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Abstract: A total of 39 bulls from Arsi (11), Boran (14) and Harar (14) breeds were used for the study. The bulls were stratified into three age groups. Blood samples were collected before loading, after unloading and at slaughter to determine the level of creatine kinase and lactate dehydrogenase. M. longissimus dorsi between 12th and 13th ribs was used to determine ultimate pH, color (L*, a*, b*), tenderness and water-holding capacity. The results of the study revealed that bulls from Arsi breed had exhibited higher (p < 0.05) level of creatine kinase and lactate dehydrogenase (351.82 ± 89.35 U/L, 1496.73 ± 81.60 U/L) before loading. Levels of the two enzymes after unloading were significantly affected (p < 0.05) by transportation. Breeds had significantly affected (p < 0.05) the instrumental tenderness with beef from Harar (19.07 ± 1.33 N) and Boran (20.56 ± 1.33 N) bulls were tender than Arsi. Boran had higher (p < 0.05) slaughter weight and carcass weight than Arsi and Harar breeds in their respective age groups. It was observed that Arsi, Boran and Harar cattle expressed different level of stress before loading, after unloading and at slaughter. Moreover, quality of beef based on tenderness differs among breeds, but were similar based on color, pH and water-holding capacity.

ABOUT THE AUTHOR
Addis Fikrie Birhanu had been working at Ethiopian Meat and Dairy Industry Development Institute since 2009 until known as Meat expert & researcher under Beef cattle, camel and small animals meat product research and processing industry development directorate. He holds a B. Sc. degree in Animal Production and an M.Sc. degree in Food Science and Technology from Haramaya University, Dire dawa, Ethiopia. Currently, he is attending B.Sc. program in Economics field at Arsi University, Faculty of Business and Economics. He is in charge of providing training and consultancy for meat producers, processors and exporters in Ethiopia for improving meat quality and safety. Moreover, he is conducting different researches in the area of pre-slaughter stress, meat quality, safety, processing and by product value addition.

PUBLIC INTEREST STATEMENT
Ethiopia currently producing and exporting 46, 120 and 19,104.7 tons of meat, respectively. This is low as compared to available cattle resource (56.7 million heads of cattle-CSA 2016/2017) in terms of production and benefits from exports due to a lot of problems. Among these, pre-slaughter stress (improper handling and transportation) is one the major concerns, which affected the welfare of the animals and meat quality. Low-quality meat not competes with domestic and international market through affecting producers and exporters income. In addition, it becomes a source of contamination through transmitting of many diseases that affects public health. Therefore good pre-slaughter management is one of the major intervention areas for better meat quality, price and more income for society as well as economic growth of the country. Other stake holder collaborations is needed to improve pre-slaughter animal handling and meat quality.
Further studies were recommended to evaluate the level of stress and quality of beef from the three breeds finished and transported under similar condition.

**Subjects:** Agriculture and Food; Animal Physiology; Food Chemistry;

**Keywords:** Beef; creatine kinase; lactate dehydrogenase and quality

1. Introduction

Ethiopia has about 28 cattle breeds with a population of 59.5 million (Central Statistics Agency, Central Statistical Authority [CSA], [2016]-2017; Ethiopian Biodiversity Institute, 2016). All except Sheko cattle breeds are humped (Food & Agricultural Organization, 2015). Most of the breeds (98.2%) are indigenous to the country (Mummed and Webb, 2014). Per capital meat consumption, annual meat production and carcass weight per head of cattle were low. The average Ethiopian carcass weight per cattle was 135 kg, which was by far less than the average 146 kg for Africa, and 205 kg for the whole world (Mummed and Webb, 2015). Ethiopia is one of the lowest per capita of meat consumption in the world which is 8 kg, of which about 5.3 kg comes from beef (Mummed and Webb, 2015).

Quality of beef was reported as one of the major constraints in satisfying the local demand and export market (Mummed and Webb, 2015). Boran bulls are highly preferred due to better carcass conformation, followed by cattle from Arsi and Hararge highland (Agricultural Growth Program—Livestock Market Development, 2013). Arsi cattle breed is categorized under large East African Zebu type, which is distributed in central highlands of Ethiopia. The breed has dual purpose (milk and meat) with main use for draft purpose. Harar breed is categorized under small east African zebu type, which is distributed eastern and western Hararghe high land. The breed categorized under mixed crop livestock production system and characterized by prominent dewlap, small short horns, extremely active and have very aggressive temperament. Boran breed is categorized under large East African Zebu which is distributed in Borana range land of Ethiopia (EBI, 2016).

One of the major challenges in Ethiopia facing the domestic consumption and meat export abattoirs has been that quality of livestock supply which was aggravated by lack of proper pre-slaughter handling and transportation of animals (Mummed, 2015). The meat supply chain is an important aspect in meat industry that includes various critical stages such as loading, transportation, unloading and slaughter of the animals. The slaughtering industry in many countries is becoming centralized into fewer locations; thus, the distance animal traveled to slaughtering plant increased (Ljungberg, Gebresenbet, & Aradom, 2007). Animals are exposed to various stresses during transport from farm to abattoirs. They are exposed to unusual noise, vibration, restraint, deprivation of feed and water, adverse weather conditions, breakdown of social grouping, mixing with unfamiliar animal, overcrowding, increased human contact and stunning (Adzitey, 2011).

