Indian Journal of Animal Sciences **88** (3): 295–299, March 2018/Article

Oxidative stress and imbalance of serum trace mineral metabolism contribute to bovine respiratory disease in dairy calves

VIVEK JOSHI[1], A G BHANUPRAKASH[2], R S K MANDAL[3], S ALAM[4], V K GUPTA[5] and UMESH DIMRI[6]

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 17 August 2017; Accepted: 12 September 2017

ABSTRACT

Bovine respiratory disease (BRD) is the most common infectious cause of clinical disease and death in young calves. The study was undertaken to scrutinize a relationship between tissue damage by oxidative stress, concentrations of serum trace minerals and clinical status of calves during BRD. The method of clinical scoring and thoracic auscultation were used to screen and select infected calves. In this study, comparison of lipid peroxides (LPO), antioxidant enzymes and serum trace minerals was done between BRD infected and healthy subjects (18 calves in each group). The infected group was further divided into 3 subgroups according to clinical scores (CS) (5, 6, 7). The blood LPO levels were significantly higher in BRD infected calves. The antioxidative activities of superoxide dismutase, reduced glutathione, catalase and serum uric acid were significantly lower in BRD infected calves. The concentrations of trace minerals (copper, zinc, selenium) were significantly reduced during BRD. All hematobiochemical parameters varied proportionately with the CS of infected calves. These findings demonstrated simultaneous occurrence of increased oxidative stress and depletion of antioxidative trace minerals during BRD in calves. A strong correlation exists between severity of oxidative stress and CS of calves.

Key words: Bovine respiratory disease, Calves, Clinical scores, Trace minerals, Oxidative stress

Bovine respiratory disease is the most significant and costly disease in calf-rearing herds worldwide. It has a high morbidity (65–80%) and mortality (45–75%) rate and leads to reduced growth rate in calves (Miller 2016, Wilson *et al*. 2015). BRD is an important infectious disease on account of high costs involved in the treatment, and care of chronically affected calves and a risk for the development of antimicrobial resistance due to long term antibiotic therapy (Schott *et al*. 2014). The clinical disease is characterized by a high rectal temperature, cough, dyspnea, tachypnea and nasal discharge (Kiser *et al*. 2016). BRD has a complex etiology and in susceptible cattle is caused by a combination of viruses and bacteria along with environmental stressors (Joshi *et al*. 2017, Hay *et al*. 2016, Autio *et al*. 2007).

Oxidative stress plays a key role in the initiation and maintenance of BRD and is a probable determinant of disease severity in calves (Ranjan *et al*. 2006, Lykkesfeldt and Svendsen 2007, Sordillo and Aitken 2009). It is an important molecular mechanism which comprises all the cellular and tissue injuries occurring due to excessive production of reactive oxygen species (ROS) and/or depleted antioxidants. As lungs are continuously exposed to the highest levels of oxygen, they are the most affected organs by oxidative stress and show severe lesions like fibrosis (Cheresh *et al*. 2013). In respiratory tract infections, neutrophils are recruited to the lungs to assist in removal of invading microorganisms by their phagocytic activity. However, in BRD, the response becomes excessive and results in a number of tissue damaging products like ROS (Ranade *et al*. 2014). Higher erythrocytic malondialdehyde (MDA) (an indicator of LPO) levels indicate insufficient antioxidant defense mechanisms to neutralize oxidative stress. The isolated granulocytes of calves, during BRD, produce 10 times as much ROS and have lower plasma superoxide dismutase (SOD) (Grewal *et al*. 2005).

Micronutrients, such as minerals, are essential components of the antioxidant defense system against free radical-induced damage to tissues for the maintenance of health and a deficiency of any of them leads to poor immune response in cattle. Zinc (Zn), copper (Cu) and selenium (Se) are utilized for the synthesis of antioxidant enzymes to counteract oxidative metabolism (Evans and Halliwell 2001). Serum trace minerals are required for adequate immune response as they directly affect both redox status and antioxidant function. While Cu is a component of SOD and ceruloplasmin, Zn and Se are found in SOD and glutathione peroxidase, respectively. These antioxidant

Present address: [1,2,3,4]Ph.D. Scholar (joshignet@gmail.com, vetbhanu@gmail.com, dr.ravi0911@gmail.com, dr.shahjahanalam@gmail.com), [2]Principal Scientist (vinodgupta1288@rediff.com), [3]Head and Principal Scientist (udimrimesh@rediff.com), Division of Medicine.

enzymes scavenge superoxide and alter functions of immune cells (Overton and Yasui 2014). To the authors' knowledge, the ability of serum trace minerals to influence the level of antioxidants and their interaction during clinical infection of BRD have not been studied in calves. The present study aimed at the quantification of MDA, key antioxidant enzymes (SOD, catalase, reduced glutathione, serum uric acid) along with serum trace minerals (Zn, Cu, Se) and their correlation with clinical scores of calves.

