

Full Length Research Paper

## Xanthine oxidase activity during transition period and its association with occurrence of postpartum infections in Murrah buffalo (*Bubalus bubalis*)

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Accepted 3 May, 2013

The aim of this study was to quantify xanthine oxidase (XO) levels during the transition period in Murrah buffalo (*Bubalus bubalis*) and determine its association with certain postpartum infections. For this, six healthy buffaloes were selected from the National Dairy Research Institute (NDRI) herd and managed under standard managerial practices as followed at the institute. Blood samples were drawn weekly from each buffalo from day -21 to +21 relative to parturition by jugular vein puncture. Additional blood samples were collected from buffaloes suffering from metritis (n=5), endometritis (n=6) and mastitis (n=8) on alternate days. XO activity followed a defined pattern with values gradually declining from day -21 up to the day of calving followed by an increase to day +21, but the difference was statistically not significant between pre- and post-partum stages. The activity significantly declined on the day of calving when compared to the pre-partum mean value ( $p < 0.05$ ). The activity was significantly enhanced in buffaloes with bacterial infections, endometritis, and mastitis compared to healthy controls ( $p < 0.05$ ). However, the levels were not significantly altered among buffalo with metritis. The results indicate that increased XO activity during the postpartum period was associated with some bacterial infections in buffaloes, which could be due to increased phagocytic activity as a part of the innate defense system.

**Key words:** Xanthine oxidase, transition period.

### INTRODUCTION

The transition period is the most interesting stage of postpartum health. During this period, host defense mechanisms can be compromised directly because of numerous genetic, physiological and environmental factors that can affect the cow's immunological defenses (Sordillo, 2005). The physiological stress associated with rapid differentiation of secretory parenchyma, intense mammary gland growth and the onset of copious milk synthesis and secretion during this period are accom-

panied by a high energy demand and an increased oxygen requirement (Gitto et al., 2002). Increased oxygen demand augments the production of oxygen-derived reactive oxygen species (ROS) and result into oxidative stress to the animal. A relationship between the physiological changes associated with parturition and a loss in overall antioxidant potential was established in both humans and dairy cows (Bernabucci et al., 2005; Sordillo et al., 2007). The oxidative stress therefore plays

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a pivotal role in the pathophysiology of inflammation (Kabe et al., 2005). It is well known that inflammatory events involve the generation of free radicals through NADPH oxidase and myeloperoxidase in immunoparticipating cells for the purpose of bacterial defense and phagocytosis (Hellsten et al., 1997). Superoxide radicals ( $O_2^-$ ) are also of importance in neutrophil attraction and adherence to endothelium. The enzyme xanthine oxidase is one of the proposed sources of superoxide radicals during inflammatory, an event which in most of the tissues, is localized in the vascular walls. In diabetic rats, xanthine oxidase was reported to play an important role in the development of oxidative stress (Desco et al., 2002). Xanthine oxidase also converts nitrate to nitrite under anoxic conditions and therefore, acts as an alternate source of nitric oxide (NO) generation which are comparable to those produced from nitric oxide synthase (Li et al., 2003). In this process, nitrite and reducing substrate concentrations constitute important regulators of XO catalyzed NO generation.

There is no information available on XO activity in buffaloes and its quantitative importance in biological system or as a marker for detection of bacterial infections. Thus, the present investigation was aimed to quantify XO activity during transition period in Murrah buffaloes and its association with certain postpartum infections in buffaloes.

## MATERIALS AND METHODS

### Selection and feeding management of buffaloes

Six apparently healthy pregnant Murrah buffaloes were selected during the dry period from NDRI herd. All these buffaloes were maintained under general managemental practices as followed for the herd. The feed and water was available *ad libitum* to these buffaloes throughout the day. 19 symptomatic buffaloes were also selected from same herd, with symptoms indicative of metritis ( $n=5$ ), endometritis ( $n=6$ ) and mastitis ( $n=8$ ). The diagnosis of metritis, endometritis, and mastitis was confirmed by the institute's herd veterinary officer based on symptoms of metritis and endometritis as described by Sheldon et al. (2006). Metritis was diagnosed by the presence of systemic signs of sickness, including fever, red-brown watery foul-smelling uterine discharge, dullness, elevated heart rate, and low production, whereas clinical endometritis was diagnosed by the presence of purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) uterine exudates in the vagina, 21 days or more post partum. Clinical mastitis was diagnosed by an elevated somatic cell count in milk and visual signs of inflammation such as clumpy, watery, bloody or yellowish milk.

