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Particle-Induced X-ray Emission Analysis of Serum Trace and Major Elements in Cattle with Acute Coliform Mastitis

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Abstract

The aim of the present study was to examine the applicability of the direct determination of trace and major element concentrations in serum samples collected from Holstein dairy cattle with acute coliform mastitis (n = 53) compared with a healthy control group (n = 39). Twenty-eight elements (Na, Mg, Al, Si, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Ce, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, and Pb) were detected by particle-induced X-ray emission (PIXE). Significant differences were observed in serum K, Fe, Zn, and Br concentrations, but not in those of the remaining twenty-four elements. Furthermore, serum Fe concentrations (0.751 ± 0.583 μg/m*l*, n = 18) were significantly lower in dairy cattle with a poor prognosis than in those with a good prognosis (0.945 ± 0.393 μg/m*l*, n = 35, $P < 0.05$) and healthy controls (1.458 ± 0.391 μg/m*l*, n = 39, $P < 0.01$). We proposed a diagnostic cut-off point for serum Fe concentrations of <0.82 μg/m*l* based on receiver operating characteristic (ROC) curves in order to identify cattle with a poor prognosis. The results of the present study indicated that assessing the elemental composition of serum, particularly iron, is a promising prognostic tool for determining the outcomes of cattle with severe acute coliform mastitis.

Key Words: Acute coliform mastitis, Dairy cattle, Iron, Particle-induced X-ray emission, Prognosis

Introduction

Bovine mastitis, which is an inflammation of the mammary glands that often develops following intra-mammary bacterial infection, is one of the most prevalent and economically costly diseases to the dairy industry[20,21]. Mastitis causes substantial milk production losses that translate into an annual loss of approximately $2 billion in the United States[20,21,26]. Among the different pathogenic causes of mastitis, Gram-negative coliform pathogens such as *Escherichia*

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coli and *Klebsiella pneumoniae* typically cause severe inflammation and heavy losses in milk production. Coliform mastitis may result in bacteremia and septicemia as the blood-milk-barrier is destroyed[16]. Septicemia resulting from coliform mastitis is rare, but often fatal. Approximately 25% of cattle with severe Gram-negative intra-mammary inflammation (IMI) will either die or be culled[6]. Non-specific, but potent factors that are important during the pathogenesis of *E. coli* and *K. pneumoniae* are endotoxin and lipopolysaccharide (LPS). Endotoxin is the primary virulence factor of Gram-negative bacteria responsible for damage to the cow and is released from bacteria at the time of cell death, thereby initiating an inflammatory response[6]. The outcome of coliform mastitis depends on the severity of the case, which is typically dependent on the balance between endotoxin and the ability of the cow to respond immunologically. The ability to identify inflammation in its early stages is crucial for a clinical diagnosis, herd health, and animal welfare.

Inflammation is a complex response to cell or tissue injury. Acute inflammation causes a non-specific systemic reaction denoted as the acute phase response. Previous studies on hamsters[12] suggested that acute phase stimuli caused by an endotoxin challenge induced the release of interleukin-l, which, in turn, stimulated an increase in serum Cu concentrations. Serum Cu concentrations have been shown to increase during acute phase reactions in other species, in part because of elevations in the serum concentrations of the Cu-binding protein, ceruloplasmin[12]. Furthermore, selenium modulates the functions of many regulatory proteins in signal transduction is advantageous for animals with inflammatory diseases. Zhang *et al*.[31] showed that the LPS-induced expression of cyclooxygenase-2 and tumor necrosis factor-α was significantly decreased in Se-deficient mouse mammary epithelial cells treated with Se. Many chronic diseases in human and animals have been associated with modifications to extracellular matrix metabolism that lead to the accumulation of several elements. Therefore, the relationship between coliform mastitis and the status of trace and major elements needs to be investigated in order to improve food animal health care. However, to date no comparative studies have been conducted on trace and major elements in serum collected from cattle with coliform mastitis. The aim of the present study is to investigate the serum of dairy cattle with acute coliform mastitis, and how that may be reflected in the concentrations of trace elements measured using Particle-Induced X-ray Emission (PIXE) analysis. Receiver operating characteristic (ROC) curves were used to describe the performance of serum in screening for acute coliform mastitis and propose diagnostic cut-off values for cattle.