Pre-slaughter stress causes rapid release of enzymes such as creatine kinase and lactate dehydrogenase into the blood which depletes glycogen store in the body ending up in lower rate of post-mortem lactic acid synthesis, high ultimate pH, undesirable color, and greater water-holding capacity (Adzitey & Nurul, 2011). Moreover, pre-slaughters stress due to inappropriate handling and transportation causes carcass damage through bruising, dark firm dry and reduction in live weight. Dark firm dry or dark cutting is defined as meat having value of ultimate pH which is higher than normal and has a dark color. Bruised and Dark Firm dry meat is less likely to be acceptable by consumers and poorly processed. It has to be trimmed and condemned resulting in discount in value of cuts or carcass. These defects were a serious problem for the fresh meat market and causes large economic losses to suppliers, meat industry and country (Adzitey & Nurul, 2011; Gupta, Earley, & Crowe, 2007). They have been receiving a lot of attention from researcher and the beef industry due to such substantial economic burden and possible animal welfare implications.
2. Materials and methods

2.1. Description of study area

The study was conducted on Arsi, Boran and Harar cattle breeds purchased from their in situ production systems; West Arsi (kofelle), Borena Zones (Yabello) and West Hararge (Chiro), respectively, and slaughtered at Elfora Bishoftu Export Abattoir. Arsi and Harar cattle breeds were purchased from cattle market, while Boran was purchased from ranch. West Arsi is located at 7° 19’ N to 7° 40’ N and 35° 38’ 30” E to 38°. West Hararge zone is located at altitude between 970 and 1,410 meter above sea level. The annual rainfall ranges from 650 mm and 950 mm and means temperature range is between 17.5°C and 27°C. Mixed crop livestock production practiced in the Arsi and Haraghe regions. Grazing and crop residue feeding practiced in these regions. Borana zone is semi-arid with an average rainfall ranging from 300 to 600 mm and average daily temperature from 19°C to 26°C. Extensive grazing on natural pasture practiced in these areas which are dominated by perennial grasses (Cenchrus, Pennisetum, and Chrysopogon spp.). The area has a bimodal rainfall distribution, with the long rains “ganna” extending from March to May and short rains” Hagaya” from September to November.

The abattoir is located at Bishoftu town which is 47 km south east of Addis Ababa. It is found at 9° N and 40°E with an altitude of 1880 m above sea level in the central high land of Ethiopia. It has an annual rainfall of 1151.6 mm of which 84% falls down during the long rainy season that extends from June to September and the remaining during the short rainy season that extends from March to May. The mean annual minimum and maximum temperature are 8.5°C and 30.7 °C, respectively, and the mean humidity is 61.3% (National Meteorology Service Agency, 2003).

2.2. Sample procedure and experimental animals

A total of 39 beef cattle samples consisting of three breeds (Boran, Harar and Arsi) were used for the study. The bulls were grouped in to three age groups, group 1 (2–3 years), group 2 (3–6 years) and group 3 (6-9 years) according to Torell, Bruce, and Kvasnicka (2003). Blood samples and live weight of bulls were taken before loading for transport to abattoir, after unloading and at slaughter. Due inadequate skills of veterinarian, blood was not collected from Harar breed before loading and due to absence of weighing scale at Kofale livestock market live weight was not taken from Arsi breed before transportation. Boran bulls were purchased from a ranch, while bulls from Arsi and Harar cattle breed were purchased from respective markets. Isuzu trucks were used to transport the bulls to Elfora Bishoftu abattoir for slaughter. Transportation time, distance traveled and ambient temperature were recorded. Bulls were handled and slaughtered according to animal handling procedure of the abattoir after letting them off feed for 18 h.

2.3. Experimental design

The experiment laid out in factorial arrangements with three factors (breed, age and stage of blood collection) in CRD (completely randomized design). The composition of treatment is described in Table 1.

2.4. Blood collection and serum separation

A total 103 blood samples (42 from Boran, 28 from Harar and 33 from Arsi) were collected. Blood collection and serum separation were conducted following the procedure followed by Muchenje and Chulayo (2017). Four milliliter of blood per animal was collected from jugular vein using vacutainer tubes and needles (21 gauges). The samples were labeled and allowed to coagulate for 30 min at room temperature. Then after, it was centrifuged at 3550 revolution per minute, 21°C for 10 min. Then, the serum was collected and inserted into 1.5 ml labeled Eppendorf tubes using pasteur pipettes and stored at (−20 °C). Furthermore, the serum was transported by using icebox to Ethiopian Public Health Institute, Biochemistry Laboratory, for the determination of the level of creatine kinase and lactate dehydrogenase.
2.5. Level of creatine kinase and lactate dehydrogenase determination

The level of CK and LDH was determined by Cobas Integra 400 Plus machine (fully computerized machine) with ready use reagents. The content of Reagent one (R1) for CK included Imidazole 58 mmol/L, PH 6, N-Acetylcysteine 40 mmol/L, ethylene diamine tetra acetic acid 3 mmol/L, Adenosine mono phosphate 10 mmol/L, Diadenosine pentaphosphate 24 mmol/L, NADP⁺ 9.50 mmol/L, Mg²⁺ 20 mmol/L, D-Glucose 40 mmol/L and non-reactive stabilizer while Reagent two (R2) for CK contain ethylene diamine tetra acetic acid 3 mmol/L, pH 9.1, Hexokinase (yeast) ≥ 600 μkat, Glucose-6-Phosphate Dehydrogenase (microbial) ≥ 600 μkat, ADP 12 mmol/L, creatine phosphate 180 mmol/L, N-methyl diethanolamine 69 mmol/L, preservative, non-reactive stabilizer and ingredient. The content of Reagent one (R1) for LDH consisted of phosphate buffer 68 mmol/L, pH 7.5, pyruvate 0.73 mmol/L, stabilizers, preservative, whereas Reagent two (R2) contain NADH 1.1 mmol/L, stabilizers and preservative.