### Blood sampling and processing

Blood samples (15 ml) were drawn in sterile heparinized vacutainer tubes from each buffalo at 6.00 A.M. in the morning by jugular venipuncture on days -21, -14, -7, 0, +7, +14, +21 relative to parturition. Immediately after collection, the tubes were transported to the laboratory in an ice box for further processing. Blood samples

from infected buffaloes postpartum were collected on the day of confirmation of metritis, endometritis and mastitis respectively followed by one more sample on an alternate day. The heparinized tubes were centrifuged at 3000 rpm for 15 min, plasma aliquoted and stored at  $-20^\circ\text{C}$  till analysis of Xanthine oxidase (XO) activity. XO was determined using the xanthine oxidase colorimetric assay kit from BioVision. It involves oxidation of xanthine to hydrogen peroxide ( $H_2O_2$ ) by XO, which reacts with OxiRed Probe to generate 570 nm light. The detection range of the test was 1-100 mU/ml. Kit reagents were prepared and stored in accordance with the manufacturer's instructions.

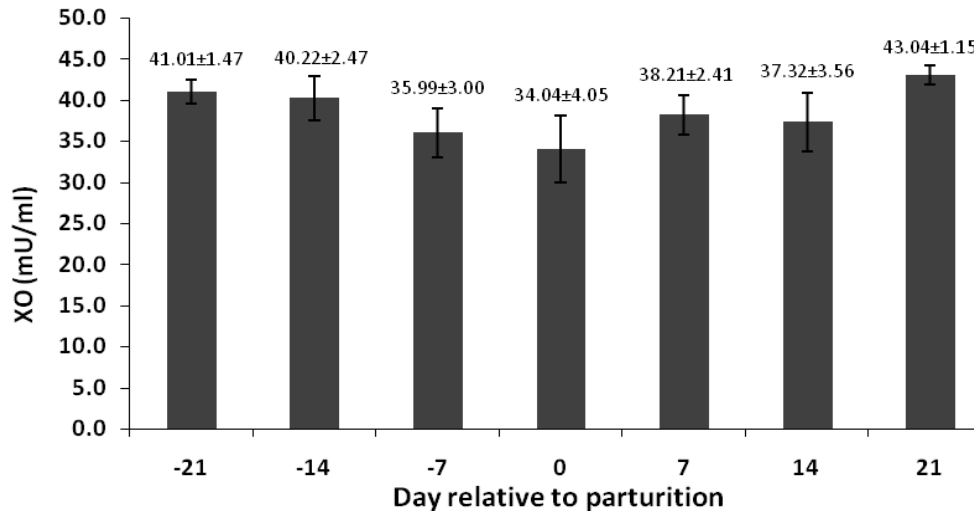
### Statistical analysis

The data was presented as mean  $\pm$  standard error (SE) in mU/ml units. The unpaired and paired student "t" test was performed in graph prism version 5. The student "t" test was applied to determine the significant changes in XO activity during transition period. The unpaired "t" test was used to compare the normal post partum XO activity with the activity during different bacterial infections. The normal XO activity was computed by grouping the data from day 7 to 21 postpartum.

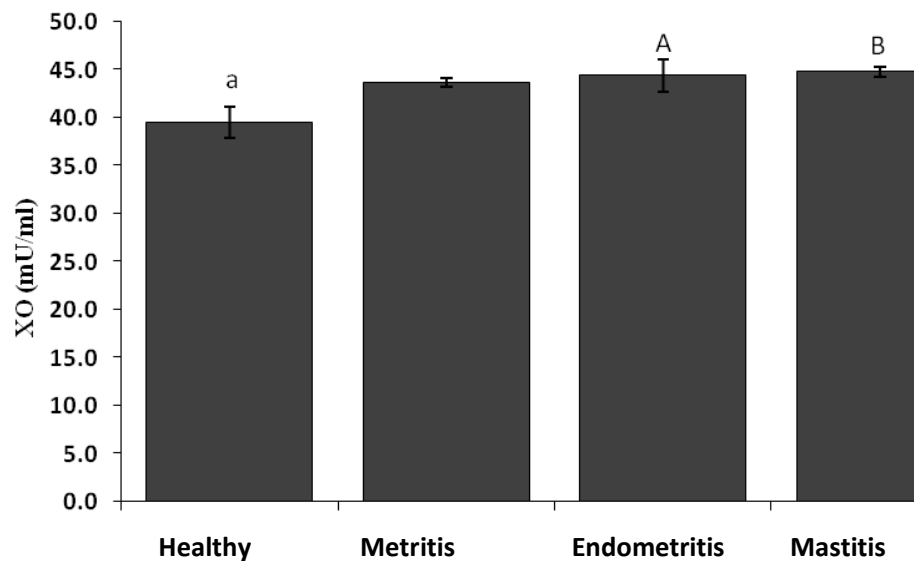
## RESULTS AND DISCUSSION

Changes in plasma XO activity during transition period in healthy buffaloes and in those with postpartum bacterial infections are shown graphically in Figures 1 and 2 respectively. Although plasma xanthine oxidase activity constituted a definite pattern with values gradually declining from  $41.01 \pm 1.47$  mU/ml on day -21 relative to parturition to  $34.04 \pm 4.05$  mU/ml on the day of calving followed by a rise to 43.04 mU/ml on day +21 postpartum, this difference did not achieve statistical significance during transition period in these buffaloes. The decline did not reach statistical significance on the day of calving in relation to the pre-partum day 21 value ( $p < 0.05$ ). The xanthine oxidase activity was significantly elevated in buffaloes suffering from endometritis ( $44.31 \pm 0.48$  mM/ml) and mastitis ( $44.74 \pm 0.51$  mM/ml) compared against  $39.52 \pm 1.59$  mM/ml in healthy ones. However, there was no significant difference in mean xanthine oxidase activity between metritis and healthy buffaloes.

