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Correlation of Minerals and Enzymes in Blood Serum and Milk of Healthy and Mastitic Cows

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Abstract: Levels of minerals (Ca, Na, K, Mg, P, Fe, Zn and Cu) and enzymes (LDH, GOT and ALP) from 45 cows; 15 cows from healthy, 15 with subclinical mastitis and 15 from cows with clinical mastitis were estimated during the present study. The milk samples from clinical mastitis infection revealed positive correlation when comparing sodium and each of calcium, potassium and copper; potassium and each of iron and GOT; calcium and each of copper and iron; and copper and iron. However significant negative correlation were found between zinc and each of sodium, copper and iron. The subclinical milk samples revealed positive correlation between iron and each of calcium and LDH; and Magnesium and ALP, while significant negative correlation were found between sodium and potassium. Correlation between serum minerals and enzymes of blood serum from cows with subclinical mastitis revealed positive correlation between AP and potassium, and LDH and phosphorus, while significant negative correlation were found between LDH and zinc. In subclinical mastitis, magnesium was found to correlate positively with calcium and negatively with sodium. The present study concluded that minerals and enzymes from milk and blood serum of cows can be used as indicator of mastitis infection.

Key words: Minerals, enzymes, mastitic milk, blood serum, correlation, dairy cows, Sudan

INTRODUCTION

Mastitis is a widely occurring and costly disease in the dairy industry[27]. The major causative agents of mastitis in modern dairy herds are *Escherichia coil*, *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalaciae*[2]. Milk may contain varying amounts of materials originating from blood secretory tissue cells or from polymorphonuclear leukocytes depending upon the balance between pathological and physiological factors[14]. Moreover he stated that the enzymes levels were the most suitable candidates for the diagnosis of secetory abnormalities of the bovine mammary glands. Bacterial infection of the udder results in damage to the ductal and secretory epithelium, an opening up of the tight junctions between secretory cells and an increased permeability of blood capillaries. Thus sodium and chloride poor into the lumen of the alveolus and in order to maintai ned osmalrity, potassium level decreased proportionately[19,4,18]. Similarly Rao[21] stated that mastitis milk shows evidence of direct passage of blood into the milk as indicated by the presence of high concentration of sodium, chloride, blood enzymes and red blood cells.

El Deeb and Hassan[6] reported significantly higher concentrations of ash, sodium, chloride, zinc, copper and iron. while significantly lower calcium, magnesium, citrate and phosphate concentrations were detected as a result of mastitis infection. However, Mahran *et al*.[16] reported that clinical and subclinical mastitis milk samples had higher sodium, magnesium and calcium and lower potassium concentrations than those of normal milk.

Inger *et al*.[13] have shown that changes in absolute values for sodium, potassium and chloride can be used successfully to monitor milk samples for mastitis. Similarly Bogin and Ziv[3] concluded that minerals profile in secretions from inflamed udders may have some clinical diagnostic and prognostic values. Mohamed *et al*.[18] also concluded that the changes in macrominerals levels in individual milk samples can be indictor of udder or quarter infection. Moreover Mohamed *et al*.[18] concluded that the level some minerals (Ca, Na, K, Mg, P, Fe, Zn and Cu) and

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of enzymes (LDH, GOT and ALP) in milk samples can be efficient procedure for monitoring abnormalities of the bovine mammary glands. Pednekar *et. al.*[20] found the activities of lactate dehydrogenase (LDH), Aspertate aminotrasferase (AST) and alkaline phosphatase (ALP) were increased in cows with subclinical mastitis. Moreover, Fruganti *et. al.*[10] found that the increase i n LDH and ALP activities were associated with clinical mastitis and to lesser extent with subclinical mastitis. On the other hands Grun *et al.*[12] found that the activities of LDH was highest in blood, plasma and udder lymph, while ALP was highest in milk.

Wagner and Stull[26] found differences in potassium and calcium concentrations in blood serum of mastitic and normal cows. Moreover, Erskine and Bratelett[9] found that experimental *E. coli* infection resulted in mean serum concentrations of zinc, iron, and copper of 28, 35 and 52 % of prechallange concentrations. Mastitis resulted in a decrease in mean serum Cu, Zn and Fe concentration compared with preinfection level[17].

In the present study, the level of some minerals and enzymes from blood serum and milk of cows with subclinical and clinical mastitis were estimated and correlated.