For both tests, R1 was placed in position B and R2 was in position C of cassette section of the Cobas Integra 400 Plus machine simultaneously. Before starting sample analyzing, the machine was calibrated and quality control was checked. Then, the serum, which is stored at (−20°C) and thawed for 30 min, was transferred to sample port of Cobas Integra 400 plus machine using pipette in their order. Then, information about sample code and type of test was fed to the computer. Later on, Cobas Integra analyzers automatically begin to determine CK and LDH activity of units per liter (U/L) in serum. The level of CK and LDH activity before loading after unloading and at slaughter indicated the level of stress at each stages.

### Table 1. Experimental animals’ treatment combination and stage of blood collection

| Breeds with their respective ages | Before loading | After unloading | At slaughter |
|----------------------------------|----------------|----------------|-------------|
| Arsi 2-3                         | 11             | 5              | 5           |
| Arsi 3-6                         | 4              | 4              | 4           |
| Arsi 6-9                         | 2              | 2              | 2           |
| Boran 2-3                        |                |                |             |
| Boran 3-6                        |                |                |             |
| Boran 6-9                        |                |                |             |
| Harar 2-3                        |                |                |             |
| Harar 3-6                        |                |                |             |
| Harar 6-9                        |                |                |             |
| Total                            | 39             | 39             | 39          |

2.6. Determination of pH of meat

The carcass pH measurement was conducted using procedure used by Viljoen, DeKock, and Webb (2002). The pH and temperature were measured by using Portable HANNA pH meter (model number-HI99163) at M. longissimus dorsi (LD) between 12th and 13th rib. The initial pH was measured at 45 min post-slaughter before chilling by directly inserting the probe into carcasses, while carcasses were at hanging position. The ultimate pH was measured at 24 h after chilling at 0–4°C by removing the samples.

2.7. Determination of color of the meat samples

The color of meat was measured in triplicate 24 h post-slaughter by cutting 3 cm thick meat from M. LD between the 12th and 13th ribs (loin) from right side of the carcass. Measurements were
made after 30-min exposure to air (bloom time) on the different locations of the surface of the muscle using a Mini Scan EZ machine (model number - 4500 L) with a 20 mm diameter measurement area and illuminant D65-day light, 10° standard observer. The machine was calibrated before taking measurements using the black and white color standard samples, provided for this purpose. Three readings were taken on each sample by rotating the Color Guide 90° between measurements so as to obtain the average value for the color. Meat color was expressed using the CIELAB color space ($L^*$ = lightness, $a^*$ = redness, and $b^*$ = yellowness) according to the CIE system (Chulayo & Muchenje, 2013b; Commission International De I’Eclairage, 1976).

2.8. Determination of water-holding capacity of meat
The water-holding capacity of meat was determined 24 h postmortem in triplicate using the method suggested by Whiting and Jenkins (1981). Two Whatman number-1 filter papers were weighed (A) and 0.5 grams of meat sample (C) was placed between two filter papers, this in turn was placed between two glass sheets. Over it, a weight of 2.015 kg was placed, while the glass sheet weighed 0.8278 kg sheet, giving a total compression weight of 2.8428 kg load for 5 min. Then the weight was removed; the meat was separated from the filter papers and weighed (D). At the end the filter paper was dried and the weight was recorded (B). After that, the amount of protein attached to the filter paper and the actual weight of meat after pressure treatment was determined. Amount of protein attached to the filter paper was calculated as ($E = B - A$), actual weight of meat after pressure treatment ($F = E + D$) and % drip loss = ($C-F)/C*100$. WHC was calculated as the difference between 75% and % drip loss.

2.9. Determination of carcass weight and dressing percentage
After dressing carcass were weighed individually to obtain hot carcass weight for each animal and was chilled for 24 h at 0–4 °C. Subsequent to chilling, the weights were taken again to obtain cold carcass weight. The dressing percentage was calculated by dividing hot and cold carcass weight by slaughter weight and multiplying the obtained result by 100 (Chulayo 2015).

2.10. Determination of instrumental tenderness of meat
Meat samples collected from $M. \text{LD}$ muscle between 12th and 13th ribs within 45 min after slaughter and were packed into the plastic bag being sealed into vacuum, stored in the icebox and then transported to Oda Bultum University Meat Laboratory for determination tenderness. The samples were aged in a deep freeze for 14 days before instrumental analysis. Meat samples were thawed for 24 h at room temperature (24–25°C) for steaks preparation. The steaks were prepared and Instrumental tenderness was evaluated by Warner Bratzler Shear Force based on the procedure developed by American Meat Science Association (2015). Cooking skillet heated to a constant 204°C by adding a small amount of cooking oil. Steaks cut to 1 in (2.5 cm) in thickness perpendicular to the long axis of the LD. After external heavy connective tissue is removed, the initial weight of steak was taken. Then, initial steak temperature was recorded and the steak is placed in the pre-heated 204°C skillet. The steak was flipped when the internal temperature reaches 45°C and it removed from skillet when the temperature rose to 70°C. At the end, the peak cooked temperature and final weight of cooked steak was recorded.