Materials and Methods

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the School of Veterinary Medicine at Rakuno Gakuen University. A prospective case-control study was performed on cattle with acute coliform mastitis. Fifty-three Holstein Friesian breed dairy cattle with acute coliform mastitis with IMI and systemic symptoms were included in this study.

The initial diagnosis of cattle with mastitis was made according to their milk production amount as identified using the criteria from Heyneman *et al*[15]. in addition, the cattle that showed pre-acute or acute clinical signs such as udder swelling, redness, hard quarter, or edema in one or more quarters, tachypnea, tachycardia, fever, weakness, and/or shivering were categorized as having acute mastitis. The definitive diagnosis of coliform mastitis was made in each animal by isolation cultures of *E. coli* and/or *K. pneumoniae* using raw milk obtained from the affected quarter. The poor prognosis group comprised of cattle that died within one week or were culled due to poor milk production within 30 days of their first medical examination.

Thirty-nine healthy cattle with none of these clinical symptoms or mastitis were kept as controls at the School of Veterinary Medicine, Rakuno Gakuen University. Ten ml of whole blood was collected via jugular venipuncture into tubes for trace and major element analyses and centrifuged at 3,000 *g* for 10 min at room temperature. The serum samples obtained were separated and stored at −80°C until assayed.

The mean concentrations of trace and major elements in serum were measured using the PIXE method. A detailed description of the experimental arrangement is shown elsewhere[27,28]. Briefly, 100 μ*l* of the serum supernatant was placed on a subtlety Myler membrane and desiccated, then directly irradiated with proton beams. A small (baby) cyclotron used for positron nuclear medicine at the Nishina Memorial Cyclotron Center (Iwate, Japan) provided a 2.9 MeV-proton beam on a target after passing through a graphite beam collimator. A Si (Li) detector (0.0254 mm Be window) with 300- and 1,000-μm-thick Mylar absorbers was used to select X-rays with energy higher than that of K-K alpha. Another Si (Li) detector (0.008 mm Be) was used without absorbers for lower-energy X-rays.

Statistical analyses were performed using a commercial software package (IBM SPSS Statistics, v.21, IBM Co, Somers, NY, USA). Normally distributed data were reported as the mean ± standard deviation (SD) and non-normally distributed data were expressed as a median and range. Regarding normally distribution data, the mean values for each dependent variable were compared between the control and acute mastitis groups using the Student's *t*-test, and were compared among groups using the Tukey test after ANOVA with the *F* test. Regarding non-normally distributed data, the Mann−Whitney U-test and Kruskal-Wallis test were employed for comparisons between the control and acute mastitis groups, and among groups, respectively. ROC curves were used to characterize the sensitivity and specificity of a parameter for a poor prognosis. The optimal cut-off point for a test was calculated by the Youden index[1,22]. The Youden index (J) is defined as the maximum vertical distance between the ROC curve and diagonal or chance line and is calculated as J = maximum [sensitivity + specificity − 1]. The cut-off point on ROC curves that corresponded to J was selected as the optimal cut-off point[1,22]. The significance level was set at $P < 0.05$.

Results

Fifty-three Holstein Friesian breed dairy cattle with acute coliform mastitis were enrolled in this clinical trial. Among the 53 cattle, the good and poor prognoses groups comprising 35 and 18 cattle, respectively. The mean concentrations of trace and major elements in serum collected from these cattle with acute coliform mastitis are summarized in Table 1. The PIXE method allowed for the detection of 28 elements: Na, Mg, Al, Si, S, Cl, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Br, Rb, Sr, Y, Zr, Nb, Mo, and Pb. Except for Si, all serum trace and major element values obtained from cattle with or without acute coliform mastitis were normally distributed. Significant differences were observed in the serum concentrations of K, Fe, Zn and Br, but not in those of the remaining 24 elements.

