EFFECTS OF TRANSPORTATION, LAIRAGE AND STUNNING SHOTS ON THE EXPRESSION OF STRESS BIOMARKERS AND MEAT QUALITY IN BOVINES

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Abstract – The objective of the study was to determine the effects of transportation, lairage and number of stunning shots on the concentration of bovine heat shock proteins 70 (HSPA1A) in plasma, as well as cortisol (CORT) and glucose (GLU) levels in relations hips with beef quality. A negative correlation was observed between HSPA1A concentration and other parameters with the exception of (L*), GLU and CORT. Warner Bratzler Shear Force (WBSF) was negatively correlated with L*, redness (a*), yellowness (b*) HUE (HUE angle), HSPA1A, GLU and CORT. WBSF was positively correlated with ultimate pH (pH_u) and meat temperature (T_m). Cattle that travelled between 200 < 400 km had lower HSPA1A concentration (10.01 ± 3.149 ng/ml). Lairage duration had a significant effect on the expression of HSPA1A (P < 0.001), GLU (P < 0.05) and CORT (P < 0.001) levels. Cattle that were shot more than once had the highest expression of HSPA1A (15.085 ± 1.734 ng/ml) and CORT level (101.16 ± 1.734 nmol/L). It was concluded that long hours of transportation with shorter lairage duration increased the expression of HSPA1A and CORT while glucose levels were reduced. In addition, HSPA1A, CORT and GLU were related to beef quality. Stunning of animals more than once increased the levels of blood CORT and HSPA1A.

Key Words – Heat Shock Protein, lairage duration, proteins.

I. INTRODUCTION

The use of energy and protein imbalances during exposure of animals to a novel environment leads to the depletion of glucose and inversely increases cortisol production [1, 2]. Welfare requirements dictate that animals should be insensitive to noxious and potentially harmful stimuli prior to post slaughter. Animals are stunned to render them insensible to pains during throat-cutting in order to reduce stress [3, 4]. However, improper use of the captive bolt machine (increased number of shots) may lead to an increase in the production of stress proteins, hormones and reduce muscle glycogen which is responsible for lactic acid production [5]. Transportation of live animals to the abattoirs and places where they are auctioned has been reported to cause stress in the life of animals [4, 6]. It is a known fact that stress in the life of animals increases cell death due to the result of the accumulation of the damaged proteins and denaturation of enzymes in the organisms [7]. Research has been conducted on body temperature, blood pressure, heart rate and different markers of stress such as cortisol, hormones and heat shock proteins (HSPs) [8, 9, 10]. However, considering transportation of cattle as well as lairage duration at the abattoir, animals and genotype effects on the expression of HSPs, glucose and cortisol levels vary thereby limiting how they are related to meat quality. Hence, the objective of the study was to determine the expression of HSPA1A, glucose and cortisol as affected by transportation, lairage duration and stunning shots in slaughtered bovine animals as well as correlations associated with the quality of beef.

II. MATERIALS AND METHODS

The study was conducted at a high-throughput commercial abattoir in the Eastern Cape Province of South Africa. The permission to conduct the study was approved by the Research Ethics Committee of the University of Fort Hare, (UFH/UREC, MUC012 1SCHU01).

Animal management and sampling

One-hundred (100) cattle including 39 Bonsmara, 28 Nguni and 33 Non-descript genotypes of

different classes (heifers, bulls and cows) were identified on arrival at the abattoir. At the lairage, the animals were given *ad-libitum* access to water. At the stunning box, the number of times an animal was shot was recorded. The captive bolt method of stunning was used and the cattle were slaughtered according to the commercial abattoir slaughter procedures [11].

Blood sample collection and storage

Blood samples were collected during cattle exsanguinations using the yellow top tubes for easy separation of plasma. The samples were centrifuged at 1000 g, 15 min at 2 °C and stored at -20 ^{0}C until the assay to avoid loss of bioactivity and contamination.

