http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (4): 571-576, 2008
ISSN 1028-8880

Fibrinogen and Ceruloplasmin in Plasma and Milk from Dairy Cows with Subclinical and Clinical Mastitis

[1]A. Davasaz Tabrizi, [1]R.A. Batavani, [1]S. Asri Rezaei and [2]M. Ahmadi
[1]Department of Clinical Science, [2]Department of Microbiology,
Faculty of Veterinary Medicine, University of Urmia, P.O. Box 1177, Urmia, Iran

Abstract: The potential using of Acute Phase Proteins (APPs) in the assessment of mammary gland health was studied by examining the levels of Fibrinogen (Fb) and Ceruloplasmin (Cp) in plasma and milk from dairy cows with different grades of mastitis. Plasma samples were taken from jugular vein and milk samples were collected from quarters of cows with subclinical and clinical mastitis, as well as healthy controls. California Mastitis Test (CMT) were performed on each udder quarter of cows for detection of CMT2+ and CMT3+ quarters. CMT (0) and culture negative cases were considered healthy cows. Clinical mastitis, was graded as mild (clots in milk) or moderate (clots in milk and visible signs of inflammation in the mammary gland/s). The concentrations of Fb in the plasma of the cows with subclinical and clinical mastitis were higher than in the plasma of the healthy cows ($p<0.01$). There was no significant difference in plasma concentration of Cp between healthy and subclinical groups ($p>0.05$), but differences between clinical and healthy groups were significant ($p<0.05$). The concentrations of Fb and Cp in the milk of the cows with subclinical and clinical mastitis were higher than in the milk of the healthy cows ($p<0.01$). The results indicated that measurement of Fb in plasma and milk and Cp only in milk might be suitable for early diagnosis of mastitis in dairy cows.

Key words: Acute phase proteins, udder inflammation, blood, milk, cow

INTRODUCTION

Mastitis is the most frequent and expensive disease associated with current intensive dairying (Whitaker *et al*., 2004). It has adverse effects on the economics of milk production by reducing the quantity and quality of milk (De Graves and Fetrow, 1993) and increasing expenses through the imposition of financial penalties by milk purchasers for high numbers of somatic cells in milk as a result of mammary infections (Booth, 1997). Per acute clinical mastitis has also been recognized as the major cause of mortality in adult dairy cows (Menzies *et al*., 1995). Mastitis is caused by several species of common bacteria, fungi, mycoplasma and algae. Subclinical infections are those for which no visible changes occur in the appearance of milk or the udder, but milk production decreases, somatic cell count increases, pathogens are present in the secretion and composition is altered. Clinical mastitis is recognized by abnormal milk, varying degrees of mammary gland inflammation (redness, heat, swelling, pain) and with presence or absence of illness in the cow. Milk production declines, bacteria are present in the milk and the milk can vary from having a few milk clots to serum with clumps of fibrin in the secretion (Tyler and Cullor, 2002). Early identification of udder health problems is essential for dairy farmers and veterinarians to ensure not only animal well-being but also milk quality and dairying productivity. Economic aspects interfere with the routine application of bacteriologic examination of quarter milk samples. For this reason, alternative parameters are used to identify trends in the development of the udder health in a dairy herd, although these parameters indicate inflammation. Acute Phase Proteins (APPs) are a group of serum proteins which undergo substantial changes in concentration following infection, inflammation or trauma (Gruys *et al*., 1994). Fb is involved in homeostasis, providing a substrate for fibrin formation and in tissue repair, providing a matrix for the migration of inflammatory related cells (Thomas, 2000).

Fb is used in cattle as a reliable indicator of the presence of inflammation, bacterial infection or surgical trauma (Hirvonen *et al*., 1996; Cheryk *et al*., 1998; Hirvonen and Pyorala, 1998). Cp is a copper-containing ferroxidase that oxidizes toxic ferrous iron to its nontoxic ferric form (Patel *et al*., 2002). It protects tissues from iron-mediated free radical injury and is involved in various antioxidant and cytoprotective activities (Inoue *et al*.,

Corresponding Author: Afshin Davasaz Tabrizi, Department of Clinical Science, Faculty of Veterinary Medicine, University of Urmia, P.O. Box 1177, Urmia, Iran Tel: +98-441-2774737 Fax: +98-441-2777099

1999). The application of Cp to diagnosis remains less common than that of other APPs, but there have been a number of studies confirming that this ferroxidase is an indicator of infection in cattle (Chassagne *et al*., 1998; Sheldon *et al*., 2001).