Fig. 1 shows the K, Zn, Br, and Fe concentrations in serum obtained from the examined cattle in the control group and for those with good and poor prognoses. The average serum K, Zn, and Br concentrations in control cattle were 93.1 ± 18.8, 1.322 ± 0.580, and 26.6 ± 5.6 μg/m*l*, respectively. The mean K concentration (125.2 ± 36.9 μg/m*l*, $P < 0.05$) in serum was significantly higher, and mean Zn (0.897 ± 0.651 μg/m*l*, $P < 0.05$) and Br concentrations (10.4 ± 7.4 μg/m*l*, $P < 0.01$) in serum were significantly lower in dairy cattle with acute coliform mastitis than in the controls, whereas no significant differences were noted between the good and poor prognoses groups ($P > 0.05$).

Table 1. The mean concentrations of trace and major elements in serum from the control and with acute coliform mastitis cattle (μg/ml)

	Control (n = 39)	Acute Coliform Mastitis		
		Total (n = 53)	Good prognosis (n = 35)	Poor prognosis (n = 18)
Na	380.2 ± 97.0	564.4 ± 281.6	591.1 ± 312.5	512.3 ± 207.3
Mg	5.03 ± 4.66	8.09 ± 6.81	8.10 ± 4.59	8.07 ± 9.99
Al	4.98 ± 2.41	2.32 ± 2.56	2.12 ± 2.41	2.71 ± 2.86
Si	3.06 (0–16.8)	5.34 (0–20.9)	5.33 (0–20.9)	5.56 (0–14.0)
S	500.6 ± 105.5	573.0 ± 157.4	595.9 ± 175.7	528.3 ± 103.9
Cl	1523.2 ± 337.2	2033.5 ± 764.0	2156.2 ± 880.2	1795.0 ± 380.8
K	93.1 ± 18.8	125.2 ± 36.9[a]	133.0 ± 40.0[a]	109.9 ± 24.6
Ca	67.6 ± 13.0	62.8 ± 19.3	65.4 ± 21.8	57.9 ± 12.5
Ti	0.049 ± 0.136	0.033 ± 0.066	0.041 ± 0.072	0.017 ± 0.050
V	0.037 ± 0.054	0.047 ± 0.063	0.052 ± 0.070	0.036 ± 0.046
Cr	0.076 ± 0.053	0.080 ± 0.055	0.078 ± 0.052	0.085 ± 0.061
Mn	0.011 ± 0.034	0.017 ± 0.038	0.022 ± 0.044	0.007 ± 0.023
Fe	1.458 ± 0.391	0.879 ± 0.470[b]	0.945 ± 0.393[b]	0.751 ± 0.583[b,c]
Co	0.009 ± 0.017	0.009 ± 0.017	0.011 ± 0.020	0.004 ± 0.009
Ni	0.022 ± 0.026	0.018 ± 0.026	0.019 ± 0.028	0.017 ± 0.022
Cu	0.736 ± 0.184	0.706 ± 0.279	0.709 ± 0.320	0.701 ± 0.182
Zn	1.322 ± 0.580	0.897 ± 0.651[a]	0.910 ± 0.527[a]	0.872 ± 0.861[a]
Ga	0.031 ± 0.030	0.021 ± 0.025	0.022 ± 0.026	0.021 ± 0.025
As	0.001 ± 0.005	0.003 ± 0.013	0.002 ± 0.008	0.007 ± 0.019
Se	0.097 ± 0.062	0.067 ± 0.046	0.068 ± 0.047	0.065 ± 0.044
Br	26.6 ± 5.6	10.4 ± 7.4[b]	10.5 ± 7.7[b]	10.2 ± 7.1[b]
Rb	0.001 ± 0.007	0.060 ± 0.070	0.072 ± 0.112	0.037 ± 0.051
Sr	0.065 ± 0.044	0.098 ± 0.073	0.106 ± 0.076	0.084 ± 0.057
Y	0.023 ± 0.043	0.032 ± 0.052	0.039 ± 0.062	0.018 ± 0.021
Zr	0.062 ± 0.073	0.073 ± 0.115	0.083 ± 0.129	0.053 ± 0.078
Nb	0.019 ± 0.042	0.023 ± 0.052	0.023 ± 0.057	0.022 ± 0.044
Mo	0.045 ± 0.066	0.066 ± 0.095	0.068 ± 0.106	0.061 ± 0.071
Pb	0.052 ± 0.076	0.053 ± 0.057	0.055 ± 0.056	0.049 ± 0.060

a: $P < 0.05$ vs control, b; $P < 0.01$ vs control, c: $P < 0.05$ vs good prognosis

