

Full Length Research Paper

A comparison of stress hormone concentrations at slaughter in Nguni, Bonsmara and Angus steers

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Concentrations of stress hormones at slaughter were determined in 15 of each of 18-month old Angus, Bonsmara and Nguni steers. The steers were slaughtered at the East London Abattoir, which is about 120 km from the University of Fort Hare farm, where the animals had been raised. Serum creatinine and packed cell volume (PCV) concentrations were determined before cattle transportation. Blood was also collected along the slaughter line immediately after exsanguination for the determination of cortisol, creatinine and PCV. Urine samples were collected for the measurement of creatinine, adrenaline, noradrenaline and dopamine concentrations. Estimated glomerular filtration rate was also determined. Bonsmara had the highest concentrations of adrenaline (10.8 nmol/mol), noradrenaline (9.7 nmol/mol) and dopamine (14.8 nmol/mol) concentrations, whereas the Nguni had the least concentrations of adrenaline (6.5 nmol/mol), noradrenaline (4.6 nmol/mol) and dopamine (4 nmol/mol) concentrations. The Nguni had the highest serum cortisol concentrations (2.3 nmol/mol), while Angus had the least concentrations (1.3 nmol/mol). There were no ($P > 0.05$) breed differences in serum and urea creatinine concentrations, and on estimated glomerular filtration rate (eGFR). Bonsmara steers had higher ($P < 0.05$) levels of catecholamines and dopamine compared to Nguni and Angus steers after transport and handling stress. The Bonsmara was therefore the most stress responsive breed at slaughter.

Key words: Stress, cortisol, dopamine, adrenaline, animal welfare, breed.

INTRODUCTION

The Nguni is increasingly attracting international interest, mainly due to its resistance to ticks and tick-borne diseases, high reproductive performance and good walking and foraging ability (Strydom et al., 2001; Muchenje et al., 2007a; 2007b). For efficient production and for improving their marketability, the nutritional, health status and response to stress of the Nguni cattle needs to be monitored (Chimonyo et al., 2002). The Bonsmara is a medium-sized breed which competes favourably with European beef cattle while withstanding subtropical conditions, such as high temperatures, ticks and most tick-related illnesses (Porter, 1991). It is not clear whether the Bonsmara are as adapted to harsh conditions as the Nguni. The Angus, on the other hand, is more susceptible to

ticks and tick-borne diseases (Muchenje et al., 2007b). Although information on the growth performance, blood profiles and meat quality of the Nguni relative to the Bonsmara and Angus is available (Muchenje et al., 2007a; b; Ndlovu et al., 2007), levels of stress hormones at slaughter are not available.

Besides adaptation to local production conditions, the levels of stress hormones at slaughter have been reported to influence meat quality (Gupta et al., 2007; Pineiro et al., 2007). In addition, poor handling causes economic losses to farmers, transporters and slaughterhouses (Gupta et al., 2007; Saco et al., 2007). There is increasing interest in the measurement of stress at slaughter as an indicator of animal welfare status and disease (Odore et al., 2004; Gupta et al., 2007).

Therefore, there is need to compare the responsiveness of Nguni cattle to stress, as it affects not only growth performance, but also meat quality (Odore et al., 2004;

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Pineiro et al., 2007). Age, health status, genotype, and previous experiences of animals can influence the way they cope with stress (Grandin, 1997; Knowles and Warriss, 2000). Rough handling may be more detrimental and stressful to animals with an excitable temperament compared to animals with a more placid temperament. Temperament in cattle, with a heritability of about 0.5 (Stricklin et al., 1980), may affect the animal's reaction to handling (Grandin, 1997).