MATERIALS AND METHODS

Animals and experimental design: The present study was conducted at Cattle and Buffalo Farm of the Institute, and in adjoining farms of Bareilly where BRD is a prevalent disease in calves during winter (October-February). An infected group comprising 18 calves naturally infected with BRD, was framed and sub-divided into 3 groups based on the values of clinical scores (5, 6, 7). The same number (18) of healthy calves served as negative control. Calves suspected to be suffering from BRD were visually examined for the presence of nasal or ocular discharge, cough, respiratory distress, depression and inappetence. When 2 or more of these clinical signs were observed, rectal temperature of calf was recorded. Using the clinical scoring system (McGuirk 2008) summarized in Table 1, a calf with a score of 5 or more was classified as morbid and included in the study. Calves were not included in the study if there was a presence of concurrent diseases. In addition, physical examination of calf, thoracic auscultation, and hematological examination were undertaken for disease confirmation along with necropsy, if mortality occurred.

Sample collection: Jugular blood was collected from all the calves for the estimation of hematological parameters, hemolysate preparation to evaluate oxidative stress index and for harvesting serum. The serum samples were stored at –20°C till further analysis.

Hemolysate preparation: Using 10% RBC hemolysate, MDA and activities of antioxidant enzymes were estimated. The blood samples were centrifuged at 3,000 rpm for 5 min, plasma separated and RBC pellets were collected and washed with normal saline solution thrice. The washed RBCs were then mixed with nine parts of ice-cold distilled water to prepare 10% hemolysate.

Hematological analysis: Total leucocyte count (TLC), differential cell count (DLC), total erythrocyte count (TEC) and hemoglobin (Hb) concentration were measured as per Jain (1986). Packed cell volume (PCV) was estimated by microhaematocrit method (Coles 1980).

Estimation of oxidative stress markers: LPO in 10% hemolysate was determined as thiobarbituric acid reactive substance (TBARS) by the method of Placer *et al*. (1966). LPO in 10% hemolysate was expressed as MDA nanomoles per mililitre (nmol/ml).

SOD activity in the diluted 10% RBC hemolysate was measured using nitroblue tetrazolium as a substrate according to the method of Marklund and Marklund (1974) with certain modifications suggested by Minami and Yashikawa (1979). SOD activity was expressed as U/mg Hb.

Catalase (CAT) activity was estimated in 10% RBC hemolysate after appropriate dilution by the method of Cohen *et al*. (1970). CAT activity in the assay mixture was expressed in U/mg Hb.

The reduced glutathione (R-GSH) activity was estimated in the RBC suspension by dithio-bis-2-nitro benzoic acid (DTNB) method as per the method of Prins and Loos (1969). Activity of R-GSH was expressed as mmol/l. The concentration of serum uric acid was estimated by uricase-peroxidase method (Domagk and Schlicke 1968) using a commercially available diagnostic kit.

Estimation of serum trace minerals: The serum trace mineral levels were determined after digestion of serum samples by a triple acid mixture of nitric acid, sulphuric acid, and perchloric acid (in a ratio of 4: 2: 1) followed by heating on a hot plate till thick smoke of perchloric acid ceased to come out. Zn, Cu and Se were measured using an atomic absorption spectrophotometer (Model 4141, ECIL, Hyderabad, India). Stock standard solution (1,000 µg/ml) of Zn, Cu and Se were used in estimations. Serum concentrations of trace minerals were expressed in parts per million (ppm).

