

Original Research Article

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Evaluation of Serum Creatine Kinase (CK-MB) Activity in Murrah Buffaloes and Nellore Sheep Exposed to Slaughter Stress

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ABSTRACT

The objective of the current study was to determine and compare the effects of pre slaughter stress on serum creatine kinase activity in Murrah buffaloes and Nellore sheep. Whole blood samples collected from a total of 170 buffaloes and sheep were used in this study. Samples were collected from two groups of animals which include: 1) one day before slaughter and 2) few seconds prior to exsanguination to measure the concentration of creatine kinase (CK-MB) in serum. From this study we observed that CK-MB activity was significantly higher in the samples collected just before exsanguination in both species than in samples collected one day before slaughter. Although the magnitude of increase was slightly higher in buffaloes than in sheep, however indicates that preslaughter stress response affects serum creatine kinase activity in both the species.

Keywords

Serum Creatine kinase activity, Slaughter stress, Murrah Buffalo, Nellore sheep

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Introduction

Livestock is one of the fastest growing agricultural subsectors in India as it plays a key role in mitigating the risks and uncertainties involved in income generation through crop production. India accounts for 58% of world buffalo population according to

19th livestock census. whereas sheep population is 65.07 millions and are preferred over other species in especially in developing countries like India because of its multifaceted utility for wool, meat, milk, skin and manure apart from its vast genetic resource i.e with 42 breeds of sheep in India. Due to the increased consumption and

improved marketing trends, buffaloes and sheep became the prominent species in the meat production sector. But the stress experienced due to pre slaughter handling and related operations is gaining importance because of their negative effects on the final quality of the meat (Lowe *et al.*, 2001; Miranda-de la Lama *et al.*, 2010; Vimiso and Muchenje, 2013).

A slaughter house presents a multi factorial stressor for the meat producing animals. The animals are exposed to a variety of stressors in and around slaughter i.e. climate, temperature, nutrition, during transport, in lairage and even up to exsanguination that triggers many physiological reactions which might result in variations of haematological (Kour *et al.*, 2015), biochemical (Mccorry, 2007; Werner and Gallo, 2008; Micera *et al.*, 2010) and oxidative stress parameters (Mader, 2003; Schaefer *et al.*, 2001; O'Neill *et al.*, 2006; Fazio *et al.*, 2008; Werner and Gallo, 2008; Micera *et al.*, 2010; Anil, 2012; Svete *et al.*, 2012; Ekiz *et al.*, 2013; Loudon *et al.*, 2019).

Among such factors creatine kinase (CK) is a vital and sensible indicator in the determination of muscular activity, muscle damage and fatigue caused by physical exertion in animals as it is expressed cytoplasm and mitochondria of different tissues mostly in skeletal muscle, brain and heart (Stull and Rodiek, 2000; Tadich *et al.*, 2005; Hambrecht *et al.*, 2005; Werner and Gallo, 2008; Miranda-de la Lama *et al.*, 2010; Ekiz *et al.*, 2012) and muscle damage subsequently affects meat quality (Brancaccia *et al.*, 2010; Baird *et al.*, 2012; Gebresenbet *et al.*, 2012; Koch *et al.*, 2014; Nakyinsige *et al.*, 2014). Other than CK, blood activities of other enzymes such as lactate dehydrogenase (LDH), superoxide dismutase (SOD), glutathione peroxidase (GPX), alanine aminotransferase (ALT) and aspartate

aminotransferase (AST) also are elevated after muscle damage (Valentine and McGavin, 2013). The extent of tissue damage during handling is also indicated by the increased levels of CK in the blood (European Food Safety Authority (EFSA), 2004; Tackett *et al.*, 2008; Grandin, 2010; Minka and Ayo, 2010; Nakyinsige *et al.*, 2013). Accordingly the present work was undertaken to measure the creatine kinase activity with respect to pre slaughter stress in Murrah buffaloes and Nellore sheep.

Materials and Methods

Animal sampling

In this study the blood samples were collected from the buffaloes and sheep to compare the creatine kinase activity with respect to stressful events in and around slaughter. The blood samples were collected from Murrah buffaloes brought to the slaughter house in kabela area of Vijayawada and from Nellore sheep in local slaughter house of Tirupati.

Blood samples were collected from jugular vein of each animal one day before slaughter and the other is just few seconds before exsanguination from the same group of animals. A total of 170 animals (buffaloes: n=65 and sheep: n=105) were included in this study.