Dopamine, adrenaline, noradrenaline and cortisol have been shown to be good indicators of stress in animals (Odore et al., 2004; Lopez-Olvera et al., 2006). Urine is the main elimination route for catecholamines and glucocorticoids. Excretion products in urine accumulate over several hours. Thus, concentrations in urine are more indicative of stress levels in animals than those in plasma (Hay et al., 2000; Mostl and Palme, 2002). Transport and handling stress increase PCV (Scope et al., 2002; Lopez-Olvera et al., 2006). The increase reflects both a splenic response to stress and, to some extent, dehydration. Creatinine has also been used to assess the effect of stress on the functioning of kidneys (Scope et al., 2002; Lopez-Olvera et al., 2006). Breed differences on stress hormones are scarce, and the majority of reports tend to ignore possible breed differences on stress hormone concentration. Ndlovu et al. (2007) found that there were breed differences in blood metabolite concentrations. However, there is no information on how Nguni, Bonsmara and Angus cattle respond to pre-slaughter stress. Therefore, the objective of the current study was to determine the effect of breed on PCV, creatinine catecholamines, cortisol and dopamine at slaughter. The hypothesis tested was that the Nguni breed, which is indigenous to Southern Africa, was not highly responsive to stress.

MATERIALS AND METHODS

Animals and sample collection

Blood samples were collected into red-topped vacutainer tubes with no anticoagulant and into EDTA-containing tubes by jugular-venipuncture from 45 steers at Honeydale farm 24 h before transportation of the animals to the abattoir. The steers were transported to East London, which is about 120 km away from the farm. On the day of transportation, the minimum temperature was 13.5°C and the maximum was 24.0°C. The steers were kept overnight at the abattoir holding pens without food for 24 h. Water was available at all times. Cattle were slaughtered after stunning with a captive bolt suspended by a hind leg and exsanguinated.

Blood was sampled along the slaughter line into vacutainer tubes containing EDTA anticoagulant and the other one with no anticoagulant. This was done immediately after exsanguination. Urine samples were collected from the slaughter line from the bladder of each animal using a syringe into sampling bottles. The sample bottles contained 6M hydrochloric acid to stabilize the catecholamines. The samples were then frozen at -20°C awaiting analysis.

Hormone and metabolite analysis

Creatinine concentrations were analyzed using a colorimetric

quantitative reaction (Boehringer PAP method). Packed cell volume for each steer was measured by the standard microhematocrit method with a hematocrit centrifuge at 1 000 × g for 5 min. Serum concentrations of cortisol were quantified using an immunoassay, as described by Odore et al. (2004). The interassay coefficient of variation (CV) was 4.6%. Urinary dopamine, noradrenaline and adrenaline were assayed using an ion-exchange purification procedure followed by liquid chromatography with electrochemical detection (Ruis et al., 2002). The interassay CVs for dopamine, noradrenaline and adrenaline were 5.2, 4.7 and 4.1%, respectively.

Estimated glomerular filtration rate was determined using Liu et al. (1999) formulae. Creatinine was used to correct for urine dilution (Klante et al., 1997; Hay et al., 2000). Therefore, catecholamine concentrations were expressed relative to creatinine concentrations.

Statistical analysis

The effects of breed on cortisol, catecholamines and dopamine were analyzed using Generalised Linear Models procedures of the Statistical Analysis Systems (SAS, 2003). Pair-wise comparisons between least-square means were compared using the PDiff test of SAS (2003). A repeated-measures analysis of variance was performed for creatinine and PCV concentrations to detect statistical differences between breeds before and after slaughter, using the PROC MIXED procedure of SAS (2003).

RESULTS

Table 1 shows PCV and serum creatinine concentrations of Nguni, Bonsmara and Angus. The PCV concentrations increased ($P < 0.05$) after transportation in all the breeds. No significant differences ($P > 0.05$) were observed in serum creatinine concentrations when comparing levels before and after transportation. There were no breed differences ($P > 0.05$) in creatinine and PCV concentrations before and after transportation and handling the animals in preparation for slaughter. Figure 1 shows the breed differences in adrenaline, noradrenaline and dopamine concentrations. Bonsmara had the highest ($P < 0.05$) concentrations of adrenaline, noradrenaline and dopamine concentrations whereas the Nguni had the least ($P < 0.05$) concentrations. Figure 2 shows the breed influence on serum cortisol concentrations. The Nguni had the highest ($P < 0.05$) serum cortisol concentrations and Angus had the least ($P < 0.05$) concentrations. As shown in Figure 3, there were no breed differences ($P > 0.05$) in estimated glomerular filtration rate (eGFR).

