Veterinary Research 6 (2): 27-31, 2013
ISSN: 1993-5412
© Medwell Journals, 2013

Detection of Oxidative Stress in Pregnant and Non-Pregnant Murrah Buffaloes During Extreme Ambiences

[1]A. Joshi, [1]N. Kataria, [2]S. Sharma, [4]A. Sharma, [5]S. Arora, [1]Abhimanu,
[1]R. Maan, [5]S. Asopa, [5]N. Arya, [5]S. Khan and [3]A.K. Kataria
[1]Department of Veterinary Physiology, College of Veterinary and Animal Science,
Bikaner, RAJUVAS, 334 001 Bikaner, Rajisthan, India
[2]Department of Veterinary Gynaecology and Obstetrics,
[3]Apex Centre for Animal Disease Investigation, Monitoring and Surveillance,
College of Veterinary and Animal Science,
Rajasthan University of Veterinary and Animal Sciences,
334 001 Bikaner, Rajasthan, India
[4]Department of Animal Husbandry, Haryana, India
[5]Department of Animal Husbandry, Rajasthan, India

Abstract: Present investigation was launched to detect oxidative stress in pregnant and non-pregnant Murrah buffaloes during extreme ambiences. Serum oxidase and peroxidase profiling was used as a tool to detect oxidative stress. Blood samples of apparently healthy adult female Murrah buffaloes were collected to harvest sera during extreme hot cold and moderate ambiences. Moderate mean value of serum oxidase was 50.32±1.21 UL^{-1} and of serum peroxidase was 49.51±1.11 mUL^{-1}. Extreme hot and cold ambiences resulted into a significant ($p \leq 0.05$) rise in the activities of serum oxidase and peroxidase. Animals were classified as non pregnant milch, pregnant milch and pregnant dry and activities of both the enzymes were compared. It was observed that mean values of both the enzymes in non-pregnant milch, pregnant milch and pregnant dry differed significantly ($p \leq 0.05$) from each other, respectively in all the ambiences. Non pregnant milch animals showed lowest activities whereas pregnant dry animals exhibited highest activities of both the enzymes. Multipara animals showed significantly ($p \leq 0.05$) higher activities than primipara. Serum oxidase and peroxidase are considered as important markers to assess oxidative stress. Increased activities of both the enzymes reflected the presence of oxidative stress in the buffaloes. It was concluded that extreme ambiences resulted into oxidative stress affecting the animals of all the physiological states. In each ambience the magnitude of increase was greater in pregnant dry animals. Trend was similar in all the ambiences but impact was maximum during hot ambience. Multipara animals showed greater changes than primipara. It can be stated that multipara and pregnant dry animals require proper care and supplementation of immuno-nutrients in order to protect them from the ensuing risk of oxidative stress. Further serum oxidase and peroxidase activities can be used effectively in detection of oxidative stress.

Key words: Murrah buffalo, oxidase, peroxidase, pregnant, dry

INTRODUCTION

Oxidative stress results from an increased exposure to oxidants. Therefore, requirement to understand the role of oxidants in physiological conditions is gaining significance. Research in the area of oxidative stress in milch aniamls have been patchy and mainly with mastitis, pneumonia and retained placenta. Scientists are focusing on metabolic diseases that affect dairy animals during the peripartum period (Celi, 2011). Determination of oxidative status in buffaloes is carried out by researchers to assess health and recovery status from diseases like clinical endometritis and ovarian activity (Hanafi *et al.*, 2008). Oxidative stress can influence the cell metabolism of the vital organs (Kataria *et al.*, 2010a). Ambience related oxidative stress has received minimum attention. Designing of specific antioxidant therapies needs an understanding of the underlying mechanisms of oxidative stress. Hence, establishment of a reference panel of biomarkers to detect oxidative stress in veterinary

Corresponding Author: Nalini Kataria, Department of Veterinary Physiology, College of Veterinary and Animal Science, Bikaner, RAJUVAS, 334 001 Bikaner, Rajisthan, India

medicine should be the centre point of research. From time to time researchers have proposed the development of an appropriate oxidative stress index as a part of practical application. Investigation of oxidant-antioxidant status will also allow a rapid detection of oxidative stress in animals to formulate in-time strategies for health monitoring. Assessment of the effect of extreme ambience on the physiological processes of the animals requires validation of existing biomarkers of oxidative stress as well as the creation of new indices. Heat stress generally increases the production of free radicals, leading to oxidative stress. In dairy animals, oxidative stress has a negative impact on immune and reproductive functions. Measures of oxidative stress make the assessment of real standing of physiological defenses possible and also help in taking preventing aspects of the correlated diseases (Piccione *et al*., 2007).