The aim of this study was to evaluate the potential of using APPs in the assessment of mammary gland health by examining the levels of Fb and Cp in relation to different grades of subclinical and clinical mastitis in dairy cows.

MATERIALS AND METHODS

Animals were selected during the year 2006 from two Holstein dairy herds located around the city of Tabriz in East Azerbaijan province of Iran. Cows Were milked three times daily by machine milking. All cows were subjected to post-milking teat disinfection, those were dried off approximately 2 months before expected calving and all quarters of cows were infused with an antibiotic preparation approved for use in non lactating cows following the last milking of lactation. Milk samples were collected from cows just before morning milking. Teats were washed thoroughly and dried with a single-use paper towel. The first three streams of milk from each teat were discarded. The teat end and orifice was sanitized with cotton swabs soaked in 10% ethyl alcohol and approximately 10 mL foremilk sample were collected from each quarter of cow in a sterile tube held horizontally. Clinical mastitis was recognized by the dairymen on each farm in the usual way, by observation and palpation of the udder. In 25 of the cases, mastitis was diagnosed by the presence of clots in the milk (defined as mild mastitis) and in 25 other cases by the presence of clots and observable inflammation in the infected quarter such as heat, pain, redness or swelling (defined as moderate mastitis). Subclinical mastitis was determined by CMT. The CMT results were interpreted as negative (O), 1+ (traces), 2+(gel) and 3+(clumps), (Busato *et al*., 2000). CMT1+ cases were eliminated from this study. CMT2+ and MT3+ cases (25 case from each score) were submitted in the research. CMT0 and culture negative cases were considered healthy (Control). Milk samples were also taken from an unaffected, non-mastitic, diagonally opposed quarter of the udder of the healthy and mastitic cows, as intra-animal controls. The number of samples in each group were 25 cases. Jugular blood samples were taken from each dairy cow. Venoject tubes with EDTA and without additive were used. All milk and blood samples were tested at mid lactation and none of the cows were sampled twice in the study. Samples were immediately placed in crushed ice and submitted to the laboratory within 2-4 h. Somatic cell count were determined by a fluro-opto-electronic method (fossomatic 250®, Foss electric, HillerØd, Denmark). For bacteriological examinations, standard procedures were performed according to the guidelines described by Sears and Mccarthy (2003) and Quinn *et al*. (1994). Milk serum (whey) was prepared at a two-step centrifugation procedure. At first milk samples were centrifuged at 3000 rpm for 10 min to remove their creams and cells. Samples were then treated with 0.1 M hydrochloric acid at controlled pH for 20 min for casein precipitation. Treated samples were recentrifuged and the supernatants (Whey) were collected. Fb in plasma and milk was determined using the heat precipitation technique (Millar *et al*., 1971). Cp in plasma and milk was determined by its P-phenylendiamine oxidase activity (Sunderman and Nomoto, 1970).

Data were analyzed by using the Minitab statistical programme, version 14.0. One-way ANOVA was carried out to find out the differences between the results of mastitic and non-mastitic plasma and milk. Student's t-test with $p<0.01$ was used to evaluate differences between mastitis quarter and healthy diagonally apposed quarter of cases.

RESULTS

Bacteria isolated from the mastitic cows included the usual range of pathogens. The isolates from subclinical mastitis cases were coagulase negative staphylococci (40%), *Staphylococcus aureus* and *Corynebacterim bovis* (each 12%), *Streptococcus* sp. (6%), *Serratia marcescens*, *Enterobacter aerogenes* and *Proteus* sp. (each 4%). Eighteen percent from subclinical mastitis cases showed no growth on bacteriological examination.

