

# ACTIVITIES OF CREATINE KINASE AND LACTATE DEHYDROGENASE AS WELFARE INDICATORS IN SLAUGHTER CATTLE

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**Abstract** – The objective of the study was to determine the activities of creatine kinase and lactate dehydrogenase in slaughtered cattle as welfare indicators. The study was conducted at a commercial abattoir where 209 cattle from four different breeds (Beefmaster, Charolaise, Holstein-Friesian and Non-descript) from different farms were slaughtered. Information on distance travelled (DT) while transporting cattle from the farm to the abattoir was obtained from the drivers upon arrival. Ambient temperatures ( $T_a$ ) were obtained from the data loggers used at the abattoir. Blood samples were collected during exsanguination using disposable vacutainer tubes to determine the activities of plasma creatine kinase (CK) and lactate dehydrogenase (LDH). There were significant ( $p < 0.001$ ) differences on CK from the breeds considered in the study; non-descript cattle had the highest CK levels ( $3.20 \pm 0.252$  U/l) while Charolaise had the lowest CK levels ( $1.47 \pm 0.338$  U/l). There were no significant breed differences ( $p > 0.05$ ) on LDH levels. A positive correlation was observed between DT and CK (0.48),  $T_a$  and CK (0.26) and between CK and LDH (0.18). There were no significant relationships ( $p > 0.05$ ) between  $T_a$  and CK, DT and LDH. In slaughter cattle, CK activity was elevated with increase in DT whereas LDH was not affected. Therefore, CK can be used as an indicator of animal welfare in slaughter cattle.

**Key Words** – Abattoir, Animal welfare, Enzymes

## I. INTRODUCTION

Stress factors in slaughter animals include distance travelled, dehydration, exercise, fear, heat and cold; all of which emanate from bad or poor pre-slaughter handling. During animal handling animals must be treated in a manner that will bring about good welfare [1]. Animal welfare is described by five familiar freedoms that explain the fundamental needs of an animal [2; 3]. There is an ongoing debate, discussion and re-framing of

the concept of animal welfare to understand what is good or acceptable or poor as far as welfare is concerned [4]. Due to the fact that transport is one of the prime factors inducing stressful environment in the life of an animal, it therefore compromises their welfare [3, 5]. When animals experience changes in the environment, they become stressed. Environmental stressors include climate, temperature and nutrition [6, 7]. Transportation stress experienced by animals stimulates body responses that are aimed at re-establishing homeostatic conditions [8]. Body responses can either be physiological and or biochemical. Biochemical responses include hormonal and enzymatic secretions while physiological responses include avoidance behaviour. Examples of enzymatic secretions include activity of creatine kinase and lactate dehydrogenase [9]. Creatine kinase (CK) and lactate dehydrogenase (LDH) are enzymes that are released into the blood when there is muscle damage such as bruising and when there is a vigorous exercise [10]. Transportation stress causes welfare, economic and ethical concerns to the livestock industry [11]. Research has been conducted in cows to determine correlation of creatine kinase and aspartate aminotransferase associated with severity of endometritis [10]. Research has been conducted on the changes of elevated CK and LDH levels on cerebrospinal fluid and tissue damage of the central nervous system [12]. In addition, reduction on average daily gain in grazing cattle [13], nutritive value and enzymes present in cattle blood at slaughter [14] has been carried out under experimental design. Little has been done under practical conditions with the aim of monitoring the activity of CK due to sheep handling [15], logistic transport chain and pre-slaughter handling of cattle and pigs on animal welfare [16; 17]. Despite

challenges that are associated with unconventional research at the abattoir [18], protocols developed under such conditions are easy to implement as they promote good animal welfare [4, 19]. Nowadays, production processes that are aimed at increasing productivity and profitability ignore animal welfare and basic health needs [20] especially under practical conditions. Transportation and slaughter of farm animals are the final stages of an animal production [21]. This implies that any inappropriate handling procedures will determine the quality and quantity of the product. Therefore, the objective of the study was to determine the activities of creatine kinase and lactate dehydrogenase in slaughter cattle and how they are they related to each other.

## II. MATERIALS AND METHODS

### *Study site*

The study was conducted at a high-throughput commercial abattoir in the Eastern Cape Province of South Africa. The climate of the area is mild with an average rainfall of 850mm. Permission to conduct the study was obtained from the Ethics Committee of the University of Fort Hare, South Africa. Ethical principles were considered in the study to conform to the national and international standards governing research with usage of animals.

### *Animal management*

Two-hundred and nine ( $n = 209$ ) cattle composed of 4 different (Beefmaster, Charolaise, Friesian-Holstein and Non-descript) breeds that were brought to the abattoir for slaughter were used in the study. Upon arrival at the abattoir, distance travelled by the animals was obtained from the driver. The minimum and maximum distance recorded ranged from 35 – 1400 km. Ambient temperatures were obtained from the data loggers used at the abattoir. Low and high temperatures ranged from 14.5 – 19.5 °C. Animals were allowed to rest at the lairages while having *ad-libitum* access to water for 24hours (overnight).

