



Effect of lameness due to claw disorders on oxidative and mineral profile of crossbred cattle

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ABSTRACT

To evaluate the effect of lameness due to claw disorders on oxidative parameters and mineral profile, 34 crossbred dairy cattle were selected from 11 dairy farms. Lamé animals were categorized based on locomotion score, hoof lesions and severity of disease. Significant increase in MDA level (25.90 ± 1.32 nmol MDA/ml) along with significant decrease in SOD level (35.30 ± 0.98 U/mg of Hb) was recorded among lame animals compared to control (2.55 ± 0.36 nmol MDA/ml and 49.53 ± 0.54 U/mg of Hb, respectively). GPx activity decreased non-significantly (3.82 ± 0.31 U/mg of Hb) compared to healthy animals (4.65 ± 0.64 U/mg of Hb). Analysis of plasma mineral profile revealed significant decline in the average levels of Cu, Ca and Pi (6.34 ± 0.25 μ mol/l, 8.94 ± 0.25 mg/dl and 3.44 ± 0.13 mg/dl, respectively) compared to control (22.06 ± 0.77 μ mol/l, 10.66 ± 0.50 mg/dl and 4.47 ± 0.21 mg/dl, respectively). Study concludes that the antioxidant defence system is compromised in lame cows and such animals need mineral supplementation especially Ca, P and Cu.

Key words: Claw disorders, Crossbred cattle, Lameness, Oxidative stress, Trace minerals, zinc

Oxidative stress is involved in the pathogenesis of many diseases in dairy animals. The elevated levels of reactive oxygen species (ROS) leads to enormous lipid peroxides production causing toxic damage to the tissues in the form of rapid injury and necrosis. Minerals play an important role in hoof development and oxidative process. Copper (Cu), Zinc (Zn) and Manganese (Mn) are essential constituent of enzyme-superoxide dismutase (SOD) which catalyses superoxide ions into H_2O_2 and oxygen. Calcium is required for enzyme activation involved in production of mature cells of horn. Enormous drainage of Ca in milk from high yielders causes lower plasma levels thereby leads to dyskeratotic horn as epidermal cells are sensitive to this imbalance. Zn plays role in formation of the structural keratin proteins, epidermal cell differentiation and keratinization (Mülling *et al.* 1999). Copper helps in the formation of the chemical bonds between keratin filaments and gives rigidity to the horn cells. Decline in the reserves of body's elements is expected if the animal is deprived of good mineral sources and/or supplements. However, these declines may not result in a disease condition with distinct clinical signs. Oxidative stress also leaves no clinical signs.

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So, both mineral deficiencies and oxidative stress may be left undiagnosed under routine management practices. Not much of the research work has been done to evaluate oxidative parameters during lameness. Therefore, present study was designed to evaluate potential impact of antioxidant activity and plasma mineral status on hoof health of crossbred cattle.

MATERIALS AND METHODS

HF crossbred dairy cattle (34) suffering from lameness from 11 dairy farms at various locations were selected and to compare the levels of various parameters, 10 healthy animals were sampled.

Hoof examination: Each lame animal was properly restrained and different foot lesions were identified after performing hoof trimming as per standard procedure (Toussaint 1989).

Categorization of lame animals: Lame animals were categorized under various groups based on locomotion score (LS)-1, 2, 3 and 4 as per Wells *et al.* (1993). Seven animals were placed under LS 1 score, 5 animals in LS 2, 16 animals in LS 3 and 6 animals in LS 4. Lesion-wise affected animals were placed under two groups based on zone description of hoof i.e. Group I comprised 17 animals having lesions like white lie haemorrhage (WLF), heel erosion (HE) and over grown hoof (OGF) whereas Group II comprised of 17 animals having lesions like sole haemorrhage (SE), sole ulcer (SU), toe ulcer (TU) and toe haemorrhage (TH). According to severity of the lesions and the duration of

disease, lame animals were designated as acute cases (having history of lameness <4 days duration) in which 11 animals were identified and chronic cases (having history of lameness >4 days duration) in which 23 animals were identified.

Sampling: Blood samples were collected from each cattle affected with lameness. Blood samples meant for mineral and biochemical estimations were centrifuged at 3,000 rpm for 30 min to separate plasma immediately after collection. Plasma samples were stored at -10°C in deep freeze for subsequent analysis.