Clinical mastitis cases had the following bacteria isolated from them: *Staphylococcus aureus* (26%), coagulase negative staphylococcus (18%), *Streptococcus* sp. (10%), *Corynebacterium bovis* and *E. coli* (each 8%), *Pasteurella multocida* (6%), *Bacillus cereus* and *Arcanbacterium pyogenes* (each 4%). Sixteen percent from clinical mastitic cases showed no growth on bacteriological examination. The somatic cell count in milk from quarters with subclinical and clinical mastitis were significantly ($p<0.01$) greater than in the milk of the control cows, also was significant difference between each four mastitic groups ($p<0.01$) (Table 1).

The mean plasma Fb in subclinical and clinical groups were significantly different with healthy groups ($p<0.01$), While between affected cows, Moderate mastitis group only had difference with other groups ($p<0.01$). The mean plasma Cp in the control group had no difference with

Table 1: Means and standard deviation of somatic cell count (SCC), Plasma Fb and CP concentrations values in different groups (n = 25 in each group)

		Subclinical		Clinical	
Parameters	Control	CMT2+	CMT3+	Mild	Moderate
SCC (×1000 mL^{-1})	116.00±29^{A}	2800.00±1231^{B}	6279.00±1329^{C}	9347.0±2177^{D}	12766.00±2721^{E}
Plasma Fb (mg dL^{-1})	405.97±107.99^{A}	603.40±131.9^{B}	606.60±126.3^{B}	618.3±120.5^{B}	726.80±147.7^{C}
Plasma Cp (mg dL^{-1})	22.08±5.6^{a}	31.12±8.73^{a}	30.87±6.02^{a}	36.5±6.58^{b}	37.02±10.09^{b}

The mean different letters (A, B, C, D, E) in each row are significantly different ($p<0.01$). The mean different letters (a, b) in each row are significantly different ($p<0.05$)

Table 2: Milk Fb mean and standard deviations values in different groups and contra lateral quarters in each group

Groups	Mastitic quarter (mg dL^{-1})	Contra lateral quarter (mg dL^{-1})
Control	10.3±5.2^{a}	9.8±4.6
CMT2+	27.6±8.1^{b}	14.1±6.7*
CMT3+	32.9±12.2^{bc}	13.6±2.9*
Mild clinical	31.5±15.4^{bc}	14.3±4.8*
Moderate clinical	38.9±16.5^{c}	13.0±5.7*

The mean with different letters (a, b and c) in the same column are significantly different ($p<0.01$). Common letter(s) in column explain no significant difference ($p>0.05$). *: $p<0.01$ within the row

Table 3: Milk Cp mean and standard deviations values in different groups and contra lateral quarters in each group

Groups	Mastitic quarter (mg dL^{-1})	Contra lateral quarter (mg dL^{-1})
Control	2.95±1.02^{a}	2.68±1.53
CMT2+	8.02±4.52^{b}	4.07±2.17*
CMT3+	8.58±2.76^{bc}	3.88±2.05*
Mild clinical	8.64±4.87^{bc}	4.01±2.58*
Moderate clinical	11.50±5.83^{c}	3.76±3.51*

The mean with different letters (a, b and c) in the same column are significantly different ($p<0.01$), Common letter(s) in column explain no significant difference ($p>0.05$), *: $p<0.01$ within the row

subclinical groups ($p>0.05$), but differences between control group and clinical groups were significant ($p<0.05$). However, there was no difference between mild and moderate groups ($p>0.05$) (Table 1).

The concentrations of Fb and Cp were significantly higher in the milk of inflamed quarters than those in normal milk ($p<0.01$). Also Difference between samples of milk from quarters with CMT2+, CMT3+ and mild mastitis groups were no significant ($p>0.05$), but difference between CMT2+ group and clinical moderate mastitis group was significant ($p<0.01$). In each mastitic group concentrations of each two APPs was significantly greater in the infected quarter than in the diagonally opposite quarter ($p<0.01$). On the other hand, there was no difference between healthy quarter of control group and contra lateral quarter (Table 2, 3).