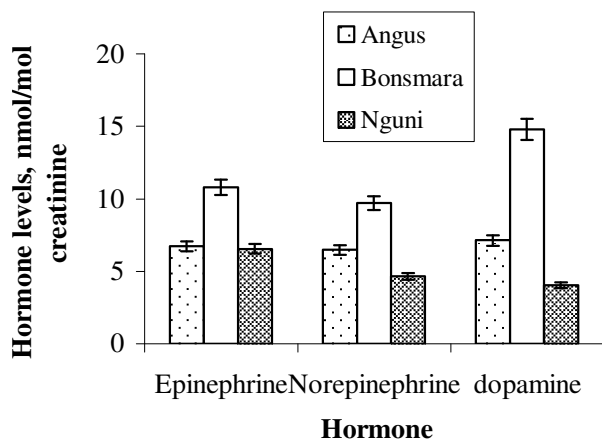
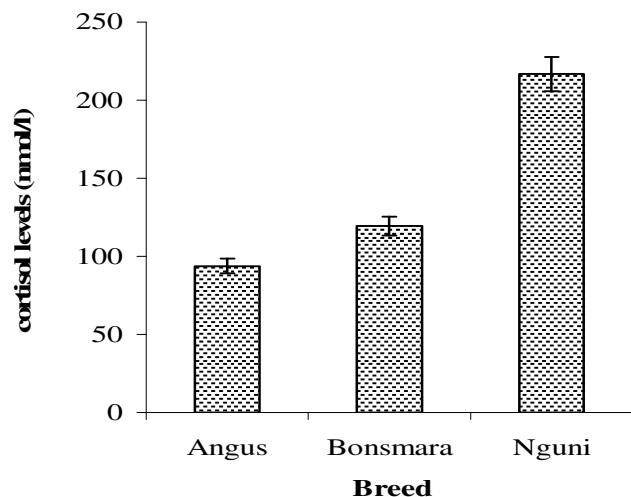
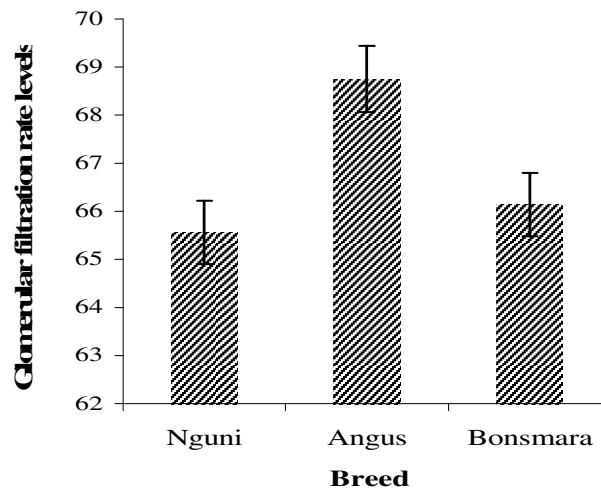
DISCUSSION

Predicting secondary physiological outcomes resulting stressors occur, often in combinations unique to a particular transportation scenario or species. The high concentrations of cortisol obtained in Nguni steers was possibly due to the breed's high response to pre-slaughter stress. Transport and handling are reported to evoke an increase in circulating cortisol concentrations (Odore et al., 2004). It has been documented that dopamine regulates cortisol secretion in ewes (Sowers et al., 1983),

Table 1. PCV and serum creatinine concentrations (mean values and standard errors) of Angus, Bonsmara and Nguni steers before and after transport and handling stress.

Parameter	Before transportation	After transportation	P value
PCV (%)			
Angus	33.33 ± 0.522 ^a	36.00 ± 0.511 ^b	P= 0.021
Bonsmara	29.93 ± 0.432 ^a	33.36 ± 0.446 ^b	P=0.013
Nguni	31.73 ± 0.401 ^a	35.71 ± 0.411 ^b	P=0.010
Serum creatinine			
Angus	98.60 ± 4.631	87.50 ± 4.222	P= 0.130
Bonsmara	106.10 ± 3.921	91.29 ± 3.544	P=0.110
Nguni	106.90 ± 3.322	92.87 ± 2.987	P=0.090

^{a,b} Values with different superscripts within each row are significantly different ($P < 0.05$) PCV- Packed cell volume.

