CHANGES IN HAEMATOLOGICAL AND METABOLIC PROFILES IN RELATION TO MASTITIS IN CROSSBRED GRAZING DAIRY COWS

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SUMMARY

Haematological and metabolic blood profiles were studied in 25 healthy and 25 mastitic grazing crossbred zebu cows under field conditions. Alkaline phosphatase, aspartate aminotransferase, ceruloplasmin, blood plasma urea, total white blood cell count, eosinophil count and serum immunoglobin were higher ($P<0.05$) in mastitic cows as compared to healthy cows. Plasma calcium, inorganic phosphate, zinc concentration, haemoglobin, packed cell volume and neutrophil count were higher ($P<0.05$) in healthy animals as compared to cows with mastitis. No statistical differences in total protein, plasma glucose, lymphocytes count and monocytes count were observed. These changes may assist the diagnosis of the disease.

INTRODUCTION

Mastitis is one of the disease conditions of the mammary gland responsible for decreased milk production (Oltenacu and Ekesho, 1994), increased treatment costs (Miller *et al*., 1993), increased labour and replacement costs because of higher culling rates (Beaudeau *et al*., 1994; De Graaf and Dwinger, 1996) and decreased sales value of infected cows (Dobbins, 1977).

Complex interactions between pathogens, immunity, milk yield, environment and genetic factors have been suggested to be involved in the disease pathogenesis (Radostits *et al*., 1994; Lam *et al*., 1997). Several pathogenic bacteria, fungi, mycoplasma and multicellular parasites are known to cause mastitis in cattle (Johnson *et al*., 1991; Torgerson *et al*., 1992; Phiri *et al*., 1998). Clinically, the infection is characterised by swelling, heat, redness and pain of the udder (Radostits *et al*., 1994). At the subclinical level, symptoms of the disease may not be evident and the dairyman may be unaware of the problem (Radostits *et al*., 1994).

Studies conducted in Tanzania by Msanga *et al*. (1989), Shekimweri, (1992), Kambarage *et al*. (1996), Phiri *et al*. (1998), Swai *et al*., (2003) and Mdegela *et al*., (2004)

showed that the average annual incidence of clinical mastitis is between 2% and 3% while subclinical mastitic incidence frequencies may be between 40% and 72%. The most common groups of bacteria isolated in these studies were *Coliform spp, Staphylococcus spp, Streptococcus spp* as well as certain fungi, i.e*. Candida albicans***.**

Several alterations in the udder defence mechanisms in cows with mastitis had been reported in temperate climate and in experimentally induced mastitis. These include change in neutrophil maturation and phagocytic activity, cortisol synthesis, increased levels of trace elements, protein and energy metabolites in the blood (Kehrli *et al*., 1989; Cai *et al*., 1994). These indirect changes may be useful in the diagnosis of the disease, as they tend to precede clinical mastitis. However there is scarce information regarding these changes in grazing crossbred grazing cows under field condition in Tanzania.

The purpose of this study was to determine the changes in blood-borne parameters in grazing crossbreed cows with mastitis as well as in healthy cows under field conditions. The animals used in this study were the ones used in the first part of this study (Phiri *et al*., 1998). The activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST), ceruloplasmin (Cp) and the content of other components of the blood including plasma urea (Plu), total protein (TP) were studied. Immunoglobulin (SIM), haemoglobin (Hb), packed cell volume (PCV), total plasma calcium (Ca), inorganic phosphate (Pi), zinc (Zn) and total white blood cell and differential count were investigated.

MATERIALS AND METHODS

Site, animals and management

The study was carried out at Abri Said Abri Salim (ASAS) dairy farm in Iringa located at 7° 48´ S and 35° 43´ E. The climate of the area is characterized by two seasons, a dry season (June – mid-November) and a rainy season (mid-November–May).

ASAS dairy farm is a medium sized farm with 380 cattle including 100 milking cows during the study. The present study was carried out in the month of April 1997 initially involving all 100 lactating cows at the farm. These cows were hand milked twice daily at 3.30 a.m - 6.00 a.m and 4.00 p.m. - 6.00 p.m.

