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# Studies on biological stress markers in a threatened spotted deer and barking deer species with myopathic changes

**Amol Rokde, Madhu Swamy, Somesh Singh, Kajal Jadav and Kshmankar Sharman**

**Abstract**
Exertional myopathy is a noninfectious, metabolic disease of wild and domestic animals that can lead to significant morbidity and mortality. The ecological studies now shown that oxidative status could be use for impact on fitness components in wild animals. The oxidative status can also predict their chances of reproduction and survival in the future in their natural habitat. In both the studied ruminants, spotted deer and barking deer, the oxidative markers in muscle tissues showed an insignificant raise in the affected animals. The condition of capture myopathy due to exertion or stress-induced muscle degeneration in affected captured wild animals is often fatal. There are very few systematic studies on the capture myopathy in wild animals. This makes early detection of this condition difficult. Therefore present study was carried out for comparative oxidative studies on wild spotted deer (*Axis axis*) and barking deer (*Muntiacus muntjak*) affected with myopathic conditions.

**Keywords:** Spotted deer, barking deer, exertional myopathy stress markers

## Introduction
Exertional or Capture myopathy is a noninfectious disease characterized by massive muscle degeneration, metabolic acidosis and myoglobinuria. This disorder has been reported in variety of species, including ruminants and wild mammals. Once the clinical sign developed the condition is difficult to treat and carries a grave prognosis. Prey species are considered the most susceptible to CM in the mammalian taxa, particularly ungulates. Literatures on capture myopathy in carnivores are rare but the disease can occur under certain conditions (Cattet *et al*., 2008) [6]. The ecological studies now shown that oxidative status could be use for impact on fitness components in wild animals. The oxidative status can also predict their chances of reproduction and survival in the future in their natural habitat. The knowledge of oxidative markers may help conservation practitioners to identify conservation threats to populations and also helpful for maximize the success of wildlife management (Beaulieu and Costantini 2014) [3]. In the last 50 years, environmental conditions have changed at an unprecedented rate resulting impacting on ecological processes (Diffenbaugh and Field, 2013) [11]. There significant effects on fitness components lead to increase the health related problems in animal populations (Beaulieu *et al*., 2013) [4]. For better management of wild species in protected areas it is essential to adopt the successful conservation strategies. Furthermore, stressful events are significantly associated with the pathological onset and different forms of myopathy, recognized as capture shock, ataxic myoglobinuric, ruptured muscles and delayed peracute, have been reported. All the stages of myopathy conditions are fatal due to cardiogenic shock, renal failure, metabolic disorders or chronic cardiac damage (Spraker 1993) [26].

In individuals variation in marker values can arise from differences genetic quality, circumstances experienced during development, past or current health status and stressful experiences that may have lead to cellular damage, increased secretion of stress hormones, and activation or suppression of the immune system (e.g. Hau *et al*. 2015; Marasco *et al*. 2017) [16, 18]. It is increasingly advocated that physiological markers of allostatic load ('stress') may provide a valuable tool for long-term environmental monitoring of animal populations to assess the effects of environmental changes on individual health and to predict how individuals will cope with these ongoing changes (Romero, 2004; Busch and Hayward, 2009; Cooke *et al*., 2013; Wingfield, 2013) [22, 5, 8, 28].

Oxidative stress constitutes crucial mechanism that leads to biological damage. This has even been implicated as the cause of several degenerative health problems, which affect the overall performance and productivity in animals (Fellenberg and Speisky, 2006) [15]. Free-radicals accumulated in response to oxidative stress impair cell membrane and mitochondrial integrity through lipid peroxidation, which considerably increase the risk of oxidative reactions (Estevez, 2015) [14].

In the present study, biological samples of spotted deer (*Axis axis*) and barking deer (*Muntiacus muntjak*) were subjected to the assessment of oxidative stress during the capture myopthy. However, there is infancy of literature regarding the oxidative stress markers in wild ruminants.