Collection and preparation of blood samples for serum creatine kinase (CK-MB) activity estimation

The whole blood samples were collected from jugular vein of both the species in the 4 ml clot activator (A-Vac tubes; Lot No: 2019/05/CA) vacutainer tubes to extract serum. A centrifuge machine (Model no: 5403; Remi Instruments) was used to centrifuge blood samples at 3000rpm for 15 minutes.

Serum was then separated carefully and inserted in to 2.0mL eppendorf tubes using pipettes and stored at -20°C until used. Serum Creatine kinase (CK-MB) activity was measured by Immuno inhibition method in A15 random access analyzer (Biosystems, Spain) using Creatine kinase detection kit (CK-MB kit; catalogue no: 12566, Biosystems, Spain) according to the manufacturer instructions.

Statistical analysis

Means and standard deviation between the samples collected one day before slaughter and just prior to exsanguination was analysed through paired t-test by using SPSS 20 software. The significant differences of creatine kinase concentrations were considered significant if value of P is less than 0.05.

Results and Discussion

The creatine kinase concentrations were evaluated in two groups of serum samples in each species. From the results it is observed that the creatine kinase concentration was significantly higher in the samples collected just before exsanguination in both species (Fig. 3.1 and 3.2). Among the two species studied the creatine kinase level was significantly higher in buffaloes than in sheep in both the groups (266.34U/L Vs 221.86U/L a day before slaughter and 530.38U/L Vs 483.50U/L at exsanguination). From this study we conclude that the creatine kinase levels increase in response to preslaughter stress in both buffaloes and sheep although the magnitude of increase was significantly higher in buffaloes among the two species studied.

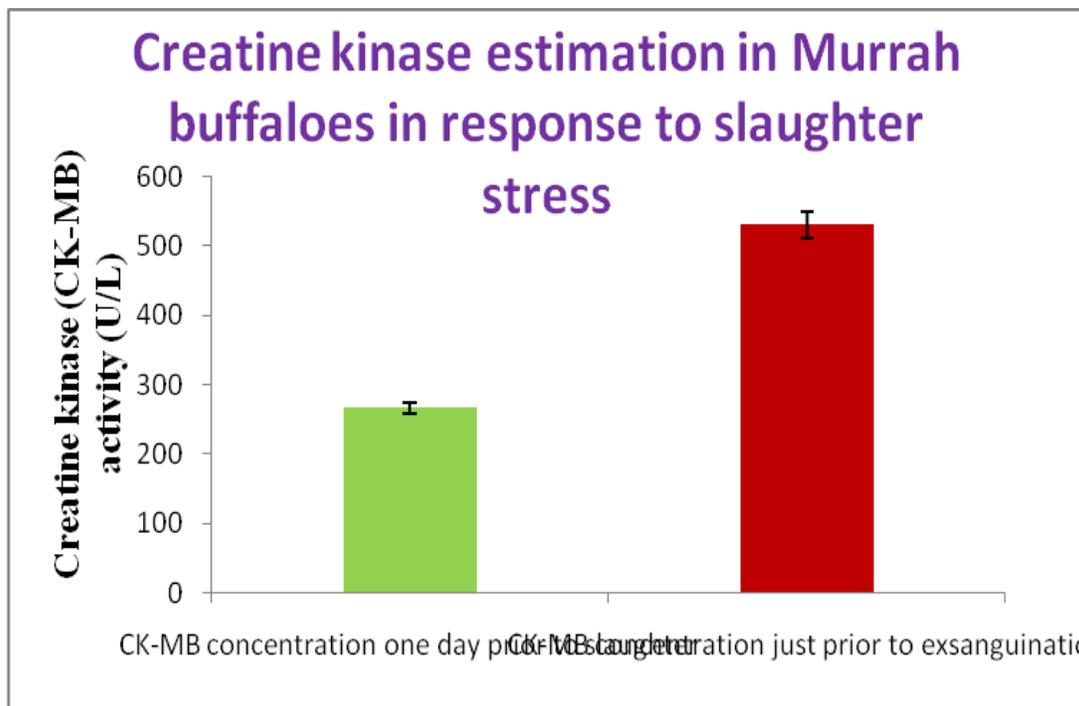


Fig.1 Bar diagram representing the creatine kinase activity in Murrah buffaloes in the serum samples collected one day before slaughter and just before exsanguination