When cellular antioxidants are not able to remove free radicals completely, free radicals attack and damage proteins, lipids and nucleic acids. The signs of these damages include loss of energy metabolism, cell signaling, transport and other major functions. Severity may result into cell death. Thus, oxidative stress have a great role in the pathogenesis of a number of diseases. Serum oxidase and peroxidase activities are gaining importance as biomarkers to assess oxidative stress (Kataria *et al*., 2010b). Buffaloes experience a variety of stressors viz. heat, cold, drought, dehydration, infection, trauma, transportation, regrouping, crowding that modify their behaviour, production and performances. To combat the stress, physiological changes occur according to the priorities of the body (Kataria and Kataria, 2005). Oxidative stress may exacerbate physiological demands brought about by stressful conditions by promoting the insurgence of serious pathologies as a result of degenerative damage of tissue structures (McCord, 2000). Murrah is the premier milking buffalo and contributes greatly in enhancing economical status of farmers. The inescapability of these animals from contact to extreme ambient temperatures of arid and semiarid tracts makes the scientists to think regarding suitable field of investigation to find out the mechanism of stress reactions. Therefore, detection of oxidative stress by employing suitable measures becomes an important route to achieve proper diagnosis of stressed animals. Due to scanty research work about normal and clinical variations in the values of serum biomarkers of oxidative stress, the present investigation was planned to carry out serum oxidase and peroxidase profiling during extreme ambiences in Murrah buffaoes of various physiological states to assess oxidative stress.

MATERIALS AND METHODS

Animals: Oxidative stress in pregnant and non-pregnant Murrah buffaloes during extreme ambiences was evaluated. Serum oxidase and peroxidase profiling was used as a tool to detect oxidative stress. Blood samples of four hundred and fifty apparently healthy adult female Murrah buffaloes between 4 and 12 years of age were collected during extreme hot, cold and moderate ambiences. All the animals belonged to private dairy farms of Nagaur district, Rajasthan, India and were managed in similar conditions of feeding and watering.

In each ambience period 150 blood samples were collected. Blood was collected in morning hours directly into the clean, dry test tubes without any anticoagulant in duplicate and test tubes were kept in the slanting position for 30 min and blood was allowed to clot. Then, the clot was separated from the walls of the each test tube with the help of sterilised stainless steel wire and then each test tube was centrifuged at 3000 rpm for 20 min. Supernatant clear serum (non-haemolysed) was pipetted out into sterilised plastic vials. Serum oxidase and peroxidase were analysed in fresh samples.

Buffaloes were broadly divided into group A and B according to physiological states. Animals of group A included non-pregnant milch (30); pregnant milch (30) and pregnant dry (30) animals. Animals of group B were classified according to pariety and included primipara (30) and multipara (30) animals. This was irrespective of states like pregnancy and milch. All primipara were between 4 and 6 years of age whereas all multipara were between 6 and 12 years of age. Maximum ambient temperatures during moderate and hot ambiences were 30.33±0.20 and 45.5±0.08°C, respectively. Minimum ambient temperatures during cold ambience was 4.88±0.20°C.

Analysis: Serum oxidase was determined by the colorimetric method as described by Snell and Snell (1954) with little modification (Kataria *et al*., 2010b). Serum peroxidase was determined by the colorimetric method of Giuseppe and Ronzoni as described by Snell and Snell (1954) and modification as per Kataria *et al*. (2010b).

Statistics: The main parameters of the present investigation were serum oxidase and peroxidase in the buffaloes. The main effects were classified as ambiences. The subsets of ambiences were moderate, hot and cold periods; non-pregnant milch; pregnant milch and pregnant dry animals and primipara and multipara animals. For each subset data were expressed as mean±SE of mean. The changes in the means were measured by using multiple mean comparison procedures. For this Duncan's new multiple range test (Duncan, 1955; Steel and Torrie, 1980) was used.