Determination of bovine heat shock protein 70

The concentration of HSPA1A in plasma was performed using an enzyme-linked *immunosorbent* assay kit (CUSABIO CSC-EL010821BO). The assay of the standards and samples was done in duplicates. The results were then calculated according to make a standard curve and the standards and samples were averaged per duplicate readings in order to subtract the zero standard optical density.

Determination of blood cortisol and glucose levels

The determination of blood cortisol was carried out through a competitive assay (EIA, RADIM, Pomezia, Italy). Glucose concentrations in blood serum samples were determined by using the calibration curve in nmol/L. Reference measurements of the blood serum glucose concentration were conducted by the classical colorimetric method using an enzymatic kit (Diaglue from L'viv Plant of Bacterial preparation, 1994 and the well-known biosensor analyzer, Eksan-G from Panevezhis Plant of precision mechanics, 1990).

Beef quality analysis

A sample was extracted from the *longissimus thoracis et lumborum* (LTL) muscles, removed from the 4th and 6th ribs of the loin region at 48 hours after animals were slaughtered.

This sample was used to measure color (L* = lightness, a* = redness and b* = yellowness) and pH_u. The color coordinates were measured using a color-guide 45/0 BYK-Gardener GmbH machine with a 20 mm diameter measurement area and illuminant D65-day light, 100 standard observer. Ultimate pH was also measured from the same muscles using a portable pH meter (CRISON pH 25, CRISON Instruments, SA Spain). Tenderness of beef was measured using an Instron - Warner-Bratzler Shear Force (WBSF) machine. The samples were sheared perpendicular to the fibre direction using a Warner Bratzler (WB) shear device mounted on an Instron 3344 Universal Testing. The mean maximum load was recorded for the three cores represented the average of the peak force in Newtons (N) for each sample.

Statistical analysis

A principal component analysis was performed to determine the correlations between HSPA1A, GLU, CORT and beef quality using JMP 9.0 [12] The mixed model procedure (PROC MIXED) [13] was used to determine the effect of distance and lairage categories, number of shots at stunning on the expression of HSPA1A, cortisol and glucose. Genotype was used as a random variable.

III. RESULTS AND DISCUSSION

Bovine heat shock protein 70, cortisol glucose and meat quality

Figure 1 indicates the relationship between meat quality, HSPA1A, GLU and CORT of bovine species. The negative relationship between HSPA1A and pH_u is as a result of the binding that occurs between hsps and adenosine triphosphate (ATP) during the period of stress resulting in depleted muscle glycogen and increased pH_u [14].

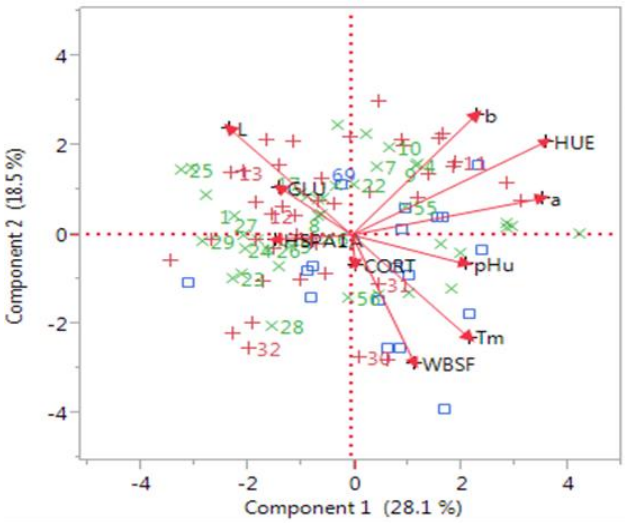

Figure 1 The relationship between the expression of HSPA1A, glucose, cortisol and beef quality. T_m - Meat temperature, pH_u - Ultimate pH, L* - Lightness, a* - Redness, b* - Yellowness, HUE - HUE angle, WBSF - Warner Braztler Shear Force, HSPA1A – Bovine heat shock protein 70, GLU - Glucose, CORT - Cortisol

Table 1 The parameter estimates and standard errors of HSPA1A, glucose and cortisol as affected by pre-slaughter conditions