DISCUSSION

The bacteriological results from the subclinical cases demonstrated that Coagulase Negative Staphylococci (CNS) were the most frequently isolated bacteria. CNS organisms, group of staphylococcal species, have become the predominant pathogens causing bovine mastitis in many countries (Pittkala *et al*., 2004; Rajala-Schulz *et al*., 2004). Although the CNS usually cause only subclinical or mild clinical mastitis (Honkanen-Buzalski *et al*., 1994), they are harmful since they increase Somatic Cell Count (SCC) in milk (Chaffer *et al*., 1999) and may slightly decrease milk production (Timms and Schultz, 1987). Mastitis caused by CNS responds well to antimicrobial treatment (McDougall, 1998; Waage *et al*., 2000; Taponen *et al*., 2003). *Staphylococcus aureus* was the second most causative organism of subclinical mastitic cows. *Staphylococcus aureus* is still regarded as one of the most infectious agents which produces mastitis in cattle. The other bacteria frequently was isolated *Corynebacterium bovis*. *C. bovis* are generally considered as opportunistic pathogens and inhabitants of teat canals (Rainard, 1987). The infection causes minor histopathological alterations in the udder parenchyma without affecting the secretory function of the tissue (Sordillo *et al*., 1989). Environmental streptococci from 6% of subclinical and 10% of clinical mastitis were isolated. In countries where the prevalence of intramammary infections due to the contagious pathogens has been reduced or eradicated, the proportion of intramammary infections associated with environmental streptococci has increased markedly (Radostits *et al*., 2007). The bacteriological results from the clinical cases of mastitis shows the *Staphylococcus aureus* is the most frequent organism. *S. aureus* is ubiquitous in the environment of dairy cattle. The infected mammary gland of lactating cows is the major reservoir and source of the organism. Transmission between cows occurs at the time of milking by contaminated milker's hands and teat cup liners (Radostits *et al*., 2007). The mean SCC was greater in the cows with moderate mastitis than those with mild mastitis, so SCC of subclinical and clinical groups were significantly higher than the SCC of the normal cows. Cell counts are used routinely for the diagnosis of subclinical mastitis but are not used for diagnosis of clinical mastitis because the visible alterations in the milk which usually accompany the increase in cells and clots and flake in the milk, make automated counting difficult. Inflammation of the mammary gland leads to a variety of compositional changes in milk either because of local effects or because of serum components entering the milk and the movement of some normal milk components out of the alveolar lumen into the perivascular space (Harmon, 1994). Theoretically,

all changes in mammary secretion during inflammation might be used to measure the effects of mastitis, but problems of instrumentation and standardization have prevented farm application of most tests. In dairy herds, mastitis is a production disease of major importance. Cows with clinical signs of mastitis are easily spotted by farmers and proper treatment can be applied. However, subclinical infections may not be observed and remain untreated. Cow-side tests, such as the California Mastitis Test, are available but laboursome and time consuming if applied to a large number of animals (Petersen *et al*., 2004). For the screening of herds, the Somatic Cell Counts (SCC) are used despite the fact that high levels do not necessarily reflect mastitis (Salniemi, 1995). In order to detect subclinical infections, APP might be applied. For a test being useful in routine investigations for mastitis, it is important that it can be applied to milk samples which are readily available than serum or plasma.

The first acute phase proteins measured from milk and used as indicators of inflammation are bovine serum Albumin and a1 antitrypsin (Giesecke and Vilijoen, 1974; Sandholm *et al*., 1984). Also there is evidence that clinical mastitis can be revealed by high serum and milk concentration of haptoglobin and serum amyloid A (Eckersall *et al*., 2001). The C-reactive protein is not regarded as an acute phase protein in cattle (Eckersall and Conner, 1988), but has been tested as an indicator for mastitis. The concentration of C-reactive protein was shown to increase in bovine milk during mastitis (Schrodl *et al*., 1995). The capacity of the milk C-reactive protein to distinguish between healthy and mastitic quarters was found to be poor (Pyorala, 2003).

Fb is the coagulation factor I, acute phase protein and is produced more rapidly than degraded during the inflammation. Another important function of Fb is the formation of fibrin matrix that enables the movement of fibroblasts and other cells and stimulates their production during the healing of damaged tissue (Bakes and Illek, 2006). Fb specifically binds to CD11/CD18 integrins on the cell surface of migrated phagocytes, thereby triggering a cascade of intracellular signals that lead to enhancement of degranulation, phagocytosis, antibody-dependent cellular cytotoxicity and delay of apoptosis (Sitrin *et al*., 1998; Ruble *et al*., 2001). Cp acts as an anti-inflammatory agent by reducing the number of neutrophils attaching to the endothelium and acting as an extra cellular scavenger of peroxide (Segelmark *et al*., 1997).