RESULTS AND DISCUSSION

Serum oxidase: Mean±SEM values of serum oxidase during different ambiences and physiological states are presented in Table 1. The overall mean value of oxidase was 80.17±2.55 UL^{-1} which was obtained from 450 adult buffaloes irrespective of ambiences and physiological states. The range was 31-132 UL^{-1}. Mean values were also determined according to ambiences and physiological states. Moderate mean value was 50.32±1.21 UL^{-1}. The range and mean value of serum oxidase in present study was close to the earlier recordings (Kataria *et al*., 2010b).

The mean value of serum oxidase was significantly ($p \leq 0.05$) higher during hot and cold ambiences as compared to moderate mean value. These findings corroborated the earlier observations (Kataria *et al*., 2010c). Many earlier researchers theorized that rate of oxidative reactions increases during hot and cold ambiences resulting in increased oxidase activity culminating in oxidative stress (Kataria *et al*., 2010b). They specified serum oxidase level as one of the markers of oxidative stress. Pattern of variation of serum oxidase activity during extreme ambiences and in buffaloes of various physiological states explicitly suggested development of oxidative stress. Kataria *et al*. (2010d) also reported higher serum concentration of oxidase in brucella infected cows and related it with oxidative stress. The increase in activity of serum oxidase has been reported in stress (Aprison *et al*., 1959).

Table 1: Mean±SEM values of serum oxidase and peroxidase in Murrah buffalo

	Enzymes ($U L^{-1}$)	
Effects	Oxidase	Peroxidase
Overall (450)	80.17±2.55	63.01±1.60
Ambiences		
Moderate (150)	50.32±1.21[b]	49.51±1.11[b]
Group A		
Non-pregnant milch	33.50±1.22[d]	40.22±1.12[d]
Pregnant milch (30)	50.25±2.11[d]	48.12±1.21[d]
Pregnant dry(30)	67.22±2.30[d]	60.21±1.32[d]
Group B		
Primipara (30)	31.34±1.60[f]	38.50±0.80[f]
Multipara (30)	69.30±2.24[f]	60.52±1.10[f]
Hot (150)	103.54±2.50[b]	77.34±1.50[b]
Group A		
Non-pregnant milch	74.81±2.20[f]	48.48±1.70[d]
Pregnant milch (30)	103.50±2.00[d]	72.10±1.50[d]
Pregnant dry (30)	132.31±3.00[d]	111.45±2.00[d]
Group B		
Primipara (30)	80.66±2.02[f]	45.54±2.50[f]
Multipara (30)	126.42±3.00[f]	109.14±1.10[f]
Cold (150)	86.66±3.20[b]	62.19±2.22[b]
Group A		
Non-pregnant milch	60.00±3.00[d]	45.14±2.55[d]
Pregnant milch (30)	80.00±3.10[d]	62.17±2.32[d]
Pregnant dry (30)	120.00±3.21[d]	79.26±2.20[d]
Group B		
Primipara (30)	74.33±3.11[f]	50.11±2.00[f]
Multipara (30)	98.99±3.31[f]	74.27±2.46[f]

Figures in the parenthesis indicate number of animals; [b]Marks significant ($p \leq 0.05$) differences among ambience overall mean values of a parameter; [d]Marks significant ($p \leq 0.05$) differences among non-pregnant milch, pregnant milch and pregnant dry mean values of a parameter within an ambience; [f]Marks significant ($p \leq 0.05$) differences between mean values of primipara and multipara of a parameter within an ambience

In group A, oxidase mean value of non pregnant milch, pregnant milch and pregnant dry differed significantly ($p \leq 0.05$) from each other in all the ambiences. In each ambience the mean value of oxidase of non pregnant milch animals was lowest whereas it was highest in pregnant dry animals. In group B, the mean value of oxidase was significantly ($p \leq 0.05$) higher in multipara animals than primipara in each ambience. Higher serum oxidase activity in pregnant dry animals and in multipara signaled towards the presence of oxidative stress. Age effect was evident as in each ambience the mean value was significantly ($p \leq 0.05$) higher in multipara. Wynne *et al*. (1988) did not observe age effect in humans on the oxidase activity. There is paucity of literature on the activity of this enzyme.