2.11. Determination of body weight loss
The weight loss determined as follows:

\[
\text{Weight loss (\%)} = \frac{\text{weight before transportation} - \text{weight after transportation}}{\text{Weight before transportation}} \times 100
\]

2.12. Statistical analysis
The data were analyzed by Statistical Analysis Software, version 9.3 (SAS, 2011). General Linear Model procedure (PROC GLIMMIX; SAS, 2011) was used to analyze the effect of breed and age on creatine kinase, lactate dehydrogenase and beef quality parameters ($pH$, temperature, WHC, $L^*$, $a^*$ and $b^*$). Significant differences among least-squares means group were compared at $p < 0.05$. 
Pearson’s correlation was used to see the linear relationship between CK, LDH concentration and meat quality parameter (pH, WHC, L*, a* and b*).

The model used was as follows:

\[ y_{ij} = \mu + \alpha_i + \beta_j + \lambda_k + \epsilon_{ijk}, \]

where

- \( y_{ij} \) is the response variable (creatine kinase, lactate dehydrogenase, pH, temperature, WHC, L*, a*, b*, carcass weight);
- \( N \) is the constant mean common to all observations;
- \( \alpha_i \) is the breed effect (Harar, Boran, Arsi);
- \( \beta_j \) is the age effect (group 1, group 2 and group 3);
- \( \lambda_k \) is the stage of blood collection;
- \( \epsilon_{ijk} \) is the random error.

3. Results and discussion

3.1. Level of creatine kinase (CK) and lactate dehydrogenase (LDH) before loading, after unloading and at slaughter of study bulls

3.1.1. Level of CK and LDH before loading for the three breeds under the study

Level of CK and LDH of study bulls before loading for transportation is presented in Table 2. Arsi bulls had higher (\( p < 0.05 \)) CK and LDH (351.82 ± 89.35 U/L, 1496.73 ± 81.60 U/L) before loading for transportation than Boran bulls (186.64 ± 89.35 U/L, 1113.07 ± 78.93 U/L). The higher level of enzymes in Arsi bulls might be due to level of exposure to environmental stress such as market related stress or due to breed difference. Arsi bulls were produced under mixed crop-livestock production system were purchased from a market. Boran was purchased directly from ranch production system in which the bulls were not exposed to heat and noise stimuli usually anticipated in livestock market like bulls from Arsi breed. Animals exposed to livestock market are highly stressed due to exposure to sun, cold, noise, unfamiliar environment, social regrouping, dehydration and starvation (Adzitey & Huda, 2012). The mean of CK and LDH before loading for Boran and Arsi bulls in this study were higher than White Fulani (126.24 ± 0.26, 763.66 ± 1.13 U/L), Red Bororo (125.82 ± 0.26, 63.01 ± 1.13), Sokoto Gudali (125.77 ± 0.26, 763.59 ± 1.13 U/L) and Adamawa Gudali (126.03 ± 0.26, 763.64 ± 1.13 U/L) breed of Nigeria Kubkomawa, Okoli, Tizhe, and Nafarnda (2015). The differences between the present finding and the latter report might be due to difference in genetic and environmental condition under which animals were managed.

| Enzymes | Boran (n = 14) | Arsi (n = 11) | P-value |
|---------|---------------|---------------|---------|
| CK      | 186.64 ± 89.35<sup>a</sup> | 351.82 ± 89.35<sup>b</sup> | 0.04    |
| LDH     | 1113.07 ± 78.93<sup>ab</sup> | 1496.73 ± 81.60<sup>b</sup> | 0.0001  |

<sup>a,b</sup> Means within different letters in the same row are significantly different (\( p < 0.05 \))

CK, creatine kinase; LDH, lactate dehydrogenase
3.1.2. Level of CK and LDH after unloading and at slaughter for the three breeds

Level of CK and LDH after unloading and at slaughter is presented in Table 3. Transport had significantly affected the level of CK and LDH enzymes in this study. The mean CK and LDH significantly increased after transportation for Boran breed (186.64 ± 89.35 U/L to 562.07 ± 89.35 U/L for CK, 1113.07 ± 78.93 to 1389.93 ± 78.93 U/L for LDH). However, the level of the CK and LDH before and after loading didn’t show significant difference in case of Arsi bulls. The differences in the trend of enzymes before and after loading for the two breeds might be associated with genetics of the breeds and/or previous level of exposure to external stimuli. Boran bulls were purchased directly from the ranch, while Arsi bulls were purchased from market. Moreover, Boran bulls had traveled longer distance for more number of hours (517 km, 16 h), while Arsi bulls were travelled shorter distance for less hours (218 km, 5 h).

The level of stress during transport varies depending on factors such as duration of transport (Dela Fuente et al., 2010). Long distance increased the level of CK and LDH enzyme that were used to assess stress and physiological responses of animals during transportation (Chulayo & Muchenje, 2013a). The longer distance and duration of transportation caused greater muscle fatigue characterized by high CK, indicating that Boran cattle have experienced significant stress during transportation. Wickham et al. (2012) reported the high levels of CK and LDH enzyme in the blood plasma or serum which is an indicative for physical fatigue and muscle damage during handling and transport. The impact of stress due to transport also depends on prevailing conditions during the journey. During transport of Boran bulls from the ranch to abattoir, the bulls were exposed to fasting for longer duration (16 h). Moreover, as the truck had no cover from the above, bulls were faces heavy rain and cold weather, which might have contributed to the rise in CK & LDH level after transportation.