Blood sampling

Samples were collected from 25 cows, which were identified as having mastitis. Methodology of identifying mastitis has been described earlier (Phiri *et al*., 1998). The animals comprised of crossbred Zebu, Ayrshire and Friesian in their 2^{nd} and 3^{rd} parity and in their early mid and late lactation. Susceptibility of individual cows to mastitic is known to vary, for which reason each cow having mastitis was paired to a healthy cow (control) with similar characteristics, i.e breed, stage of lactation and lactation number. Blood samples were collected between 7.00 - 9.00 a.m. from each cow *via* the jugular vein

into both plain, heparinised as well as EDTA containing vacutainer tubes. Whole blood in the EDTA tubes was used immediately for total WBC and differential count, Hb and PCV determinations. Sodium fluoride was added to another portion of EDTA whole blood to prevent cellular glycolysis for glucose determination. Whole blood in plain vacutainer tubes was used to prepare serum for immunoglobulin determination. Whole blood samples in heparinised tubes and in tubes for glucose determination were centrifuged within two hours after collection at 2000 x g for 10 minutes to obtain plasma. Plasma for determination of plasma Ca, Zn and Pi was collected in tubes washed in dilute nitric acid to prevent exogenous mineral contamination. The plasma and serum samples were stored at a temperature of −20°C until analysis.

Laboratory analysis

All the analyses were done in the Department of Veterinary Physiology, Biochemistry Pharmacology and Toxicology

Enzyme bioassays:

Akaline phosphatase (ALP) was measured using p-nitrophenyl phosphate as substrate according to Bowers and McComb (1975). Ceruloplasmin (Cp) activity was measured using O - diasidin dihydrochrolide (4-4'- diamino −3-3 dimethoxybiphenyl) as a substrate as described by Schosinky *et al*. (1974). Aspartate aminotransferase (AST) was determined using a commercial test kit (RANDOX Laboratories, Northern Ireland). The analyses were performed in a single batch. The intra assay coefficients of variation for the methods were 2%, 4% and 1%, respectively.

Haemoglobin, packed cell volume, total protein, plasma urea and glucose

Haemoglobin (Hb) concentration was determined by the cyanmethaemoglobin method using Drabkin's diluent whereas (PCV) was determined by the microhaematocrit method as described by Baker and Silverton (1976). Total plasma protein (TP) and glucose (Glu) were determined using commercial kits (RANDOX Laboratories, Northern Ireland). Plasma urea (Plu) was determined using a commercial kit (Boehringer Mannheim, Germany). The intra assay coefficients of variation for TP, Glu and Plu were 2%, 1% and 1%, respectively.

Mineral concentrations

Total plasma Ca was assayed using the hydroxyquinoline cresolpthalein complexone method as described by Kessler and Wolfman (1964) and Gitelman (1967). Plasma Pi was assayed by measurement of vanado-phospho- molybdate complex formed in acid as described by Fiske and Subarrow (1925). Intra assay precision was 1% for Ca and Pi, respectively. To ensure further precision of analytical level and accuracy of the measurements, at least one control sample (2.5 mmol Ca /l and 2.00 mmol Pi /l) was analyzed together with a test sample batch. Plasma Zn concentration was determined by atomic absorption spectrophotometry as described by Milner and Whiteside (1984).

White blood cell count and immunoglobulins

Whole blood for determination of total WBC was diluted 1:20 with diluting fluid () and transferred to a haemocytometer and leukocytes were counted in each of four representative squares as described by Baker and Silverton (1976). Slides for WBC differential count were stained by Giemsa and counted using the battlement method as described by Schalm *et al*. (1975). Serum immunoglobulin (SIM) concentration was measured using a selective turbidity produced by means of a zinc sulphate (208 mg /l) solution as described by McEwan *et al*. (1970). Intra assay and inter assay coefficients of variation for SIM determination was 2% and 5%, respectively.

Statistical Analysis.

Data were analysed statistically using SAS general linear model (SAS Institute Inc., 1990). Comparison of observed means was done using the t test as described by Dilorio (1991) using the modal below

$R = \mu + X_1 + X_2 + \ldots X_m$

Where:

R = Dependent variable.