Serum Fe concentrations were lower in dairy cattle with acute coliform mastitis (0.879 ± 0.470 μg/m*l*) than in those without mastitis (1.458 ± 0.391 μg/m*l*, $P < 0.01$). Furthermore, serum Fe concentrations (0.751 ± 0.583 μg/m*l*) were significantly lower in dairy cattle with a poor prognosis than in those with a good prognosis (0.945 ± 0.393 μg/m*l*, $P < 0.05$).

Fig. 2 shows the ROC curves for serum Fe concentrations in detecting poor prognosis cattle with acute coliform mastitis. The proposed diagnostic cut-off point for serum Fe concentrations in order to identify dairy cattle with acute coliform mastitis with a poor prognosis based on analyses of ROC curves was set at <0.82 μg/m*l*. The sensitivity and specificity of the proposed diagnostic cut-offs for serum Fe concentrations were 77.8% and 77.0%, respectively. The area under the ROC curve for Fe concentrations was 0.713 μg/m*l* ($P < 0.05$).

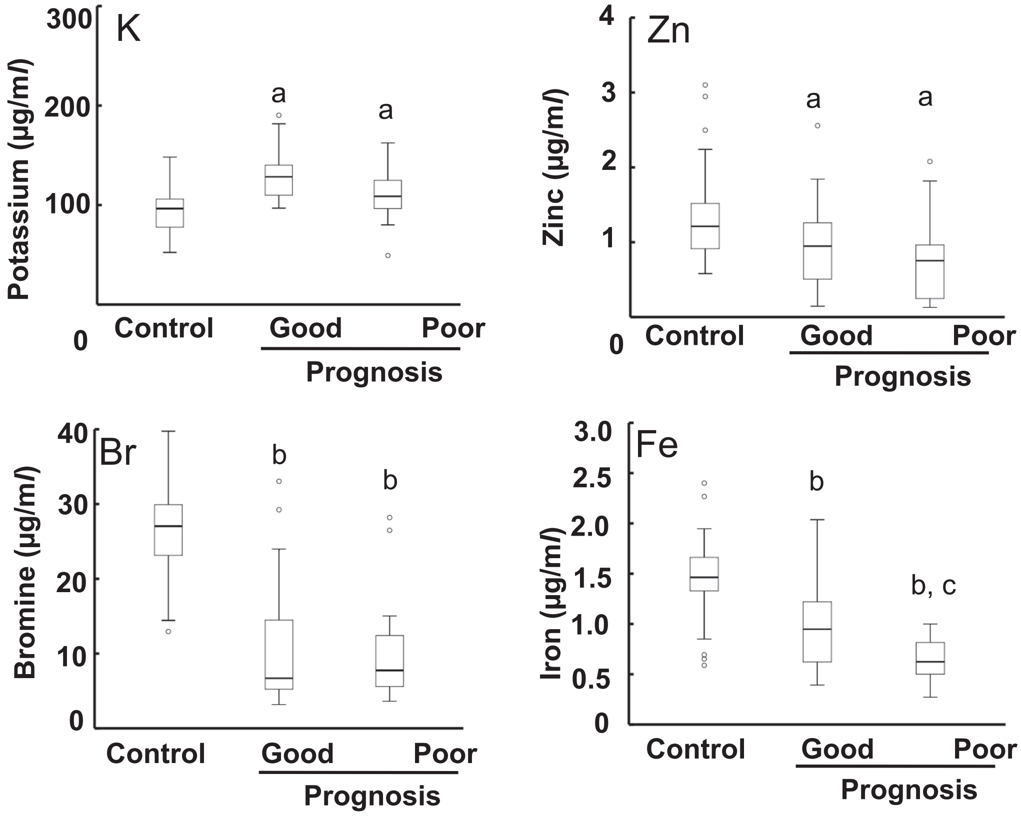

Fig. 1. Medians of serum potassium (K), bromine (Br), zinc (Zn) and iron (Fe) concentrations in cattle with acute coliform mastitis. The horizontal line in each box represents the median value. The boxes represent the interquartile range (25 to 75 percentiles). Outliers are plotted separately as dots. *a*: $P < 0.05$, b: $P < 0.01$ vs the control group, and c: $P < 0.05$ vs the good prognosis group.

Discussion

In the present study, we elucidated the linkage between acute coliform mastitis in dairy cattle and the serum concentrations of some trace and major elements. Serum concentrations of Br, Fe, and Zn were lower in dairy cattle with acute coliform mastitis with IMI than in those without mastitis. Furthermore, serum Fe concentrations were significantly lower in dairy cattle with a poor prognosis than in those with a good prognosis. Therefore, the proposed diagnostic cut-offs for serum Fe concentrations based on an ROC curves analysis to detect a poor prognosis was set at <0.82 µg/m*l*.

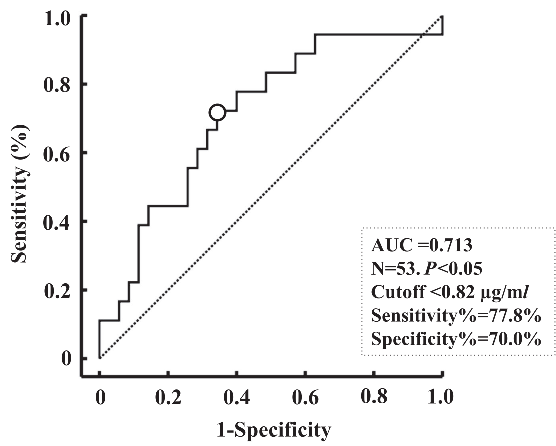

Fig. 2. The mean area under the ROC curve (AUC) is shown for the ROC curve. The optimal cut-off point for the test was calculated by the Youden index. Open Circle: Cut-off point.