Increased levels of CK and LDH were not only affected by transportation but also deprivation of feed and lack of comfort during the journey (O’Neill, Webb, Frylinck, & Strydom, 2006). Some parts of the road Boran bulls were transported in this study were under construction. This has exposed the animal to further stress as the vehicle detoured to temporary road, which had much more bumps and vibrations and possibly could create discomfort by letting the animal spent more energy to maintain their balance on the truck. During transportation of the Boran bulls, temperatures were shifting from 18.90 °C to 32.5 °C. Davis and Mader (2001) reported that physiological stress that could occur in cattle as the temperature gets higher than 20°C. Boran cattle were deprived of feed for long periods during transportation, stressed more by truck vibration and environmental condition during travel. These might be the cause for the increase in the level of CK and LDH after unloading compared to the level before loading for Boran bulls in the study.

| Breeds | CKBL       | CKUL       | CKAS       | P-value |
|--------|------------|------------|------------|---------|
| Boran  | 186.64 ± 89.35a | 562.07 ± 89.35a | 286.29 ± 89.35b | 0.0144  |
| Arsi   | 351.82 ± 89.35 | 249.45 ± 89.35 | 339.73 ± 89.35 | 0.5497  |
| Harar  | -          | 529.79 ± 86.02 | 445.29 ± 86.02 | 0.4597  |
|        | LDHBL      | LDHUL      | LDHAS      |         |
| Boran  | 1113.07 ± 78.93b | 1389 ± 78.93 | 1159.14 ± 7 78.93b | 0.0389  |
| Arsi   | 1496.73 ± 81.60a | 1519.09 ± 81.60a | 1122.18 ± 81.60b | 0.0023  |
| Harar  | -          | 1303.57 ± 77.45 | 1125.07 ± 77.45 | 0.0669  |

Means within different letters in the same rows are significantly different (p < 0.05).

CKBL, creatine kinase before Loading for transportation; CKUL, creatine kinase after unloading; CKAS, creatine kinase at slaughter; LDHBL, lactate dehydrogenase before loading; LDHUL, lactate dehydrogenase after unloading; LDHAS, lactate dehydrogenase at slaughter.
Absence of significant difference or the numerical reduction in the level of CK and LDH for Arsi bulls in this study might be associated with the level of exposure of these bulls to environmental stress at livestock market compared to Boran bulls. Despite the possible importance of genetics of the breed to respond to environmental stress, the possibility of contribution of market environment to level of stressed enzymes for Arsi bulls should not be overruled. Possibly, the Arsi bulls were already stress to the maximum by market environment and this might have overshadowed the effect of stress during transport on the breed yielding none significant difference on the level of the two enzymes before loading and after unloading. The possible scenario of contribution of market stress to animal purchased from Ethiopian livestock market can be illustrated as follows. In most cases of Ethiopian livestock market, cattle transported longer durations by foot (trekking) from producer to the primary market and/or secondary market, usually without proper provision for adequate rest, water and feed. By the time the animals reached the markets they exhausted and their physical condition had greatly deteriorated (Hailemariam, Tekle wold, Getachew & Dawit, 2011), which might raise the stress level further. The same could happen for Arsi bulls in the study, which might be reflected on higher levels of stress enzymes after market instead of after transport. The travel of most cattle in Ethiopia from primary or secondary markets to terminal market for sale in the hand of several traders were also reported by Phytosanitary Standards and Livestock and Meat Marketing Program (2010). Cattle moved in the hands of several traders through market were subject to potential stressors such as sun, cold, noise, dust, novel environment, social regrouping, starvation and dehydration (Adzitey, 2011). Arsi bulls were transported under smooth road conditions, shorter distances and durations, which might not exposed the animal for further transport stress compared to Boran bulls under the study.

The levels of CK and LDH have significantly decreased at slaughter for Boran bulls compared to the level after unloading. This further illustrates the importance of stress due to transport for Boran bulls which, travelled longer distances and durations compared to the other bulls from Arsi and Harar cattle. It seemed that the former bulls (Boran) recovered from transport stress by the time they were slaughtered. Possibly, slaughtering procedure might have similar impact of stress on the three breed as they were slaughtered under similar condition. The recovery of animals from transport stress such as creatine kinase at the end of their life in the lairrage was reported by European food safety authority (2011). The level of the LDH at slaughter for Arsi bulls was significantly decreased compared to the level after unloading while the level of the CK did not. The differences in the genetics of the breeds, distance and duration travelled and level of previous exposure to environmental stimuli (experience) might explain the difference in stress enzymes (after unloading and at slaughter) of the three breeds under study.

Breed had shown significant difference ($p < 0.05$) in the level of creatine kinase and lactate dehydrogenase at slaughter with Boran bulls having lower value of CK over the other two breeds. Chulayo and Muchenje (2013a) reported significant difference in CK at slaughter among Beefmaster, Friesian-Holstein and Charolaise breeds. The current mean of CK for three breed was lower than Bonsmara (705.3 ± 80.5 U/L), Beef master (657.3 ± 73.8 U/L) and Brhaman (461.8 ± 80.6) breeds of South Africa. This might due to difference in breed and environment.