Variations in the oxidase activity in the buffaloes of various physiological states clearly helped in understanding the coupling of the oxidative stress due to pregnancy and extreme ambience. Animals face physiological challenge during pregnancy period with special emphasis to metabolism. Increasing demand for high energy results into increased oxygen requirement. This prompts aerobic environment which is largely responsible for the increased production of reactive oxygen species resulting in the development of oxidative stress. Higher serum oxidase activity in pregnant dry animals showed the greater oxidative stress to the animals. Earlier research suggest that pregnancy and parturition lead to oxidative stress and subsequently cause DNA damage (Pathan *et al*., 2011). Treatment with antioxidants like vitamin E during dry period can help in reducing oxidative stress (Aggarwal *et al*., 2012). On the basis of results of present study and the available literature, it can be stated that serum oxidase can be used to detect oxidative stress in buffaloes effectively.

Peroxidase: Mean±SEM values of serum peroxidase during different ambiences and physiological states are presented in Table 1. The overall mean value of oxidase was 63.01±1.60 mUL^{-1} which was obtained from 450 adult buffaloes irrespective of ambiences and physiological states. The range was 40-111 mUL^{-1}. Mean values were determined according to ambiences and physiological states. Moderate mean value was 49.51±1.11 mUL^{-1}. The range and mean value of serum peroxidase in present

study was close to the earlier recordings in cattle by Kataria *et al*. (2010d) who used it to detect oxidative stress.

The mean value of serum peroxidase was significantly ($p \leq 0.05$) higher during hot and cold ambiences as compared to moderate mean value. Its increased activity during hot ambience signified oxidative stress (Kataria *et al*., 2010b). Kataria *et al*. (2010d) also attributed higher activity of peroxidase to oxidative stress. Serum peroxidase activity is considered as the main indicator of the antioxidant activity (Podil'chalk *et al*., 1996). Increased nitric oxide activity during heat stress in buffaloes have been observed to cause impairment of cell functions (Abou-Zeina *et al*., 2009). Scientists have observed increased levels of peroxidase in heat stressed buffaloes along with increased levels of serum cortisol and free radicals. They also recommended antioxidant treatment before the beginning of extreme hot period (Megahed *et al*., 2008). In a succinct way the extreme ambiences produced oxidative stress in the buffaloes in the present study.

In group A, peroxidase mean value of non pregnant milch, pregnant milch and pregnant dry differed significantly ($p \leq 0.05$) from each other in all the ambiences. The mean value of peroxidase of non pregnant milch animals was lowest whereas it was highest in pregnant dry animals. In group B, the mean value of peroxidase was significantly ($p \leq 0.05$) higher in multipara animals than primipara.

Higher serum peroxidase activity in pregnant dry animals and in multipara signaled towards the presence of oxidative stress. Age effect was evident as in each ambience the mean value was significantly ($p \leq 0.05$) higher in multipara. Ciesielska and Szerszen (2002) observed higher value of serum peroxidases in females and in adult humans. Stress effect on antioxidant enzyme peroxidase was referred to as a function of time by Das and Banerjee (1993). Researchers have suggested that pregnancy is associated with oxidative stress and supplementation of vitamin E and selenium may be beneficial by alleviating oxidative stress in water buffaloes (Dimri *et al*., 2010). Higher activities of serum peroxidase due to ambience effect and pregnancy stress clearly indicated its importance as an important tool to detect oxidative stress.

CONCLUSION

It was concluded that extreme ambiences resulted into increased activities of the enzymes exhibiting oxidative stress. Extreme ambiences affected the animals of all the physiological states. The magnitude of increase was greater in pregnant dry animals. Trend was similar in all the ambiences but impact was maximum during hot ambience. Multipara animals showed greater changes than primipara. It can be stated that multipara and pregnant dry animals require proper supplementation of immune-nutrients in order to protect them from the ensuing risk of oxidative stress. Further serum oxidase and peroxidase activities can be used effectively in detection of oxidative stress.

REFERENCES

Abou-Zeina H.A.A., S.G. Hassan, H.A. Sabra and A.M. Haman, 2009. Trials for elevating adverse effect of heat stress in buffaloes with emphasis on metabolic status and fertility. Global Vet., 3: 51-62.

Aggarwal, A., Ashutosh, G. Chandra and A.K. Singh, 2012. Heat shock protein 70, oxidative stress and antioxidant status in periparturient crossbred cows supplemented with α-tocopherol acetate. Trop. Anim. Hlth. Prod., 45: 239-245.