Endotoxin, which is released from bacteria at the time of cell death, thereby initiating an inflammatory response, refers to the lipopolysaccharide protein of the Gram-negative bacterial wall and is the primary virulence factor of Gram-negative bacteria responsible for damage to cattle. Endotoxin is known to be responsible for many pathophysiological signs observed during Gram-negative bacterial infections in ruminants such as fever, leukopenia, complement activation, the activation of macrophages, and changes in plasma levels of metabolites, minerals, acute phase reactants and hormones. Coliform mastitis may result in bacteremia and septicemia as the blood-milk-barrier is destroyed[6]. Approximately 25% of cattle with severe Gram-negative IMI will either die or be culled[6]. Although coliform mastitis is associated with acute inflammation, the arsenal of the practicing veterinarian includes only a limited number of laboratory tests for the diagnosis of inflammation. Borges *et al*.[4] investigated the diagnostic value of plasma element levels in horses with systemic inflammation and reported that the plasma Fe level was a superior marker than that of other elements in detecting systemic inflammation in horses. Therefore, serum trace and major elements have been evaluated as markers of inflammation in some species; however, limited data is available for determining whether serum trace elements may be used in predicting the prognosis of dairy cattle with acute coliform mastitis.

The results of the present study showed that average serum K concentrations were higher in cattle with acute coliform mastitis than in controls. Potassium leaks out to the extracellular fluid with epithelial cell injury because these elements are mostly contained in the intracellular fluid. Therefore, increases in the serum levels of K may strongly correlate with inflammation caused by endotoxin. We herein demonstrated that decreased serum Zn and Br concentrations in cattle with acute coliform mastitis were clearly different from those in healthy controls.