The mean concentration of plasma Fb in subclinical cases with healthy cows showed significant difference ($p<0.01$), but the mean concentration of plasma Cp between subclinical groups and healthy cows was not significant ($p>0.05$). This difference between two APP can be as a result of their discrepancy in blood as compared with inflammatory stages in cattle, so that Fb was taken major APP, but Cp is minor APP (Murata *et al*., 2004).

The mean milk Fb and Cp concentrations in mastitic groups were significantly different with healthy cows ($p<0.01$). It is probable that most serum proteins leak into milk across the blood-mammary barrier as a result of the disruption caused by the inflammation due to mastitis. However, there have been reports of the extra hepatic synthesis of acute phase proteins (Eckersall *et al*., 2001). Although the acute phase proteins have been conventionally thought to be synthesized in the liver, there have been reports of the expression of the messenger RNA for these proteins during the acute phase response in extra hepatic tissues such as lung (Yang *et al*., 1995), intestinal epithelium (Vreugdenhil *et al*., 1999) and endometrium (Timms and Schultz, 1987). For example, Cp is synthesized primarily in the liver but is also induced at extra hepatic sites (Pan *et al*., 1996; Mazumder *et al*., 1997). There is considerable potential for the use of a biological marker, Such as an acute phase protein, which is present in milk and can be measured routinely and reliably, for the objective and early diagnosis of mastitis. Such a marker could be particularly important for the continued development of robotic milking systems in which the manual examination of milk and cows is not practicable (Mottram, 1997). It might also provide a more accurate and earlier diagnosis of intramammary infection, reducing the time to treatment and thus possibly reducing the adverse effects of mastitis in both economic and welfare terms.

The results of this study show that Fb and Cp can be detected and quantified in milk from dairy cows with mastitis; the technique could have major implications for the diagnosis and treatment of this important disease. Plasma Fb for diagnosis of subclinical mastitis was suitable, but plasma Cp cannot be detected from dairy cows with subclinical mastitis.

ACKNOWLEDGMENTS

The authors thank the Urmia University for providing research funds, the management of boniad and samimy dairy farms for taking care of the cows and the sampling and Mr. F. Farhangpajooh for his technical assistance.

REFERENCES

Bakes, J. and J. Illek, 2006. Plasma ceruloplasmin and fibrinogen during enzyme therapy of mastitis in dairy cows. Acta Vet. Brno, 75: 240-241.

Booth, J.M., 1997. Is mastitis being reduced in the 1990s? Cattle Pract., 5: 61-65.

Busato, A., P. Trachsel, M. Challibaum and J.W. Blum, 2000. Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland Prev. Vet. Med., 44: 205-220.

Chaffer, M., G. Leitner, M. Winkler and A. Glickman, 1999. Coagulase-negative staphylococci and mammary gland infections in cows. J. Vet. Med., B 46: 707-712.

Chassagne, M., J. Barnouin and J.P. Chacornac, 1998. Biological predictors for early clinical mastitis occurrence in Holstein cows under filed conditions in France. Prev. Vet. Med., 35: 29-38.

Cheryk, L.A., E.K. Hooper-Mcgravy and A.P. Gentry, 1998. Alterations in bovine platelet function and acute phase proteins induced by *Pasteurella haemolytica* Al, Can. J. Vet. Res., 62: 1-8.

De Graves, F.J. and J. Fetrow, 1993. Economics of mastitis and mastitis control. Vet. Clin. North. Am. Food Anim. Pract., 9: 421-434.

Eckersall, P.D. and J.G. Conner, 1988. Bovine and canine acute phase proteins. Vet. Res. Commun., 12: 169-178.

Eckersall, P., F.J. Young, C. McComb, C.J. Hogarth, S. Safi, A. Weber, T. McDonald, A.M. Noland and J.L. Fitzpatrick, 2001. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. Vet. Rec., 148: 35-41.