3.1.3. Trend in the level of CK before loading, after unloading and at slaughter for the three age groups

Trends in the level of CK (before loading, after unloading and at slaughter) for the three age groups by breeds are presented in Figure 1. In general, the level of CK (before, after unloading and at slaughter) had decreased as the age of the bulls increased. The figure further shows the importance of transport stress in all age categories compared to the level of stress before loading and at slaughter. The decreasing trend in the level of CK from young bulls to the old bulls might be associated with the lack of experience of the young to external environment compared to the older bulls, which made them respond by high level of stress enzymes. The relatively lower level of stress experienced by the older animals compared to the younger animals was similarly reported by Njisane and Muchenje (2016). Animals show different abilities to cope with stress depending on
their ages. The figure further illustrates the stress at slaughter compared next to transport stress. Stress at slaughter might be lower compared to stress due to transport in all age groups of Boran and Harar breeds and/or possibly, bulls had recovered from transport stress at time of slaughter. But in case of Arsi breeds of 2–3 and 3–6 age groups, the stress level after transportation/unloading were similar with stress level at slaughter even though stress raised at slaughter for 6–9 age groups. The similarity possibly suggests that these age groups were not recovered from transport stress by the time they were slaughtered and/or high level of stress at slaughter.

3.2. Weight loss due to transportation
Weight loss due to transportation for the bulls under the study is shown in Table 4. The bulls under the study had lost 2-8% of their body weight during transportation from their source to the abattoirs. Bulls with live weight between 100 and 300 kg lost more weight (8-9%) over bulls heavier above 300kg which was about 2-3%. Cattle in lower body weight category were younger and were more susceptible to transportation stress. Transport stress can be reflected through weight loss. Bulls in this study lost 5 to 8% of their body weight. Despite longer distance traveled and more duration by Boran bulls, the weight loss was relatively lower (5.18%) compared to Harar bulls which traveled shorter distance and shorter duration. Adzitey (2011) reported the reduction in gut fill and breakdown of muscle glycogen due to transport stress.

| Body weight category (kg) | Weight before transportation (kg) | Weight after transportation (kg) | Weight loss (%) | P-value |
|---------------------------|----------------------------------|---------------------------------|-----------------|---------|
| 105–203                   | 160.35 ± 6.98                    | 147.50 ± 6.98                   | 8.01 ± 0.91     | 0.2008  |
| 204–300                   | 232.00 ± 9.72                    | 211.25 ± 9.72                   | 8.94 ± 0.90     | 0.1818  |
| 301–398                   | 391.50 ± 12.09                   | 377.50 ± 6.98                   | 3.58 ± 0.90     | 0.9663  |
| 399–496                   | 455.00 ± 6.37                    | 442.50 ± 6.3                    | 2.75 ± 0.58     | 0.2999  |

P-value

Breed
| Breed | Weight before transportation (kg) | Weight after transportation (kg) | Weight loss (%) | P-value |
|-------|----------------------------------|---------------------------------|-----------------|---------|
| Boran | 233.14 ± 34.67                   | 221.07 ± 34.42                  | 5.18 ± 0.85     | 0.8068  |
| Harar | 183.14 ± 10.77                   | 167.14 ± 10.08                  | 8.74 ± 0.58     | 0.2881  |

Means in the same row and column with same superscript are not significantly different (p > 0.05)
3.3. Effect of breeds and ages on meat and carcass quality

3.3.1. Effect of breeds on meat and carcass quality

Effects of breed on beef quality attribute are shown in Table 5. Instrumental tenderness of beef from Boran and Harar bulls were relatively tender, while beef from Arsi bulls was intermediate based on classification by Calkins and Sullivan (2006). The author classified beef tenderness as tender (<37.31 N (8.46 lb), intermediate (37.49–48.82 N) and tough eat (>48.82 N (10.3 lb). In this study beef from Boran and Harar bulls were relatively tender, while beef from Arsi bulls was considered intermediate tender based on categorization of tenderness of beef by Calkins and Sullivan (2006) who classified beef as tender for instrumental value <37.63 N/8.46 lb, intermediate tender (37.63–48.82 N) and tough meat for value >48.82 N/10.3 lb. The tenderness value of beef from Boran and Harar bulls in the present finding was lower than shear force values of 37.71 N and 42.66 N, reported for Brazilian cattle (Juliana et al., 2013) and for Bonsmara, Brahman, Drakensberger, Nguni and Tulli cattle breed in South Africa, which were in the range of between 29.6 and 32.5 N (Strydom, Frylinck, & Smith, 2011).

The average ultimate pH for the three breeds in the current study fall under normal meat. No significant differences in pH were observed between the three breeds studied. The absence of significant differences among breeds in ultimate pH and initial pH of carcass was similarly reported by Arik and Karaka (2017). However, breeds effect on ultimate pH was reported by Muchenje et al. (2014).