Analysis: Heparinised whole blood samples were used for the estimation of blood glutathione peroxidase (GPx) as per Hafeman *et al.* (1974). Superoxide-Dismutase (SOD) (Marklund and Marklund 1974), glutathione-peroxidase (GPx) (Hafeman *et al.* 1974) and malondialdehyde (MDA) (Ohkawa *et al.* 1979) were also estimated. Estimation of Ca was done as per cresolphthalein complexon method and inorganic fraction of phosphorus (Pi) was determined using kits. Plasma sample (3 ml) was analysed for micromineral analysis by digesting in distilled concentrated nitric acid (AR, 15 ml). The concentrations of micro-elements, viz. Cu, Fe, Zn, were measured by Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2300, Hitachi).

Statistical analysis: Data were analyzed using student's t-test (Independent samples test) and general linear model: multivariate analysis (Turkey) using SPSS version 16 software.

RESULTS AND DISCUSSION

Oxidative stress parameters: Significant ($P<0.05$) increase in the average MDA level (Table 1) confirmed the presence of oxidative stress in animals affected with lameness and can be attributed to pathogenesis of lameness. Locomotion score wise significant ($p<0.05$) increase in the average values of MDA was recorded among animals with LS-1, 2, 3 and 4 compared to healthy animals with LS-0. Severity-wise significant ($P<0.05$) increase in MDA levels of animals with acute and chronic lameness was observed. Various other workers have also reported elevated levels of lipid peroxidation products (Neville *et al.* 2004, Seyek *et al.* 2008, Al-Qudah and Ismail 2012 and Zhao *et al.* 2015). Excess production of reactive oxygen species (ROS) causes dyskeratosis of hoof (Tomlinson *et al.* 2004), chondrocyte apoptosis and cartilage degeneration (Heinecke *et al.* 2010). Harris *et al.* (2006) reported that elevated levels of free radicals leads to laminitis in horses as ROS cause damage to the vascular endothelium.

Significant ($P<0.05$) decrease in the SOD levels and non-significant ($P>0.05$) decrease in the average value of GPx among lame animals was observed (Table 1). SOD levels of animals with LS-1, 2, 3 and 4 were significantly ($P<0.05$) lower compared to animals with LS-0. Animals with LS 2 and 3 had significantly ($P<0.05$) declined levels of SOD compared to LS 1 and 4. GPx activity showed non-significant variation among lame animals. Animals with LS 1 were having significantly ($P<0.05$) declined levels

Table 1. Effect of lameness due to claw disorders on oxidative parameters and mineral profile in crossbred dairy cattle (Mean $\pm$ SE)

Parameter	Locomotion score				Duration of lameness*		Affected zone of hoof**		Overall Lameness* animals*	
	0	1	2	3	4	Acute cases	Chronic cases	Group I		Group II
Lipid peroxidase (nmol MDA/mg Hb)	2.55 ^{abc} ±0.36	34.29 ^a ±2.91	32.50 ^b ±1.53	21.50 ^{abc} ±1.02	30.48 ^c ±1.89	28.96 ^a ±2.87	25.8 ^b ±1.46	24.88 ^a ±1.66	26.91 ^b ±1.77	25.90 ^a ±1.323
SOD (U/mg of Hb)	49.53 ^{abcd} ±0.54	42.71 ^{ab} ±1.30	29.75 ^{ac} ±2.17	31.94 ^b ±0.87	40.34 ^{cd} ±0.16	34.80 ^a ±2.63	35.05 ^b ±1.14	32.23 ^a ±1.47	36.46 ^a ±1.29	35.30 ^a ±0.98
Gpx (U/mg Hb)	4.65 ^a ±0.64	1.38 ^{abc} ±0.09	3.01±0.27	4.79 ^b ±0.32	5.48 ^c ±0.24	3.65±0.82	4.31±0.29	4.78±0.43	3.89±0.46	3.82±0.31
Copper (μmol/L)	22.06 ^{abcd} ±0.77	8.59 ^a ±1.70	5.77 ^b ±0.71	6.19 ^c ±0.41	6.21 ^d ±0.73	6.69 ^a ±0.37	6.08 ^b ±0.36	5.72 ^a ±0.50	6.74 ^b ±0.24	6.34 ^a ±0.25
Iron (μmol/L)	57.71±4.19	58.28±12.04	73.16±14.95	55.54±5.06	54.75±7.08	64.42±7.78	57.15±4.40	55.80±0.89	57.68±5.30	60.00±3.50
Zinc (μmol/L)	22.97±1.87	16.01±3.34	22.98±5.89	16.61±1.55	25.56±8.16	18.52±1.96	19.87±2.53	17.10±1.99	22.22±3.50	19.74±1.64
Calcium (mg/dL)	10.66 ^{ab} ±0.50	9.03±0.19	8.45±0.72	8.75 ^a ±0.35	8.79±0.62	8.85 ^a ±0.42	9.02 ^b ±0.36	8.29 ^{ab} ±0.45	9.45 ^b ±0.41	8.94 ^a ±0.25
Phosphorus (mg/dL)	4.47 ^{ab} ±0.21	3.99±0.15	3.52±0.48	3.42 ^a ±0.17	3.24 ^b ±0.45	3.36 ^a ±0.22	3.39 ^b ±0.15	3.06 ^a ±0.17	3.67 ^b ±0.16	3.44 ^a ±0.130