### *Blood collection and plasma separation*

The captive bolt method of stunning was used to stun the animals. The animals were slaughtered

following slaughter procedures at the abattoir governed by Meat Safety Act [22]. During exsanguinations, disposable vacutainer tubes with anticoagulant were used to collect blood. Each blood sample was kept in ice until plasma was separated within 2 hours after collection. Blood tubes were centrifuged (Model 5403 Centrifuge, Gatenbay Eppendorf GmbH, Engelsdorp, Germany) at 21°C for 10 min at 3550 rpm and were then stored at -20°C.

### *Activity of creatine kinase and lactate dehydrogenase*

The samples of stored plasma were analysed for CK and LDH using a Model DXC 600 machine (Beckman Coulter, Ireland) at the National Health Laboratory Services, Port Elizabeth. All the ingredients added for quantitative determination of CK and LDH activities of units per litre (U/L) in plasma.

### *Statistical analysis*

The data for breed effect on the activities of CK and LDH was analysed using PROC GLM of SAS [23]. Significant differences in means were compared using Duncan Multiple Range Test. Correlations between DT,  $T_a$ , CK and LDH were computed using Pearson's correlation coefficient [23].

## III. RESULTS AND DISCUSSION

Results in Table 1 show the effect of breed on the secretion of enzymes from the muscles to the blood stream. There were significant differences ( $p < 0.001$ ) on CK secretion between the different breeds; ND breed had the highest ( $3.20 \pm 0.250$  U/l) levels of CK. increased activity of creatine kinase found in sheep was due to breed effect [15]. Enzymes are used in clinical biochemistry to perform differential diagnosis. These enzymes are mostly located in different tissues and once they are present in the blood plasma that serves as an indication of muscle damage. In this case, Non-descript breeds had elevated CK activities that the other breeds. Reason could be that they are not accustomed to transportation stress and abattoir environment. Studies reveal that gentle touching in an early life of an animal reduces avoidance

behaviour that may also reduce fear to novel environments [24, 25]. It has been revealed cattle react quickly to stressful settings resulting to increased concentration of hormones and enzymes [26]. The reason for lower levels of CK for the other breeds (Beefmaster, Charolaise and Holstein-Friesian) is that they are used to handling procedures unlike those that are raised extensively. Research revealed that intensively raised animals are performing better than those that are extensively raised [27]. There were no significant ( $p>0.05$ ) differences of LDH concentration from the different breeds. Creatine kinase can therefore be used to show the response of animals to stress caused by transportation, distance, handling, loading and off-loading, hence it is a good indicator of cattle.

Table 1 Means and standard errors for breed effect on creatine kinase and lactate dehydrogenase

		Enzyme concentrations (U/l)	
		$P < 0.001$	
Breed	<i>n</i>	CK	LDH
ND	54	3.20±0.252 <sup>a</sup>	4.57±0.169 <sup>a</sup>
BM	115	1.85±0.173 <sup>b</sup>	4.23±0.116 <sup>a</sup>
FH	10	1.70±0.586 <sup>b</sup>	4.40±0.394 <sup>a</sup>
CH	30	1.47±0.338 <sup>b</sup>	4.00±0.227 <sup>a</sup>

<sup>ab</sup>Means with the same superscript with a column are significantly different at  $P < 0.001$ .

U/l – Units per litre,  
ND – Non-descript  
BM – Beefmaster  
FH – Friesian-Holstein  
CH – Charolaise.

Table 2 presents the correlations between distance, temperature and creatine kinase and lactate dehydrogenase. A positive correlation was observed between distance and creatine kinase ( $P < 0.01$ ), temperature and lactate dehydrogenase ( $P < 0.05$ ) and between creatine kinase and lactate dehydrogenase ( $P < 0.001$ ). Long distance increases the production of enzymes that are used to assess stress and responses of animals during transportation. During transportation, a lot of activities such as truck vibrations, stoppages and truck dimensions including friction between cattle are at play [28]. Moreover, ambient temperatures that exceed the thermo neutral zone of the animal initiate a number of responses that affect the

activity of plasma enzymes. However, the correlations observed between  $T_{a}$ , CK and LDH could be due to the fact that animals have structural composition and genetic characteristics that contribute to the ability to resist heat stress [8]. Therefore, the correlations observed between distance and CK and between temperature and LDH are indications of welfare at slaughter that also determine productivity.

Table 2 Correlations between distance travelled, temperature, creatine kinase and lactate dehydrogenase

	CK	LDH	Distance	Temperature
CK	-	0.18***	0.48**	-0.28 <sup>ns</sup>
LDH	-	-	0.32 <sup>ns</sup>	0.14*

Significance difference - \* $P < 0.05$ , \*\* $P < 0.001$ , \*\*\* $P < 0.001$ , ns – not significant

#### IV. CONCLUSION

It could be inferred from the current study that the activities of the lactate dehydrogenase were not affected by the distance travelled by the animals prior to slaughter. Observation of the elevated activity of the creatine kinase was however found to be caused by the distance travelled by the animals prior to slaughter at the abattoir. Alteration in the activity of the creatine kinase can therefore be used as a good pre-slaughter welfare indicator in cattle.

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