## Material and Methods

The present study was conducted on wild animals of different tiger reserves, wildlife sanctuaries and Jabalpur Territorial Forest Division of Madhya Pradesh. The study was conducted for the period of 12 months (January 2021 to December 2021). To know the incidence of capture mayopathy in wild animals a brief detail regarding the history, clinical sign, predation, type of injuries, date of mortality, species, sex, age, approximate body weight, mode of capture, type of terrain, session/month were recorded. Selection of the animals was done on the basis of history of stress like chasing, translocation, fighting, predation, chronic injuries or with muscular dystrophy like reluctance to move, hyperthermia, swellings, lameness etc. The skeletal muscle tissues were collected from the affected as well unaffected animals.

Specimen from each organ was separated into two parts. Each part was weighed and homogenized separately. One part was homogenized in phosphate buffer saline (PBS) 50 mM pH (7.4) for GSH level, the second was homogenized in potassium phosphate buffer 10 mM pH (7.4) for estimation of MDA levels. The crude tissue homogenate was centrifuged at 10,000 rpm for 15 minutes in cold centrifuge and the resultant supernatant was used for different estimations (Noeman *et al*., 2011) [20].

Membrane peroxidative changes in tissue homogenate were determined in terms of malondialdehyde (MDA) production by the method of Shafiq-U-Rehman (1984) [23]. GSH was estimated by the 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) method as suggested by Prins and Loos (1969) [21]. The GSH concentration was calculated from the standard curve. GSH standard solution (5 mM) by dissolving GSH (15.37 mg) in phosphate buffer as method described by Anderson (1985) [1].

### Oxidative stress in spotted deer muscle tissue

Skeletal muscle tissues from spotted deer were evaluated for assessment of oxidative stress during myopathy or contributing to myopathy. Reduced glutathione (GSH) and malondialdehyde (MDA) were measured in the affected as well as in the healthy muscle tissues of spotted deer (Table 1).

**Table 1:** Indices of oxidative stress in spotted deer muscle tissues

| S. No. | Spotted deer | | MDA (nmol/g) | GSH (µM) |
|---|---|---|---|---|
| 1. | Mean | | 59.34 | 12.43 |
| 2. | Standard Error | | 7.03 | 1.18 |
| 3. | Standard Deviation | | 38.50 | 6.48 |
| 4. | Range | | 117.82 | 21.71 |
| 5. | Minimum | | 14.26 | 2.45 |
| 6. | Maximum | | 132.08 | 24.16 |
| 7. | Sum | | 1780.41 | 373.06 |
| 8. | Mean ± SE | | 59.34±7.03 | 12.43±1.18 |
| 9. | Significance variation ± SE | Affected | 69.56[a]± 9.43 | 11.86[a]± 1.58 |
| | | Unaffected | 38.90[a]± 7.66 | 14.79[a]± 2.81 |

Table 2: Oxidative stress in barking deer muscle tissue

Reduced glutathione (GSH) and Malondialdehyde (MDA) were measured in the muscle tissues of affected and unaffected barking deer (Table 2).

**Table 2:** Indices of oxidative stress in barking deer muscle tissue

| S. No. | Barking deer | | MDA (nmol/g) | GSH (µM) |
|---|---|---|---|---|
| 1. | Mean | | 61.67 | 8.83 |
| 2. | Standard Error | | 10.10 | 1.86 |
| 3. | Standard Deviation | | 24.74051 | 4.56 |
| 4. | Range | | 59.96 | 11.08 |
| 5. | Minimum | | 37.36 | 4.34 |
| 6. | Maximum | | 97.32 | 15.42 |
| 7. | Sum | | 370.06 | 52.99 |
| 8. | Mean± SE | | 61.67±10.10 | 8.83±1.86 |
| 9. | Significance variation ± SE | Affected | 69.00[a] ± 15.67 | 8.11[a]± 3.65 |
| | | Unaffected | 54.35[a]± 14.51 | 9.55[a]± 1.87 |

In the present study the concentration of GSH in the muscle tissues of spotted deer with myopathy ranged from 2.45 to 24.16 (µM) whereas, the concentration of MDA ranged from 14.26 to 132.08 (nmol/g). The concentration of GSH in the muscles of barking deer ranged from 4.34 to 15.42 (µM) and MDA ranged from 37.36 to 97.32 (nmol/g). Earlier Sharma (2019) [24] observed the concentration of GSH in the muscles of spotted deer ranged from 4.82±0.04 to 28.13±0.03 (µM) and MDA concentration ranged from 18.34±0.05 to 51.30±0.06 (nmol/g).