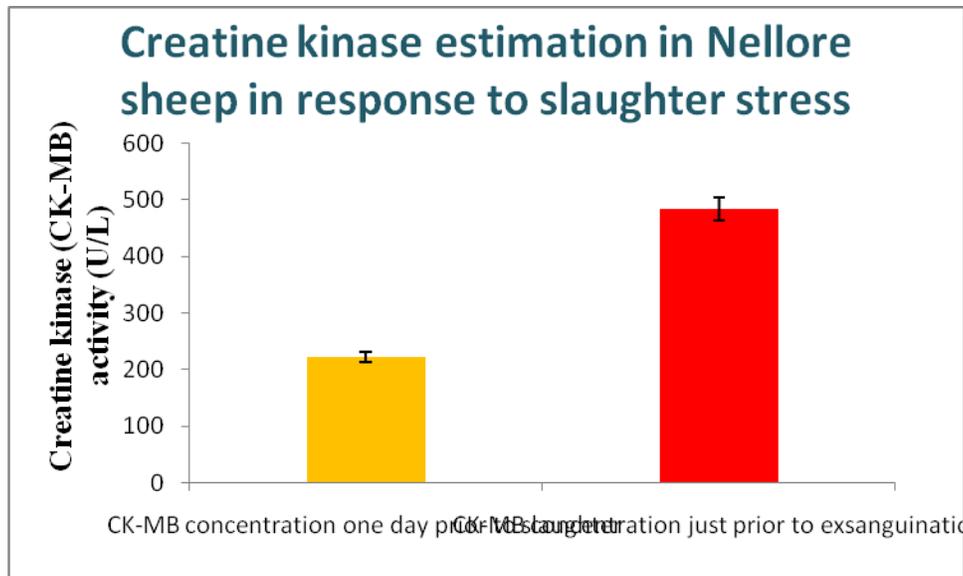


Fig.2 Bar diagram representing the creatine kinase activity in Nellore sheep in the serum samples collected one day before slaughter and just before exsanguination.

In the animal production sector preslaughter operations impose high levels of stress on the animals that leads to adverse changes in the storage life and quality of the meat (Prost 1981). Creatine kinase is a key enzyme which serves as a haematological marker of muscle damage whose activity increase significantly in response to muscle damage or injuries due to excess strain (Procajlo 2006). Such elevated levels of creatine kinase activity is a sensible indicator for the recent muscle damage due to its short half-life that can be related to the stress experienced by animals during preslaughter handling in the investigated animals.

In this study, a significant increase in serum creatine kinase activity obtained one day before slaughter in comparison with serum samples at the time of exsanguination suggests a rigorous stress response in both buffaloes and sheep. Previous reports indicated that the physical stress in and around slaughter might result from holding the animal to drive from lairage to stunning area and restraining them for stunning could lead to muscle damage and therefore subsequently increased liberation of serum

muscle enzymes (Stull and Rodiek, 2000; Van de Water *et al.*, 2003; Tadich *et al.*, 2005; Loudon *et al.*, 2019). Also not only the transportation but the feed deprivation and adverse environmental factors might infer the changes in the muscles which could bring about discharge of stress enzymes (Baird *et al.*, 2012; Teke *et al.*, 2014; Loudon *et al.*, 2019).

The elevation of CK activity observed in the current study could be ascribed to the increased permeability in the muscle membrane due to disruption of muscle due to traumatic events or physical stress (Tadich *et al.*, 2005; Bórnez *et al.*, 2009; De la Fuente *et al.*, 2010; Minka and Ayo, 2010; Baird *et al.*, 2012; Loudon *et al.*, 2019). Moreover Van de Water *et al.*, 2003 reported that mounting and butting behaviours expressed during the lairage period resulted in high CK activity determined at the time of bleeding. In this study the magnitude of increase in the creatine kinase activity in the serum samples is significantly higher values in in buffaloes than in sheep with respect to samples collected just before exsanguination which might be due to enhanced activation of the

passive fluxes via alpha 1 and 2 adrenergic receptors (Moratinos and Reverte, 1993; Chulayo and Muchenje, 2013) in buffaloes when compared to sheep (Shaw and Tume, 1992) in response to stunning and bleeding. From the current study, it is concluded that elevated levels of creatine kinase activity is due to stress response incurred as a consequence of pre-slaughter events in both buffaloes and sheep.

Out of the two species studied the creatine kinase concentration is significantly higher in buffaloes at exsanguination than in sheep which is a clear indication that buffaloes are exposed to more stressful events in and around slaughter than sheep. Further other enzymatic markers associated with stress needs to be evaluated in these breeds of buffaloes and sheep in future to undertake the proper management practices to reduce stress and therefore the quality of meat.

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