Giesecke, W.H. and M.H. Vilijoen, 1974. The diagnosis of subclinical mastitis in lactating cows: A comparison of cytological methods and a monovalent radial immunodiffusion test. J. Vet. Res., 41: 51-74.

Gruys, E., M. Obwolo and M.J. M. Toussaint, 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry. Vet. Bull., 64: 1009-1018.

Harmon, R.J., 1994. Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci., 77: 2103-2112.

Hirvonen, J., S. Pyorala and H. Jousimies-Somer, 1996. Acute phase response in heifers with experimentally induced mastitis. J. Dairy Res., 63: 351-360.

Hirvonen, J. and S. Pyorala, 1998. Acute phase response in dairy cows with surgically-treated abdominal disorders. Vet. J., 155: 53-61.

Honkanen-Buzalski, T., V. Myllys and S. Pyoralla, 1994. Bovine clinical mastitis due to coagulase-negative staphylococci and their susceptibility to antimicrobials. J. Vet. Med., 41: 344-350.

Inoue, K., T. Akaike, Y. Miyamoto, T. Okamoto, T. Sawa, M. otagiri and M. Suzuki, 1999. Nitrosothiol formation catalyzed by ceruloplasmin. Implication for cytoprotective mechanism *in vivo*. J. Biol. Chem., 274: 27069-27075.

Mazumder, B., C.K. Mukhopadhyay, A. Prok, M.R. Cathcart and P.L. Fox, 1997. Induction of ceruloplasmin synthesis by IFN-gamma in human monocytic cells. J. Immun., 159: 1938-1944.

Mc Dougall, S., 1998. Efficacy of two antibiotic treatments in curing clinical and subclinical treatments in curing clinical and subclinical mastitis in lactating dairy cows. N.Z. Vet., 46: 226-232.

Menzies, F.D., D.G. Brison, T. Mccallion and D.I. Mathews, 1995. A study of mortality among suckler and dairy cows in Northern Ireland. Vet. Rec., 137: 531-536.

Millar, H.R., J.G. Simpson and A.L. Stalker, 1971. An evaluation of the heat precipitation method for the fibrinogen estimation. J. Clin. Pathol., 24: 827-830.

Mottram, T., 1997. Automatic monitoring of the health and metabolic status of dairy cows. Prod. Sci., 48: 209-217.

Murata, H., N. Shimada and M. Yoshika, 2004. Current research on acute phase proteins in veterinary diagnosis: An overview. Vet. J., 168: 28-40.

Pan, Y., K., Katulla, M.L. Failla, 1996. Expression of ceruloplasmin gene in human and rat lymphocyte. Biochem. Biophysica Acta, 1307: 233-238.

Patel, B.N., R.J. Dunn, S.Y. Jeong and Q. Zhu, 2002. Ceruloplamin regulates iron levels in the CNS and prevents free radical injury. J. Neurosci., 22: 6578-6586.

Petersen, H.H., J.P. Nielsen and P.M.H. Heegard, 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res., 35: 163-187.

Pittkala, A., M. Haveris, S. Pyorala, V. Myllys and T. Honkanen Buzalski, 2004. Bovine mastitis in finland 2001-prevalence, distribution of bacteria and antimicrobial resistance. J. Dairy. Sci., 87: 2433-2442.

Pyorala, S., 2003. Indicators of inflammation in the diagnosis of mastitis. Vet. Res., 34: 565-578.

Quinn, P.J., M.E. Carter, B.M. Maarkey and G.R. Carter, 1994. Clinical Veterinary Microbiology. Wolfe Publication Company, UK., pp: 327-344.

Radostits, O.M., C.G. Gay, K.W. Hinchcliff and P.D. Constable, 2007. Veterinary Medicine. 10th Edn. Saunders Publication Company, UK., pp: 697-721.

Rainard, P., 1987. Should mammary infections caused by *Corynebacterium bovis* and coagulas-negative staphylococci be eliminated? Ann. Res. Vet., 18: 355-364.

Rajala-Schulz, P.J., K.L. Smith, J.S. Hogan and B.C. love, 2004. Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows. Vet. Microbiol., 102: 33-42.

Ruble, C., G.C. Fernandez, G. Dran, M.B., Bompadre, M.A. Isturiz and M.S. Parmo, 2001. Fibrinogen promotes neutrophil activation and delays apoptosis. J. Immun., 166: 2002-2010.