Statistical analysis: The packaged SPSS program for Windows version 17 (SPSS, Chicago, IL, USA) was used for statistical analysis. Data were expressed as mean and standard error of mean (Mean±SEM), and $P<0.01$ was considered as statistically significant. Differences between

Table 1. McGuirk's clinical scoring system for the screening of calves suffering from BRD

Parameter/Score	0	1	2	3
Rectal temperature (°C)	37.7–38.2	38.3–38.8	38.9–39.3	≥ 39.4
Cough	None	Induced single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs
Nasal discharge	Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
Eye score	Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
Ear score	Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop

groups were evaluated using one way analysis of variance (ANOVA) while Duncan test was used for pair-wise comparisons. Pearson's correlation (r) and linear regression (R^2) analysis was done on the paired data.

RESULTS AND DISCUSSION

A comparison between control and infected groups revealed a significant (P<0.01) decline in TEC, Hb content and PCV in calves suffering from BRD (Table 2). Leucocytosis was commonly recorded in infected calves and it varied significantly with values of CS. Low level of RBCs, Hb and PCV may be attributed to direct suppressive effect of bacterial toxins on hematopoietic system of calves, erythrophagocytosis by macrophages and RBCs destruction caused by ROS. These were in agreement with the findings of Villegas *et al*. (2014) and Esmaeilnejad *et al*. (2012). A negative correlation (r = - 0.89) was observed between CS of infected calves and PCV. Likewise, a positive correlation (r = 87.8) was recorded between CS and TLC of infected calves (Table 3). A higher CS signifies a more severe infection and to combat this, body initiates an increased inflammatory reaction by excessive production of ROS. This is a probable cause of clinical severity in calves suffering from BRD (Ranade *et al*. 2014, Sordillo and Aitken 2009).

The mean MDA values showed a significant (P<0.01) elevation in infected group of calves when compared to healthy control. A strong positive correlation (r = 0.99) existed between CS and MDA levels of BRD infected calves (Table 3). In contrast, a significant (P<0.01) decrease in SOD, R-GSH, CAT and serum uric acid, was recorded in infected group. The results showed a negative correlation between CS and activities of SOD (r = - 0.99), R-GSH (r = - 0.99), CAT (r = - 0.95), and serum uric acid (r = - 0.97) (Table 3). This rise in erythrocytic LPO and MDA levels is indicative of an increased oxidative stress secondary to severe pulmonary inflammation, whereby excessively produced free oxygen radicals bind to polyunsaturated fatty acids of membranes and cannot be removed fully by scant antioxidants like SOD, R-GSH, CAT and serum uric acid. The antioxidants get exhausted due to their overuse in the neutralization of ROS (Panciera and Confer 2010, Civelek *et al*. 2008, Lykkesfeldt and Svendsen 2007, Ranjan *et al*. 2006). Uric acid has antioxidant property on account of its ability to scavenge free radicals and chelate iron thus preventing iron-mediated oxidation and usually offers 60% of scavenging activity in serum (Fabbrini *et al*. 2014, Waring *et al*. 2001). Our findings clearly indicate that R-GSH and SOD are the major antioxidants involved in preventing oxidative damage during BRD, followed by serum uric acid and CAT.

In BRD affected calves, there was a significant (P<0.01) decrease in serum concentration of Zn and Se while an increase in Cu concentration (Table 2). Zn and Se values showed a negative correlation with CS of infected subjects. In contrast, a positive correlation was observed between Cu concentration and CS of calves during BRD (Fig. 1).

There was a decrease in serum concentration of Zn and Se due to their increased consumption for synthesis of antioxidant enzymes like Zn-SOD and Se-containing glutathione peroxidase which are involved in conversion of superoxide radical into H_2O_2 and H_2O_2 to H_2O, respectively (Esmaeilnejad *et al*. 2012, Pamplona and Costantini 2011). Reduced Zn level is also attributed to anorexia in calves and enhanced urinary excretion of Zn-amino acid complex during BRD. Moreover, it is a natural mechanism of body to lower Zn availability for growth of infectious agents (Ryan *et al*. 2015). Cu is a key component of antioxidant enzyme ceruloplasmin which plays an important part in controlling cellular oxidative injuries (Evans and Halliwell 2001). About 90% of serum Cu is firmly bound to ceruloplasmin and its concentration increases during periods of oxidative insult in BRD such that more amount of ceruloplasmin can be synthesized to neutralize ROS (Al-Qudah *et al*. 2010).