The L* value in this study (31.52–33.77) was similar to the report for Angus × Gelbvieh heifers (32.44–34.49), which were not supplemented vitamin E (Harris, Huff-Lonergan, Lonergan, Jones & Rankins, 2001). However, it was less than the value reported by other researchers (Lee, Panjono, Sun, Tae & Yeon, 2008). The difference in the reflectance value of this study from other research findings might be due to differences in genetics of the breeds of cattle, environment in they were finished and post-slaughter carcass management. Similar to the present finding, Plessis and Hoffman (2007) and Strydom et al. (2011) did not find significant differences on color values (L*, a* and b*) between breeds. Nevertheless, Mpakama, Chulayo, and Muchenje (2014) reported significant difference on color parameter between breeds. The color of meat was more associated with the vitamin within the

### Table 5. Effect of breed on beef quality attribute for Boran, Harar and Arsi breed (LSM ± SE)

| Parameters          | Breed    | P-value |
|---------------------|----------|---------|
|                     | Boran (n = 14) | Harar (n = 14) | Arsi (n = 11) |
| WBSF (N, mean ±SE)  | 20.56 ± 1.33   | 19.07 ± 1.33   | 41.83 ± 1.51   | 0.015   |
| pH45 min            | 6.30 ± 0.09   | 6.35 ± 0.09   | 6.50 ± 0.11   | 0.8933  |
| pH24 h              | 5.70 ± 0.11   | 5.55 ± 0.11   | 5.75 ± 0.13   | 0.5450  |
| Temp45 min          | 24.84 ± 0.29  | 24.75 ± 0.29  | 25.09 ± 0.32  | 0.7278  |
| Temp24 h            | 18.73 ± 0.17   | 18.92 ± 0.17   | 17.52 ± 0.19   | <.0001  |
| Lightness (L*)      | 33.77 ± 0.89  | 32.94 ± 0.89  | 31.52 ± 1.01  | 0.1963  |
| Redness (a*)        | 12.31 ± 0.41  | 12.64 ± 0.41  | 13.27 ± 0.16  | 0.3745  |
| yellowness (b*)     | 11.31 ± 0.40  | 10.95 ± 0.40  | 11.04 ± 0.45  | 0.7386  |
| WHC (%)             | 70.29 ± 0.85  | 70.86 ± 0.85  | 71.51 ± 0.96  | 0.7198  |
| SW (kg)             | 255.87 ± 5.05   | 177.62 ± 5.04   | 164.84 ± 5.83   | 0.0010  |
| HCW (kg)            | 119.38 ± 2.49   | 83.38 ± 2.49   | 76.70 ± 2.88   | 0.0050  |
| CCW (kg)            | 115.53 ± 2.42   | 81.65 ± 2.42   | 74.64 ± 2.79   | 0.0073  |
| Dressing %          | 45.15 ± 1.23   | 45.96 ± 1.36   | 45.28 ± 1.53   | 0.4119  |

* Means in the same row with different superscript are significantly different (p < 0.05).

n, total number of animals; U/L, unit per liter; CKas, creatine kinase at slaughter; LDHAs, lactate dehydrogenase at slaughter; pH45 min, pH measured at 45 min (initial pH); pH24 h, pH measured at 24 h (ultimate pH); L*, lightness; a*, redness; b*, yellowness; WHC, water-holding capacity; SW, slaughter weight; HCW, hot carcass weight; CCW, cold carcass weight.

3.3. Effect of breeds and ages on meat and carcass quality

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The L* value in this study (31.52–33.77) was similar to the report for Angus × Gelbvieh heifers (32.44–34.49), which were not supplemented vitamin E (Harris, Huff-Lonergan, Lonergan, Jones & Rankins, 2001). However, it was less than the value reported by other researchers (Lee, Panjono, Sun, Tae & Yeon, 2008). The difference in the reflectance value of this study from other research findings might be due to differences in genetics of the breeds of cattle, environment in they were finished and post-slaughter carcass management. Similar to the present finding, Plessis and Hoffman (2007) and Strydom et al. (2011) did not find significant differences on color values (L*, a* and b*) between breeds. Nevertheless, Mpakama, Chulayo, and Muchenje (2014) reported significant difference on color parameter between breeds. The color of meat was more associated with the vitamin within the
feed, which could be available in green feed or supplemented during finishing. The inclusion of vitamin E in diets for beef cattle has been employed quite successfully in the past and is viable.

Boran breed had the highest slaughter and carcass weight compared to Harar and Arsi breeds. However, no significant differences were observed in dressing percentage of the breeds under study. The value of dressing percentage of Boran bulls in current study was similarly with that reported by Mohammad and Hailu (2016), which was 47%. However, it was lower than the dressing percentage reported by Mummed (2015), which was 54.78%. The slaughter weight, hot and cold carcass weight in this study for Harar and Arsi breeds was comparable with values reported by the same author for Arado, Barka and Raya cattle breed.

3.3.2. Effect of age on meat and carcass quality
The effect of age on meat and carcass quality is shown in Table 6. Meat from all age groups were found tender based on categorization of tenderness of beef by Calkins and Sullivan (2006). However, significant differences in the tenderness values were observed between the three age groups with the age less than three years, yielding lowest value over age group between 6–9 years of ages. The relatively higher value of tenderness from older bulls might be associated with to the presence of more cross-link of collagen in older animals than younger animals. Similar to the current finding Juliana et al. (2013) reported lower value of instrumental tenderness for younger cattle.

Temperature, pH and color meat were not significantly affected by age groups of bulls in this study. Similar to the present finding Marencic, Ivankovic, Lidija, Popovic, & Cvrtla (2018) reported absence of effects of ages on ultimate pH and color parameter while Arik and Karaca (2017) reported in contrary.