Salniemi, H., 1995. Use of Somatic Cell Count in Udder Health Work. In: The Bovine Udder and Mastitis, Sandholm, M., T. Honkanen-Buzalski, L. Kaartinen and S. Pyorala (Eds.). University of Helsinki, Helsinky, Finland, ISBN, 951-834-047-1: 105-110.

Sandholm, M., T. Honkanen-Buzalski and R. Kangasniemi, 1984. Milk trypsin-inhibitor capacity as an indicator of bovine mastitis-a novel principle which can be automated. J. Dairy Res., 51: 1-9.

Schrodl, W., M. Kruger, T.T. Hien, M. Fuldner and R. Kunze, 1995. C-reactive protein as a new parameter of mastitis, Tierarzt. Prax, 23: 337-341.

Sears, P.M. and K.K. Mccarthy, 2003. Diagnosis of mastitis for therapy decisions. Vet. Clin. North Am. Food Anim., 19: 93-108.

Segelmark, M., B. Persson, T. Hellmark and J. Wieslander, 1997. Binding and Inhibition of myeloperoxidase (MPO): A major function of ceruloplasmin? Clin. Exp. Immun., 108: 167-174.

Sheldon, I.M., D.E. Noakes, A. Rycroft and H. Dobson, 2001. Acute phase protein responses to uterine bacterial contamination in cattle after calving. Vet. Rec., 148: 172-175.

Sitrin, R.G., P.M. Pan and S. Srikanth, 1998. Fibrinogen activates NF-Kappa B transcription factors in mononuclear phagocytes. J. Immun., 161: 1462-1476.

Sordillo, L.M., M.Z. Doymat, S.P. Oliver and J.T. Dermody, 1989. Leukocytic infiltration of bovine mammary parenchaymal tissue in response to *Corynebacterium bovis* colonization. J. Dairy. Sci., 72: 1045-1051.

Sunderman, F.W. and S. Nomoto, 1970. Measurement of human serum ceruloplasmin by its p-phenylendiamine oxidase activity. Clin. Chem., 16: 903-910.

Taponen, S., A. Jantunen, E. Pyorala and S. Pyorala, 2003. Efficacy of targeted 5-day combined parenteral and intramammary treatment of clinical mastitis caused by penicillin-Susceptible or penicillin-resistant *Staphylococcus aureus*. Acta. Vet. Scand., 44: 53-62.

Thomas, J.S., 2000. Over View of Plasma Proteins. In: Schalm's Veterinary Hematology, Feldman, B.F., J.G. Zinkl and N.C. Jain (Eds.). 15th Edn. Lippincott Williams, Wilkins, Philadelphia, pp: 891-898.

Timms, L.L. and L.H. Schultz, 1987. Dynamics and significance of coagulase-negative staphylococcal intra mammary infections. J. Dairy Sci., 70: 2648-2657.

Tyler, J.W. and J.S. Cullor, 2002. Mammary Gland Health and Disorders. In Large Animal Internal Medicine, Smith, B.P. (Ed.). 3rd Edn. Mosby, London, pp: 1019-1022.

Vreugdenhil, A.C.E., M.A. Dentener, A.M.P. Snoek, J.W. Greve and W.A. Buurman, 1999. Lipopoly saccharide binding protein and serum anyloid secretion by human intestinal epithelial cells during the acute phase response. J. Immun., 163: 2792-2798.

Waage, S., H.R. Skei, J. Rise, T. Roydo and S. Sviland, 2000. Outcome of clinical mastitis in dairy heifers assessed by re-examination of cases one month after treatment. Dairy Sci., 83: 70-76.

Whitaker, D.A., A.I. Macrae and E. Burrough, 2004. Disposal and disease rates in British dairy herds between April 1998 and March 2002. Vet. Rec., 155: 43-47.

Yang, F.M., W.E. Friedrichs, A.L. Navarijoashbaugh, L.A. Degraffenried, B.H. Bowman and J.J. Coalson, 1995. Cell-type-specific and inflammatory-induced expression of haptoglobin gene in lung. Lab. Invest., 73: 433-440.