Variables		*n*	Response variables		
			HSPA1A concentration (ng/ml)	Glucose nmol/L	Cortisol nmol/L
DT (km)	< 200	32	$15.13^{b} \pm 2.362$	$5.81^{a} \pm 2.362$	$95.31^{a} \pm 2.362$
	200 < 400	18	$10.05^{a} \pm 3.149$	$6.10^{b} \pm 3.149$	$88.87^{a} \pm 3.149$
	400 < 800	50	$12.11^{b} \pm 1.889$	$5.50^{a} \pm 1.889$	$103.56^{b} \pm 1.889$
Significance level			**	***	***
LD_{hr}	18	32	$15.09^{c} \pm 2.401$	$5.42^{a} \pm 2.401$	$105.77^{c} \pm 2.401$
	20	18	$-10.01^{a} \pm 4.069$	$5.89^{b} \pm 4.069$	$91.32^{a} \pm 4.069$
	24	50	$14.03^{b} \pm 3.507$	$5.67^{b} \pm 3.507$	$97.05^{b} \pm 3.507$
Significance level			***	*	***
Number of shots at stunning	1	78	$-10.01^{a} \pm 4.338$	$5.78^{a} \pm 4.338$	$94.93^{a} \pm 4.338$
	> 1	22	$15.09^{b} \pm 1.734$	$5.55^{a} \pm 1.734$	$101.16^{b} \pm 1.734$
Significance level			**	*Ns*	***

[a,b,c]Means with different superscripts within the same column are significantly different; DT - Distance travelled, LDhr - Lairage duration in hours; Significance level: *P < 0.05, **P < 0.01, ***P < 0.001, ns - not significant

The relationship between cortisol and fatness is not a new finding. Indeed, in humans, the increase in cortisol was observed to be caused by a significant increase in fatness [2]. The relationship between beef quality is based on the fact that pH_u can improve the bright red color and improve tenderness because of a higher amount of intramuscular fat [15].

Pre-slaughter stress on bovine heat shock protein 70, glucose and cortisol

As shown in Table 2, distance duration (P < 0.001), lairage duration (P < 0.001) and number of shots at stunning (P < 0.01) had significant effects on the expression of plasma HSPA1A and levels of plasma glucose and cortisol. During transportation stress, there is also an exchange of gases such as carbon dioxide (CO_2) and oxygen (O_2) [4]. However, during the 800 km transport duration truck movements and exhaustion glucose levels were reduced while cortisol was at its peak. This agrees with the reports that indicated that as transport duration increases, cortisol levels become elevated [16]. Lairage duration had a significant effect HSPA1A (P < 0.001), GLU (P < 0.05) and CORT (P < 0.001) levels. The low levels of HSPA1A during 24 hours of rest indicated that cattle were not exhausted nor in pain or stressed. The expression of HSPA1A was induced by the amount of heat shock transcription factor (HSF1) which functions during heat stress [17]. The highest expression of HSPA1A and CORT were also observed in cattle that were stunned more than once (Table 1). On the other hand, the glucose levels were reduced in animals that were stunned more than once (5.55 ± 1.734). The elevation of the proteins and cortisol has been reported to be due to the averseness of the stimulus which interrupts the proper functioning of the brain. In addition, the last 15-20 minutes prior to slaughter caused detrimental effects on the animal`s physiology [18]. The low levels of glucose for cows in not clear considering the fact that they were not stressed. In addition, cortisol levels were expected to be very low as it was reported that starvation or fasting in goats led to lower cortisol levels [19].

IV. CONCLUSION

It was concluded that transport duration, lairage duration and animal-related factors led to a negative correlation of heat shock proteins, glucose and cortisol with ultimate pH which is a widely used indicator of meat quality. Longer transport duration with fewer hours of lairage duration increased the expression of HSPA1A and cortisol while glucose levels were reduced levels of glucose and higher plasma. The number of stunning shots led to an increase in the expression of HSPA1A and cortisol levels.

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