Aprison, M.H., K. Hanson and D.C. Austin, 1959. Studies on serum oxidase (ceruloplasmin) inhibition by tryptophan metabolites. J. Nerv. Ment. Dis., 128: 249-255.

Celi, P., 2011. Biomarkers of oxidative stress in ruminant medicine. Immunopharmacol. Immunotoxicol., 33: 233-240.

Ciesielska, A.S. and M.K. Szerszen, 2002. The influence of sex and age on serum catalase, peroxidase,superoxide dismutase activity and production of O2- and H2O2 by human blood neutrophils. Annals Universitatis Mariae Curie-Sklodowska Lublin Polonia, Vol. LVII.

Das, D. and R.K. Banerjee, 1993. Effect of stress on the antioxidant enzymes and gastric ulceration. Mol. Cell Biochem., 1125: 115-125.

Dimri, U., R. Ranjan, M.C. Sharma and V.P. Varshney, 2010. Effect of vitamin E and selenium supplementation on oxidative stress indices and cortisol level in blood in water buffaloes during pregnancy and early postpartum period. Trop. Anim. Health. Prod., 42: 405-410.

Duncan, D.B., 1955. Multiple range and multiple F test. Biometrics, 11: 1-42.

Hanafi, E.M., W.M. Ahmed, S.I.A. El-Moez, H.H. El-Khadrawy and A.R.A. El-Hameed, 2008. Effect of clinical endometritis on ovarian activity and oxidative stress status in Egyptian buffalo-cows. American-Eurasian J. Agric. Environ. Sci., 4: 530-536.

Kataria, A.K. and N. Kataria, 2005. Stress induced changes in serum vitamin A and enzyme levels in Rathi calves. Indian Cow. Scien. Econ. J., 2: 16-19.

Kataria, N., A.K. Kataria and R. Maan, 2010a. Correlation of serum IgE with stress in Indian dromedaries affected with skin wounds. J. Stress Physiol. Biochem., 6: 17-24.

Kataria, N., A.K. Kataria, R. Maan and A.K. Gahlot, 2010b. Evaluation of oxidative stress in brucella infected cows. J. Stress Physiol. Biochem., 6: 19-25.

Kataria, N., A.K. Kataria, N. Pandey and P. Gupta, 2010c. Serum biomarkers of physiological defense against reactive oxygen species during environmental stress in Indian dromedaries. HVM Bioflux., 2: 55-60.

Kataria, N., A.K. Kataria and R. Maan, 2010d. Evaluation of oxidative stress due to hot environmental condition in healthy Marwari goats from arid tract in India. Philipp. J. Vet. Anim. Sci., 36: 175-184.

McCord, J.M., 2000. The evolution of free radicals and oxidative stress. Am. J. Med., 108: 652-659.

Megahed, G.A., M.M. Anwar, S.I. Wasfy and M.E. Hammadeh, 2008. Influence of heat stress on the cortisol and oxidant-antioxidants balance during oestrous phase in buffalo-cows (Bubalus bubalis): Thermo-protective role of antioxidant treatment. Reprod. Domest. Anim., 43: 672-677.

Pathan M.M., A. Latif, H. Das, G.M. Siddiquee, M.M. Vaidya and A.K. Singh, 2011. Effect of pregnancy induced oxidative stress on cellular DNA in Mehsana buffaloes. Indian J. Anim. Res., 45: 211-214.

Piccione, G., M. Borruso, C. Giannetto, M. Morgante and E. Giudice, 2007. Assessment of oxidative stress in dry and lactating cows. Acta Agric. Scand. Sec. A Anim. Sci., 57: 101-104.

Podil'chalk, M.D., V.I. Vdovychenko and L.M. Terlets'ka, 1996. Lipid peroxidation and blood serum peroxidase activity in diseases of the hepatobiliary system. Lik Sprava., 1-2: 110-112.

Snell, F.D. and C.T. Snell, 1954. Colorimetric methods of analysis. 3rd Edn., D.Van Nostrand Company, New York, pp: 512-518.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometerical Approach. 2nd Edn., McGraw Hill Co., New York, pp: 180-190.

Wynne, H.A., E. Mutch, O.F. James, P. Wright, M.D. Rawlins and K.W. Woodhouse, 1988. The effect of age upon the affinity of microsomal mono-oxygenase enzymes for substrate in human liver. Age Ageing., 17: 401-405.