μ = General common mean to all observations in the study.

X1 X2 Xm = Independent variables

RESULTS

Enzyme activity and mineral concentrations are presented in Table 1. Mastitic cows had higher ALP, AST and Cp ($P<0.05$) but lower plasma Ca, Pi and Zn than healthy cows. No significant differences ($P>0.05$) on TP and Glu were observed. However, Hb and PCV were higher in healthy cows ($P<0.001$ and $P<0.01$) respectively while Plu was higher in mastitic cows ($P<0.05$) (Table 2). Total WBC ($P<0.05$), E count ($P<0.001$) and SIM ($P<0.001$) were higher in mastitic cows whereas the N count was higher ($P<0.001$) in healthy cows. No significant differences ($P>0.05$) were observed in L and M counts (Table 3).

DISCUSSION

Alkaline phosphatase, aspartate aminotransferance and ceruloplamin activities

There was an increase in plasma ALP in mastitis cows in this study than in normal cows. This is in agreement with experimentally induced mastitis studies (Bogin and Ziv, 1973; Atrosh *et al*., 1996). The increase in ALP in mastitis may reflect the increased enzyme activity associated with inflammatory changes or trauma to the udder tissue, resulting into leakage of the enzymes in the extracellular fluid (Kaneko, 1989). Alkaline phosphates activities are known to increase in other disease conditions such as hepatobilliary obstruction or bone disorders (Kaneko, 1989) and may be important in differential diagnosis.

Aspartate aminotransferase is an intracellular enzyme, widely distributed in animal tissues including the udder (Rej, 1989) and its increase in this study indicates leakage of enzymes from damaged

cells or an increased leakage of enzymes from overproducing cells due to mastitis. Therefore it can be used as a general indicator of the disease. However, its presence in various tissues precludes its use as an organ specific enzyme (Boyd, 1983).

Conner *et al*. (1986) reported higher levels of Cp in mastitis cows than in healthy ones. This study supports these findings. The reason for the higher levels of Cp in mastitis are not clear, However it has been suggested by Chassagne *et al*. (1998) that higher Cp activity in mastitis cows could be due to the activation of a Cp dependent defence mechanism against oxidative stress in mastitis cows.

Plasma total protein, glucose and urea

Normal plasma protein concentration in cattle is between 68 – 85 g /l (Schalm, *et al*., 1975). In the present study mean plasma protein was within this range in both groups of animals. The concentration of protein in the plasma was not different in the two groups of animals and this is in contrast to studies by Kehrli *et al*., (1989) and Cai *et al*., (1994) who reported an increase in serum protein in mastitis cows. The concentration of protein at any given time is a function of the hormonal balance, nutritional status, water balance, and other factors affecting the state of health (Schalm *et al*., 1975) hence a possible reason for the differences.

Normal plasma glucose concentrations in cows range between 2.5 and 4.16 mmol/l (Kaneko, 1989). In the present study all groups of animals had plasma glucose within this range but on the higher side (Table 2). Shuster *et al*. (1991) reported elevated serum glucose between 2 to 10 hours following induction of endotoxin into the udder. In the present study, there was no difference in plasma glucose between the two groups of animals possibly indicating different stages or causative agents of mastitis.

Normal range of plasma urea varies from 3.3 – 6.0 mmol /l for dairy cows (Whitaker 1998). A temporary increase in urea in moderately and severely affected mastitic cows has previously been reported (Hirvonen *et al*., 1999). Previous reports show that high urea in acute coliform mastitic indicates a poor prognosis (Katholm and Andersen, 1992). In the present study, urea was higher in mastitic cow as compared to non-mastitic cows. It has been suggested that an endotoxin shock may decrease glomerular filtration rate and thus cause prerenal azotemia by increasing serum urea (Coles, 1986). Furthermore, enhanced catabolic breakdown of proteins may also result in an increased urea concentration (Hirvonen *et al*., 1999).