Hu *et al*.[17] previously reported that intestinal mRNA levels of TLR4 and its downstream signals, including MyD88, IL-1 receptor-associated kinase 1, and TNF-α receptor-associated factor 6, were decreased, with simultaneous reductions in the expression of intestinal pro-inflammatory cytokines and chemokines in Zinc-supplemented piglets. The protective effects of zinc on intestinal integrity have been closely related to decreases in the expression of genes associated with inflammation through the inhibition of TLR4-MyD88 signaling pathways. Therefore, its protective effects against endotoxin-induced inflammation may depend on the amount of ionized zinc in the serum of cattle with acute inflammation[17]. The results of the present study support the above findings. A structurally and functionally distinct enzyme from neutrophil myeloperoxidase has been shown to exhibit the unique ability to use halides or pseudohalides (X^-) and H_2O_2 derived from the respiratory burst to generate cytotoxic hypohalous acids, especially hypobromous acid (HOBr)[5,30]. Eosinophil peroxidase (EPO), such as the EPO-H_2O_2-Br^- system, is also an effective cytotoxin for multiple targets such as multicellular worms or parasites, bacteria, viruses, and host cells[30]. HOBr and the EPO-H_2O_2-Br^- system are both involved in many of the pathophysiological features of inflammatory diseases[5]. The results for the present study revealed no significant differences between the good and poor prognoses groups, whereas significant differences were observed in these elements in cattle with acute coliform mastitis. Even if marked differences existed in these elements between cattle with mastitis and healthy control, they were not regarded as suitable for determining the prognosis of cattle based on the results obtained in this study.

We herein demonstrated that serum Fe concentrations were significantly lower in dairy cattle with acute coliform mastitis than in controls on the first clinical examination day. Iron is known to play roles in many enzymatic activities and is an essential trace element for the host and pathogen[24]. Significant decreases in Fe concentrations have been reported in the serum and/or plasma of patients and animals with acute inflammation[8,9]. This reduction has been attributed to decreased intestinal absorption and reductions in the release of Fe by reticulo-endothelial cells[2,8,9]. Iron concentrations in serum may also be used as a marker of acute inflammation in humans[29] and horses,[4,18] and previous studies reported decreases in 90% of cats[23], 60% of dogs[23], and 32–52% of cows[3,11] with inflammatory diseases. Serum Fe levels are known to decrease during infection or inflammation, and may be part of the innate host defense mechanism to limit the availability of this element to most pathogens[8,9]. Jacobsen *et al.*[19] reported that serum Fe levels decreased within 24 hrs postoperatively in horses with osteochondritic lesions, laryngeal neuropathy, and ovarian tumors. Previous studies demonstrated that plasma Fe levels decreased rapidly within 24 hrs of the initiation of inflammation[14,25]. Therefore, they may represent a useful tool for determining the prognosis of acute inflammation in cattle with acute coliform mastitis on the first clinical examination day. In the present study, the diagnostic cut-off point for serum Fe concentrations to identify a poor prognosis was set at <0.82 μg/m*l* based on an ROC analysis. Therefore, we suggested that the assessment of the elemental composition of serum, especially Fe, is a promising prognostic tool for determining the outcomes of cattle with severe acute coliform mastitis.

The PIXE method used in the present study is a fast and reliable multi-element qualitative and quantitative analytical tool that is easily accomplished[10]. In this technique, a detector analyzes characteristic X-rays emitted as a result of the inner-shell ionization of target atoms, which require a few μg of samples to analyze concentrations in the ppm range[10]. Also, this method works well especially for analyzing medium- and higher atomic weight elements in a matrix consisting of light elements. Since this method does not involve complicated sample preparation steps, the risk of contamination during the preparation of a sample for the PIXE method is markedly lower than that for other methods[27,28]. Previous studies reported that usage of ligands such as 4-[5-bromo-2-pyridyl) azo]resorcinol (Br-PAR) and some other types can determine the concentration of Fe, Cu, and Zn in serum samples[7,13]. The Br-PAR and other ligands could help to determine the concentrations of Fe, Cu, and Zn in serum samples with a range of 25–500 μg/ml[7]. This concentration range determined by Catillo *et al.*[7] in serum samples was an equivalent level with the PIXE measurement used in this study, thus usage of such ligands may help to determine the element concentrations with more simple and inexpensive spectrophotometric system in various places.