The activation of local and systemic host defense mechanisms requires cross-talk between numerous types of immune cells. One component of this response is inflammation. The host of signaling molecules released by activated immune cells includes inflammatory mediators such as nitric oxide, xanthine oxidase, prostaglandins and cytokines. While many of these molecules promote local inflammation and increased blood flow, xanthine oxidase play a key role in stimulating oxidative reactions. It is a highly versatile enzyme that is widely distributed among species (from bacteria to man) and within the various tissues of mammals. In healthy tissue, xanthine oxidase mainly exists in a dehydrogenase form not capable of producing superoxide radicals, but the enzyme may be modulated to its superoxide-generating



**Figure 1.** XO activity (mean ± SE) in healthy buffaloes during transition period.



**Figure 2.** Plasma XO activity (mean ± SE) in buffaloes exhibiting postpartum infections (Bar with superscript aA and aB differ significantly from each other).

oxidase form via oxidation of critical sulfhydryl groups (Della and Stirpe, 1972) or through limited proteolysis (Della and Stirpe, 1968).

Xanthine oxidase has been implicated in several physiological and pathological cases. It is a form of xanthine oxidoreductase that generates reactive oxygen species (Ardan et al., 2004). To the best of our knowledge this is the first report that has quantified the levels of XO during transition period. We did not find significant difference in xanthine oxidase activity during the pre- or post-partum period. However, XO activity demonstrated a defined trend with lowest value on the day of parturition. After calving, the level remained

low for first 14 days followed by a rise on day 21. Earlier reports have indicated a relationship between the physiological changes associated with parturition and a loss in overall antioxidant potential in both humans and dairy cows (Bernabucci et al., 2005; Sordillo et al., 2007). The possibility that oxidative stress during the transition period might be a major underlying cause of inflammatory and immune dysfunction in dairy cattle has been supported by various studies conducted either *in vivo* or *in vitro* (Sordillo and Aitken, 2009). Since Xanthine oxidase is involved in the generation of reactive oxygen species (Ardan et al., 2004) through NADPH oxidase and myeloperoxidase in immunoparticipating cells for the

purpose of bacterial defense and phagocytosis during inflammation (Hellsten et al., 1997), therefore it could be used as a marker of oxidative stress.

We recorded comparatively higher activity during transition period as compared to earlier study in buffaloes (24.5 and 1.4 U/L) and cattle (Chen et al., 1996). Due to versatile nature of this enzyme and its wide distribution among various species, the enhanced levels could have other benefits as well. XO being a primary enzyme in purine metabolism, higher levels might have a special role to play in nitrogen metabolism also which in ruminants has exceptional importance. Alternatively, it could also enhance substantially the substrate source (nitrite) for NO generation (Li et al., 2003; Haitao et al., 2004) under normoxic or hypoxic conditions. In an earlier study in our laboratory, the nitrite level remained elevated throughout the pregnancy in buffaloes, reaching maximum during the last trimester (Huozha et al., 2010) which further confirmed its association with NO generation.

The present study shows for the first time that buffaloes suffering from bacterial infections express an increased xanthine oxidase activity. The activity response in this investigation was significantly higher ( $p < 0.02$ ) in buffaloes suffering from endometritis and mastitis but not in metritic case. These results are in agreement with the findings of Kataria et al. (2010) who reported low activity in healthy cows ( $51 \pm 2.0$  mU/L) as compared to high ( $79 \pm 6.0$  mU/L) levels in brucellosis infected cows. Elevated activity levels of xanthine oxidase following various disease conditions were attributed to a protease-induced conversion of xanthine dehydrogenase to xanthine oxidase (Lindsay et al., 1990; Smith, et al., 1991) as demonstrated in whole-tissue homogenates and could occur during increased leukocyte activity as a part of body innate defense mechanism or through enhanced phagocytic activity during chronic bacterial infections. The higher activity was also attributed to higher rate of oxidative reactions (Kataria et al., 2010) which might contribute to the generation of reactive oxygen species thus leading to oxidative stress. In these studies, the actual sites for the increase in xanthine oxidase were not assessed. However, in muscle injury, the elevated expression occurred mainly in the endothelial cells of microvessels and also in leucocytes present in the muscle (Hellsten et al., 1997). Thus, the elevated levels could be used as a marker for detection of some bacterial infections in buffaloes during post partum period.

## Conclusion

In conclusion, the changes in XO could be used as a marker for oxidative stress during transition period and was related to total leukocytic activity. It could also be used as an inflammatory marker for postpartum bacterial

infections in buffaloes. Further studies are required to confirm it as a risk factor for uterine infections.

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