MATERIALS AND METHODS

The present study was carried out on lactating dairy cows of the Arab Company for Agricultural, Production and Processing (Dairy Land), Khartoum. The farm is located 30 km south of Khartoum. The farm was established in 1984 with a stock of Holstein- Friesian originally imported from Netherlands. According to clinical signs and the California Mastitis Test (CMT) scores, the cows were grouped as clinically, subclinically-infected and healthy cows (45 cows; 15 cows in each group). Quarter milk samples' were collected during the year 1992, in sterile bottles and the blood samples were collected in sterile vacationer from the milk vein.

Determination of minerals concentrations in milk and blood serum: The methods used for determination of minerals concentrations in milk and blood serum was obtained from the Atomic Absorption Spectrophotometer 2380. Perkin, Germany. Samples of milk and blood serum were prepared according to the procedures described in the technical manual of the Atomic Absorption Spectrophotometer.

A series of standard metals solutions in part per million (PPM) were prepared by dilution. The instrument was calibrated and the absorption of the different concentrations of the metal standards were measured at specific wave length (i.e. calcium 442.7, magnesium 285.2, sodium 589.0, potassium 766.5, zinc 213.9, iron 248.7 and copper 324.8 nm).

The samples solutions were aspirated and their values in PPM were obtained and calculated according to the procedures described in the technical manual and recorded in mg/ 100 ml.

Concentrations of blood serum phosphorus were determined according to the method of Varley[25]. The phosphorus concentrations of milk samples were estimated by colorimetric method of Chapman and Pratt[5].

Determination of enzymes activities in blood serum and milk: Enzymes activities were investigated in both whole milk and blood serum samples. The activities of Aspertate aminotrasferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined using commercial kits (Bio-Merieux, Laboratory reagents and Products, Marcy-I' Etoile, France).

Statistical analysis: The data were analyzed according to Gill[11] suing personal correlation.

RESULTS AND DISCUSSIONS

Correlation between minerals and enzymes from clinical mastitis milk samples: Table 1 presents the interrelationships between minerals and enzymes. It is clear that, potassium correlated positively with iron ($r = 0.5436$, $P < 0.05$) and GOT ($r = 0.4985$, $P < 0.05$). Calcium showed significant positive correlations with sodium ($r = 0.5599$, $P<0.05$), iron ($r = 0.5848$, $P < 0.05$) and copper ($r = 0.5349$, $P < 0.05$). Copper, similarly, showed a positive correlations with sodium content ($r = 0.5376$, $P < 0.05$). Zinc had significant negative correlations with copper ($r = -0.7481$, $P < 0.001$), iron ($r = -0.6505$, $P < 0.01$) and sodium ($r = -0.4921$, $P < 0.05$). Iron correlated positively with copper ($r = 0.8818$, $P < 0.001$). However magnesium, inorganic phosphorus, AP and LDH showed non significant interrelationships.

Correlation between minerals and enzymes from subclinical mastitis milk samples: Alkaline phosphatase correlated positively with potassium ($r = 0.5210$, $P < 0.05$) as shown in table 2. Lactate dehydrogenase showed significant negative correlation towards zinc ($r = -0.5884$, $P < 0.05$). However glutamate oxaloacetate showed a positive correlation with inorganic phosphorus ($r = 0.5501$, $P < 0.05$).

Relationships among minerals and enzymes of milk with subclinical mastitis: Correlations of minerals and

Table 1: Correlation between minerals and enzymes of clinical mastitic milk

Correlation	Sodium	Potassium	Calcium	Magnesium	Copper	Zinc	Iron	Phosphorus	AP	LDH	GOT
Sodium	1.00	0.03	0.56*	-0.34	0.54*	-0.49*	0.35	0.37	-0.29	0.18	-0.22
Potassium	0.08	1.0	-0.06	0.26	0.41	-0.42	0.54	0.27	-0.20	0.03	0.50*
Calcium	0.56*	-0.06	1.0	-0.21	0.53*	-0.30	0.58*	0.15	-0.16	-0.27	-0.18
Magnesium	-0.34	0.26	-0.21	1.0	-0.29	0.38	-0.01	0.02	0.07	0.20	0.30
Copper	0.54*	0.42	0.53*	-0.29	1.0	-0.75**	0.88***	-0.04	0.14	-0.24	0.27
Zinc	-0.49*	-0.42	-0.30	0.38	-0.75***	1.0	-0.65**	0.02	0.19	0.23	-0.45
Iron	0.35	0.54*	0.58*	-0.01	0.88***	-0.65**	1.0	-0.01	-0.09	-0.16	0.48
Phosphorus	0.37	0.27	0.15	0.02	-0.04	0.02	-0.01	1.0	-0.001	0.44	0.03
AP	-0.29	-0.20	-0.16	0.07	-0.14	0.19	-0.09	-0.001	1.0	-0.11	0.02
LDH	0.18	0.03	-0.27	0.20	-0.24	0.23	-0.16	0.44	-0.11	1.0	0.16
GOT	-0.22	0.50*	-0.18	0.30	0.27	-0.45	0.48	0.03	0.02	0.16	1.0