Thus, BRD in calves is characterized by a marked imbalance of oxidant-antioxidant status and serum trace

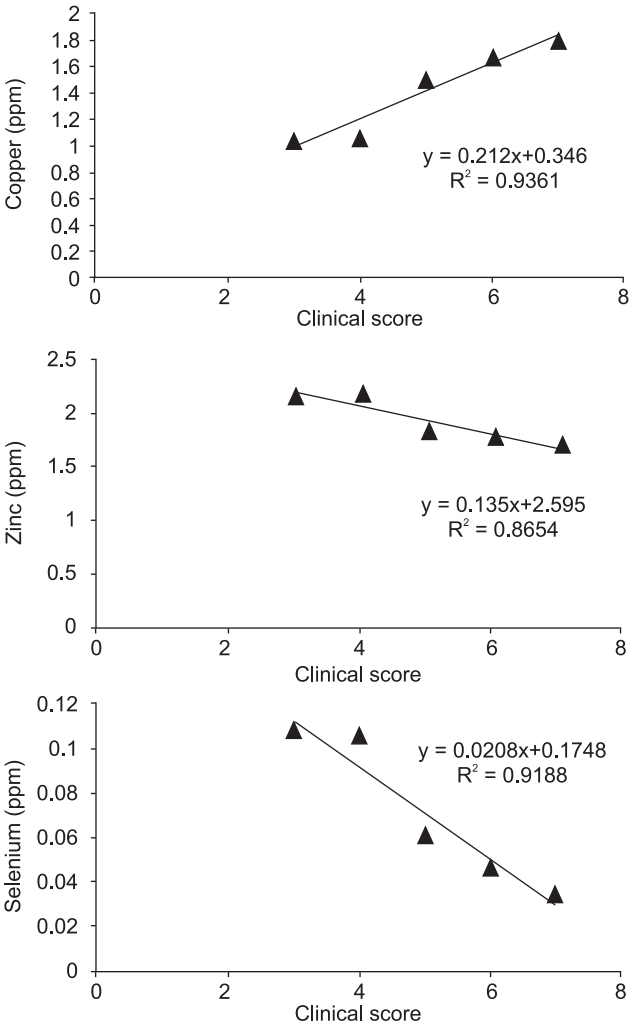

Fig. 1. Linear regression (R^2) analysis of different clinical scores of BRD-infected calves with serum trace minerals copper, zinc and selenium.

Table 2. Oxidative status and hematobiochemical values in healthy and BRD infected calves with different clinical scores

	Clinical Score	TLC (10^3/µl)	TEC (10^6/µl)	PCV (l/l)	Hb (g/dl)	LPO (nmol MDA/ml)	SOD (U/mg Hb)	R-GSH (mmol/l)	CAT (Unit/ assay)	Serum uric acid (mg/dl)	Cu (ppm)	Zn (ppm)	Se (ppm)
Control	<5 (n=18)	8.66 ±0.30^a	5.34 ±0.08^a	32.17 ±0.49^a	13.09 ±0.27^a	5.90 ±0.07^a	5.98 ±0.10^a	6.48 ±0.09^a	4.78 ±0.06^a	8.60 ±0.15^a	1.06 ±0.12^a	2.18 ±0.07^a	0.108 ±0.01^a
Infected	5 (n=6)	11.81 ±0.60^b	4.82 ±0.08^b	26.50 ±0.50^b	12.03 ±0.33^b	7.73 ±0.04^b	4.19 ±0.04^b	4.71 ±0.11^b	4.13 ±0.05^b	6.47 ±0.26^b	1.49 ±0.03^b	1.82 ±0.01^b	0.060 ±0.003^b
	6 (n=4)	12.67 ±0.46^c	3.91 ±0.05^c	22.00 ±0.58^c	10.40 ±0.11^c	8.11 ±0.04^c	3.99 ±0.01^c	4.38 ±0.02^c	3.87 ±0.03^c	6.09 ±0.02^c	1.66 ±0.01^c	1.77 ±0.01^c	0.046 ±0.001^c
	7 (n=8)	15.57 ±0.45^d	3.47 ±0.06^d	18.40 ±0.40^d	9.72 ±0.16^d	8.37 ±0.02^d	3.71 ±0.02^d	4.16 ±0.01^d	3.55 ±0.03^d	5.11 ±0.05^d	1.79 ±0.02^d	1.68 ±0.01^d	0.034 ±0.002^d

Different superscript in each column denote significant differences (P<0.01).

