro minerals and enzymatic in milk and serum as indicator of subclinical mastitis. Inflammatory conditions of mammary glands lead to alteration in milk composition because of local effects and entry of serum components into milk due to alteration in vascular permeability (Eshratkhah*et al.*, 2012). Udder infection changes the concentrations of Na and chloride; necessary for normal cellular homeostasis and ultimately leading to poor performance of mammary tissue (Rodriquez *et al.*, 2000; Guha and Gera, 2012). Moreover, increase in milk LDH and ALP has been considered as a best biomarker of udder health in mastitic animals (Kalantari *et al.*, 2013). Hence, determination of different minerals and enzymes in milk could be an efficient tool to monitor the inflammation in bovine udder (Hussain *et al.*, 2012).

Bacteria invade the teat canal and mammary glands cause mastitis. These microorganisms multiply and produce various toxins to cause injury to the milk secreting tissue. The inflammatory changes in udder lead to increase in number of leukocytes or somatic cells in the milk and thus adversely affecting the quality of milk and its byproducts. At high SSC count, level of serum albumin and immunoglobins are increased that lead to decreased heat stability of affected milk and on pasteurization, lower grade scores on storage (Hussain *et al.*, 2012). Usually milk conductivity in mastitis is increased hence both Na and Cl concentrations are elevated. While K normally declines that is a predominant mineral in milk. The breakdown of milk proteins like casein occurs due to 2-fold increased proteolytic activity of plasmin and other enzymes derived from the damaged somatic cells in the affected animals (Hamadani*et al.*, 2013). Plasmin causes extensive damage to casein and that leads to lowering of associated Ca in milk. Moreover, decreased Ca absorption from blood also results in low milk Ca level that results in impaired coagulation properties of affected milk (Hamadani*et al.*, 2013). A strong negative correlation of K with Na has already been previously reported (El Zubeir*et al.*,2005; Haron*et al.*, 2014). The increased mineral contents in milk are due to bacterial infection in udder that damages the epithelial cells of secretory alveolar ducts and results into increased vascular permeability in the area (Hamadani*et al.*, 2013). It is established that in case of infection/oxidative stress the synthetic activity of tissues is altered. In this regard variety of minerals/elements being produced by the specific tissues/cells is affected (Ghaffar *et al.*, 2017b). Therefore, there can be a positive or negative correlation among various elements as reported earlier (El Zubeir*et al.*, 2005; Batavani*et al.*, 2007). Significant positive correlation of ALP with Mg was recorded in subclinical mastitis as previously reported (El Zubeir*et al.*, 2005). The results also showed significant positive correlation among LDH, ALP and AST and among the minerals and enzymes both in milk and blood serum. These changes in levels might be due to the increased activities of enzymes in the blood serum due to damage in udder by mastitogens that usually disrupt the junctional complex of epithelium of secretory ducts that is essentially impermeable for counter-transport of minerals between milk and blood (Katsoulos*et al.*, 2010).

Sub-clinical mastitis results not only in economic losses by increase the costs of production and decrease production. The culling of potentially profitable animals due to disease is also a significant loss. On an average, a diseased quarter results in about 30% decrease in milk and an affected animal loses to about 20% of its lactation. Moreover, there is about 1% loss of total solids by alteration in milk composition like fat and casein are reduced and whey proteins and chlorine are increased that interferes with manufacturing process. The other supplementary economic losses are discarded milk from diseased and treated animals, charges of veterinary services for treatment, monitoring and cost of control measures. Thus, to maintain the udder health and to avoid all draw backs, there is a need of regular monitoring of the animals and an early diagnosis of the disease. In conclusion, the correlation of all these milk and blood parameters is a suitable clinical marker to detect the sub-clinical mastitis at an early stage.

Conclusion: From the findings of this study, it can be suggested that determination of different blood and milk biochemical changes are useful tools to detect subclinical mastitis in early stage of infection. Moreover, milk and blood electrolytes, trace minerals and enzymes have significant correlation to each other and are suitable and reliable diagnostic biomarkers for detection of sub-clinical mastitis.

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