Table 2: Correlation between minerals and enzymes of serum from clinical mastitic cows

Correlation	Sodium	Potassium	Calcium	Magnesium	Copper	Zinc	Iron	Phosphorus	AP	LDH	GOT
Sodium	1.00	0.38	0.09	-0.10	0.22	-0.24	-0.01	-0.37	0.32	-0.17	-0.03
Potassium	0.38	1.00	-0.10	-0.20	-0.15	-0.11	0.31	-0.05	0.52*	0.24	-0.07
Calcium	0.09	-0.10	1.00	0.15	0.04	-0.42	0.27	-0.02	-0.12	-0.10	0.28
Magnesium	-0.10	-0.20	0.15	1.00	0.36	-0.25	-0.17	0.13	-0.41	0.27	0.16
Copper	0.22	-0.15	0.04	0.36	1.00	-0.11	0.19	0.04	0.10	-0.02	0.02
Zinc	-0.24	-0.11	-0.42	-0.25	-0.11	1.00	-0.06	0.28	0.27	-0.59*	-0.33
Iron	-0.01	0.31	0.27	-0.17	-0.19	-0.06	1.00	-0.43	0.21	0.29	-0.36
Phosphorus	-0.37	-0.05	-0.02	0.13	0.04	0.28	-0.43	1.00	-0.23	-0.15	0.55*
AP	0.32	0.52*	-0.12	-0.41	0.10	0.27	0.21	-0.12	1.00	-0.17	-0.28
LDH	-0.17	0.24	-0.10	0.27	-0.02	-0.59	0.29	-0.15	-0.17	1.00	0.29
GOT	-0.03	-0.07	0.28	0.16	0.02	-0.33	-0.36	0.55	-0.28	0.30	1.00

enzymes of milk with subclinical mastitis are presented in Table 3. Among these, potassium showed a significant negative correlation with sodium (r= -05552, P< 0.05) and a significant positive correlation with iron (r= 0.5032, P< 0.05). Calcium correlated positively with iron (r= 0.5417, P< 0.05), LDH showed a highly significant positive correlation with iron (r= 0.7701, P< 0.001). Alkaline phosphatase correlated positively with magnesium (r= 0.5295, P< 0.05).

Correlation between blood minerals and enzymes of subclinical mastitis: As presented in table 4, only magnesium level showed a highly significant negative relationship with sodium (r= -0.8110, P< 0.01) and a significant positive relationship with calcium (r= 0.6256, P< 0.01).

Correlation between minerals and enzymes of healthy cow's milk: These data as shown in Table 5, reveal a significant positive correlation between calcium level and lactate dehydrogenase (r= 0.5210, P< 0.05).

Correlation among minerals and enzymes of healthy cows serum: As shown in Table 6, potassium is significantly correlated with LDH (r= 0.7269, P< 0.01), magnesium correlated positively with calcium (r= 0.7042, P< 0.01) and negatively with iron (r= -0.5645, P< 0.05). Copper showed a significant positive correlation with zinc (r= 0.6825, P< 0.01) and calcium (r= 0.5755, P< 0.05). Similarly, zinc correlated positively with calcium (r= 0.5755, P< 0.05).

The significant correlation between sodium and potassium, obtained during the present study supported the previous reports of Inger *et al.*[13], Mohamed *et al.*[18] and El Zubeir *et al.*[8]. Moreover, Linzell and Peaker[15] reported that potassium level can be used as a sensitive index of subclinical and clinical mastitis and the results compare favorably with the detection by bacteriology and cell counts. This is because bacterial infection of the udder results in damage to the ductal secretory cells and an increased permeability of the blood capillaries[19].