Packed cell volume and haemoglobin

Bacteria like *Staphylococcus aureus* and *Streptococcus agalactiae* are known to produce toxins which lyse red blood cells (Sandholm *et al*., 1995), hence a possible explanation for decreased packed cell volume

and haemoglobin in mastitic cows compared to healthy cow. Phiri *et al*. (1998) indicated that, most of these animals had mastitis caused by *Staphylococcus aureus* (53.3%) and *Streptococcus agalactiae* (13.3%).

Table 1. Mean ± SD, and variation range () of alkaline phosphatase (ALP), aspartate aminotransferase (AST), ceruloplasmin (Cp), plasma total calcium (Ca), inorganic phosphate (Pi) and zinc (Zn) concentration in crossbred grazing lactating healthy and mastitic cows under field conditions

Parameter	n	Healthy cows	Mastitic cows	Statistical significance
ALP (U/L)*	25	44.8 ± 9.8[b] (30.0 – 55.0)	71.3 ± 25.7[a] (35.6 – 144)	P<0.001
AST (U/L)	25	31.8 ± 6.0[b] (25.0 – 45.0)	52.2 ± 10.8)[a] (30.3 – 70.8)	P<0.001
Cp (U/L)	25	41.1 ± 6.7[b] (35.0 – 68.5)	64.6 ±23.1[a] (38.7 – 141)	P<0.001
Ca (mmol/l)	25	2.25 ± 0.08[a] (2.10 – 2.46)	2.12 ± 0.21[b] (1.56 – 2.56)	P<0.01
Pi (mmol/l)	25	1.35 ± 0.06[a] (1.30 – 1.50)	1.07 ± 0.15[b] (0.86 – 1.40)	P<0.001
Zn (mmol/l)	25	12.6 ± 0.3[a] (12.1 – 13.0)	11.8 ± 0.7[b] (10.6 – 13.2)	P<0.001

*U/L = International Units per litre. Means with the same superscript letter within a row are not significantly different (P>0.05).

Table 2. Mean ± SD and variation range of plasma total protein (TP), plasma urea (Plu), glucose (Glu), haemoglobin (Hb) and packed cell volume (PCV) in crossbred grazing lactating healthy and mastitic cows, under field conditions

Parameter	n	Healthy cows	Mastitic cows	Statistical significance
TP (g/l)	25	80.7 ± 6.1[a] (73.0 – 93.0)	78.4 ± 6.7[a] (66.4 – 90.2)	NS
Plu (mmol/l)	25	5.26 ± 0.57[b] (3.25 – 5.87)	5.90 ± 1.38[a] (3.50 – 9.20)	P<0.05
Glu (mmol/l)	25	3.94 ± 0.23[a] (3.51 – 4.50)	3.84 ± 0.35[a] (3.13 – 4.50)	NS
Hb (g/dl)	25	12.3 ± 0.5[a] (11.0 – 13.0)	11.1 ± 0.9[b] (9.6 – 12.9)	P<0.001
PCV (%)	25	32.5 ± 1.0[a] (30 – 34)	31.2 ± 1.9[b] (28 – 35)	P<0.01

Means with the same superscript letter within a row are not significantly different (P>0.05).

Table 3. Mean ± SD, and variation of total white blood cell count (WBC), neutrophils (N), lymphocytes (L), monocytes (M), eosinophils (E) counts and serum immunoglobulins (SIM) in crossbred grazing lactating healthy and mastitic cows under field conditions in Tanzania

Parameter	N	Healthy cows	Mastitic cows	Statistical significance
WBC (x 10^3 μL^{-1})	25	8.54 ± 0.96^b (7.00 – 11.95)	9.39 ± 1.57^a (6.56 – 12.35)	P<0.05
N (x 10^3 μL^{-1})	25	2.68 ± 0.30^a (2.05 – 3.16)	2.19 ± 0.94^b (0.47 – 3.84)	P<0.001
L (x 10^3 μL^{-1})	25	5.51 ± 0.26^a (5.12 – 5.89)	6.25 ± 1.09^a (4.51 – 8.17)	NS
E (μL^{-1})	25	100 ± 56^a (10 – 170)	630 ± 440^b (10 – 1780)	P<0.001
M (μL^{-1})	25	250 ± 90^a (85 – 427)	320 ± 160^a (90 – 850)	NS
SIM (ZST)	25	19.4 ± 1.0^b (18.0 – 22.0)	21.5 ± 2.3^a (16.9 – 25.2)	P<0.001

SD = Standard deviation; ZST = zinc sulphate units; NS = not statistically different. Means with the same superscript letter within a row are not significantly different (P>0.05).