Table 3. Pearson correlation coefficients between oxidative stress markers, antioxidant enzymes, haematological indices and clinical scores in calves infected with BRD

Parameter	CS	PCV	MDA	SOD	R-GSH	CAT	Serum uric acid	TLC
CS	-	–0.897**	0.998**	–0.994**	–0.996**	–0.957**	–0.975**	0.878**
PCV		-	–0.879**	0.846**	0.865**	0.981**	0.943**	–0.958**
MDA			-	–0.996**	–0.999**	–0.943**	–0.962**	0.852**
SOD				-	0.997**	0.924**	0.956**	–0.838**
R-GSH					-	0.933**	0.955**	–0.839**
CAT						-	0.988**	–0.968**
Serum uric acid							-	–0.959**
TLC								-

**Correlation is significant at the level 0.01.

mineral profile. However, these variations are usually more marked in calves with higher clinical scores. This proves the importance of antioxidant supplementation and correction of essential trace mineral concentration in calves during BRD and it requires further validation.

ACKNOWLEDGEMENTS

This work was financially supported by ICAR-Indian Veterinary Research Institute, Izatnagar.

REFERENCES

Autio T, Pohjanvirta T, Holopainen R, Rikula U, Pentikainen J, Huovilainen A and Pelkonen S. 2007. Etiology of respiratory disease in non-vaccinated, non-medicated calves in rearing herds. *Veterinary Microbiology* **119**: 256–65.

Buckham Sporer K R, Weber P S D, Burton J L, Earley B and Crowe M A. 2008. Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. *Journal of Animal Science* **86**: 1325–34.

Cheresh P, Kim S J, Tulasiram S and Kamp D W. 2013. Oxidative stress and pulmonary fibrosis. *Biochimica et Biophysica Acta (BBA)- Molecular Basis of Disease* **1832**: 1028–40.

Civelek T, Celik H A, Avci G and Cingi C C. 2008. Effects of dystocia on plasma cortisol and cholesterol levels in Holstein heifers and their newborn calves. *Bulletin of the Veterinary Institute in Pulawy* **52**: 649–54.

Cohen G, Dembiec D and Marcus J. 1970. Measurement of catalase activity in tissue extracts. *Analytical Biochemistry* **34**: 30–38.

Coles E H. 1980. *Veterinary Clinical Pathology*. 3rd edn. WB Saunders.

Domagk G F and Schlicke H H. 1968. A colorimetric method using uricase and peroxidase for the determination of uric acid. *Analytical Biochemistry* **2**: 219–24.

Esmaeilnejad B, Tavassoli M, Asri-Rezaei S and Dalir-Naghadeh B. 2012. Evaluation of antioxidant status and oxidative stress in sheep naturally infected with *Babesia ovis*. *Veterinary Parasitology* **185**: 124–30.

Evans P and Halliwell B. 2001. Micronutrients: oxidant/ antioxidant status. *British Journal of Nutrition* **85**: 57–74.

Fabbrini E, Serafini M, Baric I C, Hazen S L and Klein S. 2014.

Effect of plasma uric acid on antioxidant capacity, oxidative stress, and insulin sensitivity in obese subjects. *Diabetes* **63**: 976–81.

Grewal A, Ahuja C S, Singha S P S and Chaudhary K C. 2005. Status of lipid peroxidation, some antioxidant enzymes and erythrocytic fragility of crossbred cattle naturally infected with *Theileria annulata*. *Veterinary Research Communications* **29**: 387–94.

Halliwell B and Chirico S. 1993. Lipid peroxidation: its mechanism, measurement, and significance. *American Journal of Clinical Nutrition* **57**: 715S–24S.

Hay K E, Barnes T S, Morton J M, Gravel J L, Commins M A, Horwood P F, Ambrose R C, Clements A C A and Mahony T J. 2016. Associations between exposure to viruses and bovine respiratory disease in Australian feedlot cattle. *Preventive Veterinary Medicine* **127**: 121–33.

Jericho K W F and Kozub G C. 2004. Experimental infectious respiratory disease in groups of calves: lobular distribution, variance, and sample size requirements for vaccine evaluation. *Canadian Journal of Veterinary Research* **68**: 118–27.