^{a,b,c} Means marked with similar superscript differ significantly ($P<0.05$) in a row. *Statistically compared with values from animals with LS-0 and means marked with similar superscript differ significantly ($P<0.05$) in a row.

compared with LS 0, 3 and 4. The SOD levels were significantly ($P<0.05$) decreased in animals with acute and chronic lameness. No significant ($P>0.05$) change was observed in the average value of GPx. Analysis based on hoof lesions revealed that Gr I (WLF, OGH and HE lesions) and Gr II (SU, SH, TU and TH lesions) animals had significantly ($P<0.05$) higher levels of MDA, compared to healthy animals. SOD levels of both groups declined significantly ($P<0.05$) whereas, GPx level did not show any significant change among Group I and II compared to healthy animals. Enzymatic antioxidants including SOD and GSH-Px, represents the main form of intracellular antioxidant defence. Al-Qudah and Ismail (2012) recorded significant decline in the activity of SOD along with significant increase in GPx and catalase activity among lame cattle. Zhao *et al.* (2015) also observed significant fall in the level of SOD activity and metallothionein (MT) among lame cows.

Mineral status: Analysis of plasma mineral profile of animals with lameness due to claw disorders revealed significant ($P<0.05$) decline in the average levels of Cu, Ca and Pi compared to healthy animals (Table 1). Non significant ($P>0.05$) changes in average plasma levels of Fe and Zn among lame animals were recorded. Animals with locomotion score 3 showed significant ($P<0.05$) decline in plasma Ca and Pi levels. Cu level of animals with LS 1 to 4 was significantly ($P<0.05$) lower. Animals with various lesions and duration of lameness also showed similar changes. Kiliç *et al.* (2007) reported significant decrease in the levels of serum phosphorous and Zn in lame cows along with non-significant decrease in average value of Ca, Fe and Cu. Significantly decreased levels of Zn, Cu and Mn in the serum and hair along with non-significant difference in the levels of macro elements (Ca, Mg, P) between healthy and lame cows were reported by Zhao *et al.* (2015). Zn has been identified as a key mineral in the processes of horn production (keratinization) (Tomlinson *et al.* 2004, Seyrek *et al.* 2008) and plays a role in formation of structural keratin proteins. However, in present study, non-significant difference was observed which corroborates with the finding of Seyrek *et al.* (2008) and Nasab *et al.* (2013). Mülling (2009) and Buragohain (2012) reported that Ca, P and Mg improve density and hardness of hoof by speeding formation and regeneration. P and Ca are bound by a specific ratio for proper hoof growth and their imbalance leads to brittle hooves.

Transglutaminase (TG) is an enzyme in the epidermal layer which needs activation by the Ca for cross-linking cell envelop keratin fibers. In addition, TG is involved in initiation and regulation of the differentiation of epidermal cells (Mülling 2009). Deficiency of Ca causes lesser availability of mineral to maturing keratocytes thereby leading to formation of dyskeratotic horn manifested by hoof softness, fragmentation and disorganized collagen fibers with decreased collagen content. Copper is also involved in keratinisation of horn cells as it activates thiol oxidase enzyme, which is responsible for the formation of

disulfide bonds between keratin filaments. This process provides structural strength at cellular level, giving rigidity to the keratinized cell matrix.

Cu is required as a cofactor for lysyl oxidase which is essential for cartilage synthesis. Nasab *et al.* (2013) observed that with the increase in degree of lameness among dairy cattle serum and hoof Cu concentration reduces significantly. Cattle suffering from a subclinical Cu deficiency are susceptible to heel cracks, foot rot, and sole abscesses. Linder (1996) observed that Cu deficiency reduces SOD activity which causes heel cracks and abscess in dairy animals. Reduced Cu/Zn SOD activity enhances the cell membrane fragility as unsaturated lipids are vulnerable to oxidative damage. Contrary to present finding, Seyrek *et al.* (2008) observed non-significant variation in serum Cu level of lame cows.

Study concludes that oxidative stress is involved in lameness and the antioxidant defence system is compromised as evidenced by increased activity of MDA, along with significant decline in the levels of antioxidant (SOD). Metabolic activities of Ca, P and Cu promoted lameness in crossbred dairy cows. Declined plasma Cu level might be contributing to oxidative stress in lame animals.

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