The significant correlation which were obtained for calcium, supported Sbodia *et al.*[28], Mohamed *et al.*[18] and El Zubeir *et al.*[8]. They reported that mastitic milk contained less calcium than healthy cows milk. This could be due to damage to the mammary glands by pathogens, which is often disrupting the junctional complex of the secetory epithelium that essentially impermeable to calcium transport from milk to the blood[1].

The significant correlation of magnesium and AP in subclinical mastitis might be due as stated by El Deeb and Hassan[6] that the change in magnesium due to mastitis was transient. However Singh and Ganguli[23] and Wagner and Stull[26] concluded that magnesium content of milk remained relatively constant with the increased somatic cell counts. Similarly the changes of phosphorus level due to mastitis were in accordance to those of Bogin and Ziv[3] and El Deeb and Hassan[6].

Mastitis infection was demonstrated to alter milk trace elements e.g. copper, zinc and iron[24,6,17,8].

Blood serum minerals except magnesium showed non significant effect in the present study because of mastitis.

Table 3: Correlation between minerals and enzymes of subclinical mastitic milk

Correlation	Sodium	Potassium	Calcium	Magnesium	Copper	Zinc	Iron	Phosphorus	AP	LDH	GOT
Sodium	1.00	-0.56*	-0.14	-0.27	0.33	-0.05	-0.20	-0.24	-0.40	-0.17	-0.25
Potassium	-0.56*	1.00	0.06	0.01	-0.26	0.01	-0.50*	0.05	0.31	0.33	0.25
Calcium	-0.14	0.06	1.00	-0.34	-0.03	0.18	0.54*	-0.18	-0.30	0.35	-0.02
Magnesium	-0.27	0.01	-0.34	1.00	0.03	-0.40	-0.25	-0.09	0.53*	-0.02	0.07
Copper	0.32	-0.26	-0.03	0.03	1.00	0.32	-0.10	0.35	-0.19	-0.03	0.25
Zinc	-0.05	0.14	0.18	-0.40	0.32	1.00	0.09	0.19	-0.25	0.11	-0.24
Iron	-0.20	0.50	0.54*	-0.25	-0.10	-0.09	1.00	0.33	-0.29	0.77***	0.12
Phosphorus	-0.24	0.05	-0.18	-0.09	0.36	0.19	0.33	1.00	-0.28	0.22	0.30
AP	-0.40	0.31	-0.30	0.53*	-0.19	-0.25	-0.29	-0.28	1.00	-0.17	-0.17
LDH	-0.27	0.33	0.35	-0.02	-0.03	0.11	0.77***	0.22	-0.17	1.00	-0.05
GOT	-0.25	0.25	-0.02	0.07	0.25	-0.24	0.12	0.30	-0.17	0.05	1.00

Table 4: Correlation between blood serum minerals and enzymes from subclinical mastitic cows

Correlation	Sodium	Potassium	Calcium	Magnesium	Copper	Zinc	Iron	Phosphorus	AP	LDH	GOT
Sodium	1.00	0.17	-0.32	-0.61*	-0.25	0.40	0.21	-0.10	-0.13	0.28	-0.39
Potassium	0.17	1.00	-0.08	-0.02	-0.13	-0.04	-0.0002	0.09	0.12	-0.46	-0.05
Calcium	-0.32	-0.08	1.00	0.63**	0.03	0.03	0.13	0.008	-0.13	0.12	0.11
Magnesium	-0.61*	-0.02	0.63**	1.00	0.15	-0.25	-0.11	0.20	-0.14	0.10	0.10
Copper	-0.25	-0.13	0.03	0.15	1.00	-0.26	0.27	-0.06	0.30	-0.01	-0.25
Zinc	0.40	-0.04	0.03	-0.25	-0.26	1.00	0.21	-0.18	-0.009	-0.26	0.41
Iron	0.21	-0.0002	0.13	-0.11	0.27	0.21	1.00	0.15	0.20	-0.11	-0.14
Phosphorus	-0.10	0.09	0.01	0.20	-0.06	-0.18	0.15	1.00	-0.47	-0.06	-0.29
AP	-0.13	-0.12	-0.13	-0.14	0.30	-0009	0.20	-0.47	1.00	-0.16	0.005
LDH	-0.28	-0.45	0.12	0.10	-0.01	-0.26	-0.11	-0.06	0.16	1.00	0.007
GOT	-0.39	-0.05	0.11	0.10	-0.25	0.41	-0.14	-0.29	0.005	0.007	1.00