Joshi V, Gupta V K, Dimri U, Vinodh Kumar O R, Sharma D K and Bhanuprakash A G. 2017. Assessment of nebulisation of sodium ceftiofur in the treatment of calves naturally infected with bovine respiratory disease. *Tropical Animal Health and Production* **49**: 497–501.

Kiser J N, Seabury C M, Taylor J F, Womack J E, Hagevoort R, Lehenbauer T W, Aly S S, Van Eenennaam A L and Neibergs H L. 2016. Clinical signs associated with bovine respiratory disease diagnosis and high heritability in beef and dairy cattle. *Journal of Animal Science* **94**: 181–82.

Lykkesfeldt J and Svendsen J. 2007. Oxidants and antioxidants in disease: Oxidative stress in farm animals. *Veterinary Journal* **173**: 502–11.

Marklund S and Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47**: 469–74.

McGuirk S M. 2008. Disease management of dairy calves and heifers. *Veterinary Clinics of North America: Food Animal Practice* **24**: 139–53.

Miller S. 2016. *Current and Future Strategies of Bovine Respiratory Disease Diagnostics and Treatments.*

Minami R and Yashikawa M. 1979. A simplified assay method of superoxide dismutase activity for clinical use. *Clinica Chimica Acta* **92**: 337–42.

Nemi C J. 1986. *Schalm's Veterinary Hematology.* p. 1–19. Lea and Febiger, Philadelphia.

Overton T R and Yasui T. 2014. Practical applications of trace minerals for dairy cattle. *Journal of Animal Science* **92**: 416–26.

Panciera R J and Confer A W. 2010. Pathogenesis and pathology of bovine pneumonia. *Veterinary Clinics of North America: Food Animal Practice* **26**: 191–214.

Placer Z A, Cushman L L and Johnson B C. 1966. Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. *Analytical Biochemistry* **16**: 359–64.

Prins H K and Loos J A. 1969. Glutathione. *Biochemical Methods in Red Cell Genetics*. p. 115–137. Academic Press, New York.

Ranade R, Talukder S, Muscatello G and Celi P. 2014. Assessment of oxidative stress biomarkers in exhaled breath condensate and blood of dairy heifer calves from birth to weaning. *Veterinary Journal* **202**: 583–87.

Ranjan R, Naresh R, Patra R C and Swarup D. 2006. Erythrocyte lipid peroxides and blood zinc and copper concentrations in acute undifferentiated diarrhoea in calves. *Veterinary Research Communications* **30**: 249–54.

Ryan A W, Kegley E B, Hawley J, Powell J G, Hornsby J A, Reynolds J L and Laudert S B. 2015. Supplemental trace minerals (zinc, copper and manganese) as sulfates, organic amino acid complexes or hydroxyl trace-mineral sources for shipping-stressed calves. *Professional Animal Scientist* **31**: 333–41.

Schott C, Cai H, Parker L, Bateman K G and Caswell J L. 2014. Hydrogen peroxide production and free radical-mediated cell stress in *Mycoplasma bovis* pneumonia. *Journal of Comparative Pathology* **150**: 127–37.

Sordillo L M and Aitken S L. 2009. Impact of oxidative stress on the health and immune function of dairy cattle. *Veterinary Immunology and Immunopathology* **128**: 104–09.

Villegas L, Stidham T and Nozik-Grayck E. 2014. Oxidative stress and therapeutic development in lung diseases. *Journal of Pulmonary and Respiratory Medicine* **4**: 194.

Waring S W, Webb D J and Maxwell S R. 2001. Systemic uric acid administration increases serum antioxidant capacity in healthy volunteers. *Journal of Cardiovascular Pharmacology* **38**: 365–71.

Wilson B K, Step D L, Maxwell C L, Wagner J J, Richards C J and Krehbiel C R. 2015. Evaluation of multiple ancillary therapies used in combination with an antimicrobial in newly received high-risk calves treated for bovine respiratory disease. *Journal of Animal Science* **93**: 3661–74.

Pamplona R and Costantini D. 2011. Molecular and structural antioxidant defenses against oxidative stress in animals. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **301**: R843–63.

Al-Qudah K M, Gharaibeh A A and Maysaa M. 2010. Trace minerals status and antioxidant enzymes activities in calves with dermatophytosis. *Biological Trace Element Research* **136**: 40–47.