Oxidative stress results when reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms. Free-radicals are generated in the skeletal muscles both in rest as well as contractile activities. Many muscle disorders involve breakdown or necrosis of muscles and/or nerve tissues, a process that is likely to be associated with production of free-radicals. However, it is difficult to determine whether these free-radicals are the cause rather than the consequence of the disease (Duthie *et al*., 1993) [12]. In the present study also, the oxidative stress in muscle tissues with lesions was observed in all the spotted deer with different proportions of severity. It may be possible that the free-radicals generated due to some systemic infection and pathological condition might have led to the myopathy. Although, reports on the oxidative stress in relation to myopathy in wild mammals are lacking, however, many

workers have described different biomarkers which can be used to quantify oxidative damage. Measuring levels of oxidative stress is highly fitness relevant, because high oxidative stress levels may compromise survival and reproduction (Costantini, 2008; Monaghan *et al*., 2009) [10, 19].

Environmental stresses that favour *in vivo* free-radical formation often exacerbate nutritional myopathy in animals and thus support a role for free-radicals in the underlying disease process (Arthur, 1988) [2]. Such factors may increase endogenous production of free-radicals and thus strengthen a role for these molecules in the pathogenesis of the disorder. Whatever the aetiology, excessive free-radical production leads to the lipid oxidation and damage to the cell membranes. Malondialdehyde (MDA) is a well known secondary product of lipid peroxidation and is used as indicator of cell membrane damage.

Cellular glutathione and related enzymes such as glutathione peroxidase, glutathione S-transferase and glutathione reductase are among the principle protective mechanisms against endogenous and exogenous toxic substances and free-radicals-mediated damage in tissues (Ellah *et al*., 2008) [13]. Several research groups have measured glutathione peroxidase and reductase activity in tissues of experimental animals and calves (Hill *et al.*, 1987) [17]. However such studies are lacking in wild ruminants.

The workers have opined that oxidative stress may occur due to excessive production of reactive oxygen species such as hydroxyl radicals by activated neutrophils from the inflammed tissue causing peroxidative damage to the membranes (Sharma *et al*., 2016) [25]. In the present study also, the same pathogenesis for oxidative stress might have occurred in barking deer with myopathy.

The present results showed that in muscle damage the lipid peroxidation was raised in barking deer. Similarly, there was a decrease in the amount of reduced glutathione which showed increase in the activity of antioxidant system in response to compete reactive oxygen species (ROS). In the present study, oxidative stress was observed, as evidenced by decreased GSH and increased MDA in the muscles of barking deer after dog chasing, traumatic injuries, physical handling and transportation. Transport stress increases serum MDA concentrations and mortality has been observed in livestock (Chirase *et al*., 2004) [7]. Muscle immobilization can induce directly the release of catecholamines in CNS and also can initiate a local inflammatory response which leads to cytokine production. Catecholamines and cytokines (IL1, IL6 and TNF) contribute to the activation of hypothalamic - pituitary axis and to the glucocorticoids synthesis (Steinhardt and Thielscher, 2000) [27].

The oxidative status can also predict their chances of reproduction and survival in the future in their natural habitat. In both the studied ruminants, spotted deer and barking deer, the oxidative markers in muscle tissues showed an insignificant raise in the affected animals. The low level of GSH and increased lipid peroxidation in the muscles of spotted deer and barking deer clearly indicated direct correlation between oxidative stress and muscle damage. In the present study, oxidative stress was evidenced by decreased GSH and increased MDA in the muscles of spotted deer and barking deer after dog chasing, trauma, physical capture. Similarly, reduced level of GSH and raised level of MDA. To sum up, oxidative stress observed in the damaged muscle tissue of wild ruminants.

## Conclusion

For better understanding the wildlife conservation challenges stress is an essential component. The present study in free ranging spotted deer and barking deer the oxidative markers in muscle tissues showed an insignificant raise in the affected animals. Nevertheless the acutely low levels of GSH and raised level of MDA in the deer's muscle tissue in the study indicates that muscle tissue injury is definitely associated with lipid peroxidation and oxidative stress. Oxidative stress is observed in muscle damage caused by variable etiology can be used as indicators of the myopathy in wild ruminants.

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