**Figure 1.** Urine adrenaline, noradrenaline and dopamine concentrations and standard errors of A.**Figure 2.** Serum cortisol concentrations and standard errors for Angus, Bonsmara and Nguni steers after transportation and handling stress.**Figure 3.** Estimated glomerular filtration rate and standard errors for Angus, Bonsmara and Nguni steers after exposure to transport and handling stress.

yearling steers and heifers (Browning, et al., 2000) and cows (Ahmadzadeh et al., 2006). With this argument, cortisol and dopamine concentrations were both expected to be high in Nguni. However according to Grandin (1997), absolute comparisons of cortisol concentrations between studies should be done with caution, as cortisol concentrations can vary greatly between individual animals and breed. For example, cortisol concentrations have not been found to be positively correlated with stress in swine during transport (Brown et al., 1999).

The low cortisol concentrations in Angus and Bonsmara breeds are difficult to explain. However, in a study conducted by Koch (2003), Bonsmara had lower plasma cortisol concentration following 30 min transportation compared to the plasma cortisol concentrations prior to transportation. Blecha et al. (1984) reported similar findings in that Angus feeder steers also had decreased cortisol concentrations following 10 h of transportation.

These authors suggest that it is likely to be due to the animals acclimating to the trailer. Since the transportation time of these steers in the current study was less than three hours, it was difficult to conclude that the Angus and Bonsmara had acclimated to transportation. Acclimation is unlikely to occur during a short trip.

Bonsmara had high concentrations of adrenaline and noradrenaline compared to Nguni and Bonsmara. This confirms the findings by Grandin (1997) and Gonyou (2000) who observed that genotype can also influence the physiological changes. The effect of genetics is also well documented and an example of stress susceptibility in swine is where sudden death following stress, is inherited by a single recessive gene (*Haln*). When subjected to transport stress, the three *Hal* genotypes (*HalN/N*, *HalN/n*, *Haln/n*) had different cortisol concentrations immediately after transport (Nyberg et al., 1988). Bonsmara is more susceptible to stress before slaughter compared to Angus and Nguni because it had higher adrenaline and noradrenaline concentrations.

Estimated glomerular filtration rate was between 60 and 89 ml/min, thus indicating possibly mild renal function impairment in all the steers. However, this finding is not conclusive as the creatinine concentrations were within reference ranges (Kaneko, 1997) and there was no significant increase in creatinine concentration. Alteration of creatinine concentration in stressed animals has been reported before (Knowles and Warriss, 2000; Lopez-Olvera et al., 2006). In this study, no significant differences were found between the concentrations of creatinine before and after transportation. However, increased creatinine concentration due to muscular activity and a decrease in renal excretion because of vasospasm in the kidney produced by catecholamines has been described (Knowles and Warriss, 2000; Lopez-Olvera et al., 2006). Adrenaline (40%) and noradrenaline (20%) cause a decrease in renal blood flow, thus predisposing to renal hypoxia (Guyton and Hall, 1996). In the present study, it is difficult to quantify the adrenaline and noradrenaline percentile increase because urine was not sampled prior to subjecting the animals to stress for detection of basal adrenaline and noradrenaline concentrations.

The increase in PCV concentrations observed in this study at slaughter of animals agrees with observations that Gupta (2007) and Lopez-Olvera et al. (2006) made. Increases in PCV are associated with splenic contraction, caused by the effect of catecholamines on adrenergic receptors located in the splenic capsule (Gupta, 2007), and partly to a reduction in plasma volume (Cross et al., 1988). Scope et al. (2002), however, found no significant differences in PCV concentrations on the influence of stress from transport and handling on PCV concentrations of racing pigeons.

Conclusions

Bonsmara had high concentrations of adrenaline, noradrenaline and dopamine concentrations whereas the

Nguni had the least concentrations. The Nguni had the highest serum cortisol concentrations and Angus had the least concentrations. There were no breed differences in serum and urea creatinine concentrations as well as estimated glomerular filtration rate (eGFR). No difference in creatinine concentrations were detected in all the breeds whereas PCV concentrations were high at slaughter. From the results, it can be concluded that the Bonsmara is more susceptible to stress. In future studies, pre-transport and handling physiological events must be recorded in order to demonstrate and compare non steady-state condition experienced by cattle while being transported and handled. Understanding these events, however, has been a necessary first step in being able to make well-informed suggestions on treatment regimens to attenuate transport and handling stress.

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