Table 5: Correlation between minerals and enzymes of healthy cow's milk

Correlation	Sodium	Potassium	Calcium	Magnesium	Copper	Zinc	Iron	Phosphorus	AP	LDH	GOT
Sodium	1.00	-0.08	0.05	-0.19	-0.01	0.32	-0.09	0.13	-0.18	-0.20	-0.18
Potassium	-0.04	1.00	-0.02	0.32	-0.14	0.07	0.18	-0.32	0.29	-0.42	0.02
Calcium	0.05	-0.02	1.00	-0.20	0.001	-0.01	0.16	0.46	0.15	0.52*	-0.29
Magnesium	-0.19	0.32	-0.20	1.00	-0.10	-0.34	0.19	0.05	0.34	-0.22	0.12
Copper	-0.01	-0.14	0.001	-0.10	1.00	-0.04	-0.36	-0.20	-0.27	0.35	0.12
Zinc	0.32	0.07	-0.01	-0.34	-0.04	1.00	-0.12	-0.13	-0.07	-0.15	-0.12
Iron	-0.09	0.18	0.16	0.19	-0.36	-0.12	1.00	0.40	-0.16	-0.02	-0.20
Phosphorus	0.13	-0.32	0.46	0.05	-0.20	-0.13	0.40	1.00	-0.37	0.24	-0.03
AP	-0.18	0.29	0.15	0.34	-0.27	-0.07	-0.16	-0.37	1.00	-0.14	-0.07
LDH	-0.20	-0.42	0.52*	-0.22	.034	-0.15	-0.02	0.24	-0.14	1.00	-0.39
GOT	-0.18	0.02	-0.29	0.32	0.12	-0.12	-0.20	-0.03	-0.07	-0.39	1.00

Table 6: Correlation between minerals and enzymes of healthy cow's serum

Correlation	Sodium	Potassium	Calcium	Magnesium	Copper	Zinc	Iron	Phosphorus	AP	LDH	GOT
Sodium	1.00	-0.25	-0.25	0.10	-0.21	-0.03	0.16	-0.21	-0.10	-0.10	0.05
Potassium	-0.25	1.00	0.16	0.28	0.47	0.35	-0.40	0.06	-0.28	0.73**	-0.29
Calcium	-0.25	0.16	1.00	0.70**	0.60*	0.58*	-0.42	0.20	0.31	0.05	-0.005
Magnesium	0.10	0.28	0.70**	1.00	0.28	0.41	-0.56*	0.04	0.002	0.16	-0.15
Copper	-0.21	0.47	0.60*	0.28	1.00	0.68*	-0.09	0.10	0.06	0.43	0.001
Zinc	-0.03	0.35	0.58*	0.41	0.68*	1.00	-0.27	0.04	-0.20	0.14	-0.26
Iron	0.16	-0.40	-0.42	-0.56*	-0.09	-0.27	1.00	0.08	0.29	-0.03	0.12
Phosphorus	-0.21	0.06	0.20	0.04	0.10	0.04	0.08	1.00	0.43	0.05	0.31
AP	-0.10	-0.28	0.31	0.002	0.06	-0.20	0.29	0.43	1.00	0.12	0.40
LDH	-0.10	0.73**	0.05	0.16	0.43	0.14	-0.03	0.05	0.12	1.00	0.16
GOT	0.05	-0.29	0.005	-0.15	0.001	-0.26	0.12	0.31	0.40	0.16	1.00

This might be due as explained by Wagner and Stull[26] that either the homeostatic mechanisms are sufficiently dynamic to cope with minerals losses from the blood into mastitic glands or the magnitude of the minerals passing into the diseased udder secretion is so small as to be negligible as compared to the total pool of the blood, since milk yield is reduced greatly in mastitic gland. However Bogin and Ziv[3] stated that minerals profile in secretions from inflamed udders may have some clinical diagnostic and prognostic values. Similarly, Erskine and Bartlett[9] found that the changes in serum trace elements might occur too late in the pathogenesis of infection. The increase, as they stated, starts with the beginning of the decline phase of the bacterial counts of milk.

The significant correlation of GOT, LDH and AP might be due to the increase activities of those enzymes in blood serum[3,10,8].

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