In conclusion, we identified serum Fe level as a superior diagnostic marker for detecting poor prognosis in cattle with acute coliform mastitis compared with other elements. Based on ROC curves, we proposed a diagnostic cut-off point for serum Fe concentrations of <0.82 μg/m*l* in order to identify cattle with a poor prognosis. Our results indicate that an assessment of serum Fe concentrations is a promising prognostic tool for determining the outcomes of cattle with severe acute coliform mastitis.

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References

1) Akobeng, A. K. 2007. Understanding diagnostic tests 3: receive operating characteristic curves. *Acta. Paediatr.*, **96**: 644–647.
2) Andriopoulos, B. Jr., Corradini, E., Xia, Y., Faasse, S. A., Chen, S., Grgurevic, L., Knutson, M. D., Pietrangelo, A., Vukicevic, S., Lin, H. Y. and Babitt, J. L. 2009. BMP6 is a key endogenous regulator of hepcidin expression and iron metabolism. *Nat. Genet.*, **41**: 482–487.
3) Baydar, E. and Dabak, M. 2014. Serum iron as an indicator of acute inflammation in cattle, *J. Dairy Sci.*, **97**: 222–228.

4) Borges, A. S., Divers, T. J., Stokol, T. and Mohammed, O. H. 2007. Serum iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses. *J. Vet. Intern. Med*., **21**: 489–494.
5) Brottman, G. M., Regelmann, W. E., Slungaard, A. and Wangensteen O. D. 1996. Effect of eosinophil peroxidase on airway epithelial permeability in the guinea pig. *Pediatr. Pulmonol*., **21**: 159–166.
6) Burvenich, C., Bannerman, D. D., Lippolis, J. D., Peelman, L., Nonnecke, B. J., Kehrli, M. E. Jr. and Paape, M. J. 2007. Cumulative physiological events influence the inflammatory response of the bovine udder to Escherichia coli infections during the transition period. *J. Dairy Sci*., **90**: Suppl 1: E39–54.
7) Catillo, G. M., Thibert, R. J., Seudeal, N. D. and Zak, B., 1988. Determination of iron, copper, and zinc in a single aliquot of serum sample using 4-[(5-bromo-2-pyridyl) azo]resorcinol. *Microchemical Journal*, **37**: 99–109.
8) Cherayil, B. J. 2011. The role of iron in the immune response to bacterial infection. *Immunol. Res*., **50**: 1–9.
9) Cherayil, B. J., Ellenbogen, S. and Shanmugam, N. N. 2011. Iron and intestinal immunity. *Curr. Opin. Gastroenterol*., **27**: 523–528.
10) Chiba, M. 1994. Bioinorganic chemistry: a science in the spotlight—interface of chemistry, biology, agriculture and medicine. *Int. J. PIXE*., **4**: 201–216.
11) Erskine, R. J. and Bartlett, P. C. 1993. Serum concentrations of copper, iron, and zinc during Escherichia coli-induced mastitis, *J. Dairy Sci.*, **76**: 408–413.
12) Etzel, K. R., Swerdel, M. R., Swerdel, J. N. and Cousins, R. J. 1982. Endotoxin-induced changes in copper and zinc metabolism in the Syrian hamster. *J. Nutre*., **112**: 2363–2373.
13) Feldkamp, C. S., Watkins, R., Baginski, S. and Zak, B. 1977. Essential serum metals part I determination of iron. *Microchemical Journal*, **22**: 335–346.
14) Forsberg, C. M. and Bullen, J. J. 1972. The effect of passage and iron on the virulence of Pseudomonas aeruginosa. *J. Clin. Pathol*., **25**: 65–68.
15) Heyneman, R., Burvenich, C. and Vercauteren, R. 1990. Interaction between the respiratory burst activity of neutrophil leukocytes and experimentally induced Escherichia coli mastitis in cows. *J. Dairy Sci*., **73**: 985–994.
16) Hogan, J. and Larry Smith, K. 2003. Coliform mastitis. *Vet. Res*., **34**: 507–519.
17) Hu, C. H., Song, Z. H., Xiao, K., Song, J., Jiao, L. F. and Ke, Y. L. 2014. Zinc oxide influences intestinal integrity, the expressions of genes associated with inflammation and TLR4-myeloid differentiation factor 88 signaling pathways in weanling pigs. *Innate Immun*., **20**: 478–486.
18) Jacobsen, S., Jensen, J. C., Frei, S., Jensen, A. L. and Thoefner, M. B. 2005. Use of serum amyloid A and other acute phase reactants to monitor the inflammatory response after castration in horses: a field study. *Equine Vet. J*., **37**: 552–556.
19) Jacobsen, S., Nielsen, J. V., Kjelgaard-Hansen, M., Toelboell, T., Fjeldborg, J., Halling-Thomsen, M., Martinussen, T. and Thoefner, M. B. 2009. Acute phase response to surgery of varying intensity in horses: a preliminary study. *Vet. Surg*., **38**: 762–769.
20) Kandasamy, S., Green, B. B., Benjamin, A. L. and Kerr, D. E. 2011. Between-cow variation in dermal fibroblast response to lipopolysaccharide reflected in resolution of inflammation during *Escherichia coli* mastitis. *J. Dairy Sci*., **94**: 5963–5975.
21) Kerro Dego, O., Oliver, S. P. and Almeida, R. A. 2012. Host-pathogen gene expression profiles during infection of primary bovine mammary epithelial cells with *Escherichia coli* strains associated with acute or persistent bovine mastitis. *Vet. Microbiol*., **155**: 291–297.
22) Nakas, C. T., Dalrymple-Alford, J. C., Anderson, T. J. and Alonzo, T. A. 2013. Generalization of Youden index for multiple-class classification problems applied to the assessment of externally validated cognition in Parkinson disease screening. *Stat. Med*., **32**: 995–1003.
23) Neumann, S. 2003. Serum iron level as an indicator for inflammation in dogs and cats. *Comp. Clin. Path*., **12**: 90–94.
24) Ong, S. T., Ho, J. Z., Ho, B. and Ding, J. L. 2006. Iron-withholding strategy in innate immunity. *Immunobiology*., **211**: 295–314.
25) Ratledge, C. and Dover, L. G. 2000. Iron metabolism in pathogenic bacteria. *Annu. Rev. Microbiol*., **54**: 881–941.
26) Sordillo, L. M. and Streicher, K. L. 2002. Mammary gland immunity and mastitis susceptibility. *J. Mammary Gland Biol. Neoplasia*., **7**: 135–146.
27) Suzuki, K., Higuchi, H., Iwano, H., Lakritz, J., Sera, K., Koiwa, M. and Taguchi, K. 2012. Analysis of trace and major elements in bronchoalveolar lavage fluid of Mycoplasma