Total, differential leukocyte counts and serum immunoglobulin

It has been reported that cows with acute induced mastitis are generally depressed and the blood picture taken early in the course of the disease, that is during the first 24 – 48 hours often is marked leukopenia (Craven and Williams 1985). On the other hand the differential leukocyte count reveals a pronounced neutropenia and but normal lymphocyte percentage (Craven and Williams 1985). The present study seems to confirm this tendency (Table 3). It has been reported that leukocytes, which migrate from the blood and enter the udder play a role in preventing or eliminating infections (Sandholm *et al*., 1995). Neutrophil cells are the most important migratory response of leukocytes and their reaction is considered to be the most important defence and cleaning mechanism of the udder (Sandholm *et al*., 1995). The levels of eosinophils were higher in mastitic cows compared to healthy cows probably indicating chronic infections. Some of these mastitic cows (13.3%) had chronic type of mastitis caused by *Candida albicans* (Phiri *et al*., 1998).

The term immunoglobulin is used to describe all proteins with antibody activity as well as some proteins that have the characteristic immunoglobulin structure but do not have antibody activity (Kaneko,

1989). Immunoglobulin classes and sub-classes are important in mediating protection of the body against infectious diseases (Kaneko, 1989). In the present study mastitic cows had high immunoglobulin concentration compared to other groups of cows probably indicating a response to the infection.

Plasma calcium, inorganic phosphate and zinc

It is well known that homeostatic mechanisms maintain blood Ca and Pi concentration within a range of 2.25 to 2.70 mmol/l and 1.80 to 2.90 mmol/l respectively (Kaneko, 1989). Cows with mastitis had low plasma Ca and Pi compared to these ranges. Epidemiological studies indicate that cows with hypocalcaemia have a five to eight times greater chance of having mastitis (Curtis *et al*., 1983). It is possible that low Ca and P in these animals was a predisposing factor for mastitis. Calcium and phosphorus are important in the production of a number of binding proteins, superoxide oxidase and activation of neutrophils, which are important in the immunity of the udder (Sohnle *et al*., 1996 and 1991; Sandholm *et al*., 1995; Srivasta *et al*., 1994).

Zinc is a micronutrient that is essential for optimal immune responses (Hogan *et al*., 1996). It is involved in antioxidant systems that maintain the integrity of phagocyte cells and lymphoid tissues (Miller *et al*., 1996). Impairments of the antioxidant system can result in a higher incidence and more severe clinical signs of disease. Cows with mastitis had low Zn concentration as compared to healthy cows. Zinc deficiency has been reported to reduce incorporation of amino acids into the skin proteins resulting in parakeratosis with lesions commonly occurring at the teat skin (Kincaid *et al*., 1984). Teat canal keratin, which is composed of desquamified epidermal cells, is the primary barrier to intrammamary infections (Kincaid *et al*., 1984). Zinc deficiency may alter keratin composition and render the mammary gland more susceptible to infections (Kincaid *et al*., 1984; Aquilar *et al.,* 1988). It is possible that the low plasma Zn concentration noted especially could have resulted into disrupting the teat canal keratin rendering the cows vulnerable to intramammary infection.

CONCLUSION

Mastitis remains one of the costly diseases affecting the dairy production. Data presented in this study indicates changes in haematological and metabolic profiles, which may indirectly assist in the diagnosis of the disease under field conditions.

AKNOWLEDGEMENT

This study was supported by the SUA/MU/ENRECA Project through DANIDA. Cooperation and support from Dr. F. Sudi, Mr. Salim , and Mr. Robert Richard at the ASAS dairy farm are much appreciated.

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