bronchopneumonia in calves. *Biol. Trace Elem. Res.*, **145**: 166–171.

28) Suzuki, K., Yamaya, Y., Kanzawa, N., Chiba, M., Sera, K. and Asano, R. 2008. Trace and Major elements Status in bronchoalveolar Lavage Fluid in dogs with or without Bronchopneumonia. *Biol. Trace Elem. Res.*, **124**: 92–96.

29) Ward, C. G., Bullen, J. J. and Rogers, H. J. 1996. Iron and infection: new developments and their implications. *J. Trauma.*, **41**: 356–364.

30) Wu, W., Samoszuk, M. K., Comhair, S. A., Thomassen, M. J., Farver, C. F, Dweik, R. A., Kavuru, M. S, Erzurum, S. C. and Hazen, S. L. 2000. Eosinophils generate brominating oxidants in allergen-induced asthma. *J. Clin. Invest.*, **105**: 1455–1463.

31) Zhang, W., Zhang, R., Wang, T., Jiang, H., Guo, M., Zhou, E., Sun, Y., Yang, Z., Xu, S., Cao, Y. and Zhang, N. 2014. Selenium inhibits LPS-induced pro-inflammatory gene expression by modulating MAPK and NF-κB signaling pathways in mouse mammary epithelial cells in primary culture. *Inflammation*, **37**: 478–485.