The result of dressing percentage in current study for three age groups was similar with that reported by Bedhane and Dadi (2016). Bulls in the age category of 6–9 years had higher slaughter weight, hot and cold carcass weight, water-holding capacity, and dressing percentage as compared to younger age group.

| Parameters | 2–3 (n = 13) | 3–6 (n = 14) | 6–9 (n = 12) | P-value |
|------------|-------------|-------------|-------------|---------|
| WBSF (N)   | 20.41 ± 1.29<sup>c</sup> | 26.56 ± 1.39<sup>a</sup> | 34.49 ± 1.51<sup>a</sup> | 0.032   |
| LDHas (U/L)| 1141.27 ± 84.71 | 1187.69 ± 82.40 | 1069.45 ± 88.82 | 0.3789  |
| pH<sub>45</sub> min | 6.30 ± 0.08 | 6.39 ± 0.09 | 6.31 ± 0.10 | 0.9880  |
| pH<sub>24</sub> h | 5.59 ± 0.11 | 5.76 ± 0.11 | 5.75 ± 0.12 | 0.6413  |
| Temp<sub>45</sub> min | 24.81 ± 0.29 | 24.95 ± 0.30 | 24.92 ± 0.34 | 0.9209  |
| Temp<sub>24</sub> h | 18.53 ± 0.16 | 18.30 ± 0.17 | 18.23 ± 0.19 | 0.6550  |
| Lightness (L*) | 34.26 ± 0.92 | 32.16 ± 0.89 | 32.09 ± 0.96 | 0.1378  |
| Redness (a*)  | 12.52 ± 0.38 | 12.33 ± 0.42 | 13.53 ± 0.45 | 0.6809  |
| Yellowness (b*) | 11.52 ± 0.37 | 10.68 ± 0.38 | 11.08 ± 0.32 | 0.2956  |
| WHC (%)      | 70.81 ± 0.84 | 70.07 ± 0.84 | 71.76 ± 0.91 | 0.4675  |
| SW (kg)      | 135.33 ± 4.84<sup>c</sup> | 181.91 ± 5.36<sup>a</sup> | 281.08 ± 5.71<sup>a</sup> | <.0001  |
| HCW (kg)     | 63.00 ± 2.40<sup>c</sup> | 82.94 ± 2.65<sup>a</sup> | 133.51 ± 2.82<sup>a</sup> | <.0001  |
| CCW (kg)     | 60.87 ± 2.32<sup>c</sup> | 80.71 ± 2.57<sup>a</sup> | 130.25 ± 2.74<sup>a</sup> | <.0001  |
| Dressing %   | 44.98 ± 1.35 | 44.37 ± 1.30 | 46.34 ± 1.40 | 0.0809  |

<sup>a,b,c</sup> Means in the same row with different superscript are significantly different (p < 0.05)

n, total number of animals; U/L, units per liter; CKAs, creatine kinase at slaughter; LDHas, lactate dehydrogenase at slaughter; pH<sub>45</sub> min, pH measured at 45 min (initial pH); pH<sub>24</sub> h, pH measured at 24 h (ultimate pH); L*, lightness; a*, redness; b*, yellowness; WHC, water-holding capacity; SW, slaughter weight; HCW, hot carcass weight; CCW, cold carcass weight.
Table 7. Pearson’s correlations of CK, LDH and meat quality parameters (pH, WHC, L*, a* and b*)

|                  | CKBL  | CKUL  | CKAS  | LDHBL | LDHUL | LDHAS | pH24  | L*    | a*    | b*    |
|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| pH24             | 0.40* | -0.02 | 0.87**| -0.23 | 0.94**| 0.89**|       |       |       |       |
| Lightness (L*)   | -0.38*| 0.05  | -0.82**| 0.09  | -0.90**| -0.89**| -0.92**|       |       |       |
| Redness (a*)     | -0.64**| -0.07 | -0.72**| -0.22 | -0.72**| -0.69**| -0.80**| -0.70**|       |       |
| Yellowness (b*)  | 0.29 ns| -0.10 | -0.74***| -0.11 | -0.80***| -0.76***| -0.81***| -0.77***| 0.62**|       |
| WHC              | 0.64***| -0.07 | 0.72***| -0.22 | 0.73***| 0.70***| 0.81***| -0.70***| -0.80***| -0.72***|

Significantly correlated at *P < 0.05, **P < 0.01, and ***P < 0.001.

ns, not significant; CKBL, creatine kinase before loading; CKUL, creatine kinase after unloading; CKAS, creatine kinase at slaughter; LDHBL, lactate dehydrogenase before loading; LDHUL, lactate dehydrogenase after unloading; LDHAS, lactate dehydrogenase at slaughter; pH24, pH at 24 h (ultimate pH); L*, lightness; a*, redness; b*, yellowness; WHC, water-holding capacity.
cold carcass weight. The variation in weights among age categories could be due to differences in muscle size and fat development among the age categories (Boito & Kuss, 2018; Plessis & Hoffman, 2007).

3.4. Correlations of CK, LDH and meat quality parameter (pH, WHC, L*, a* and b*)
Correlation among serum creatine kinase, lactate dehydrogenase, and meat quality parameters (pH, WHC, C) is presented in Table 7. CKBL and CKAS were negatively correlated with L* and a* value but positively correlated with ultimate pH and water-holding capacity. No significant correlation of CKBT with b*value. CKAS, LDHUL and LDHAS negatively correlated with color parameter (L*, a*and b*) and positively correlated with ultimate pH, water-holding capacity. Creatine kinase and lactate dehydrogenase are released into the blood when there is vigorous exercise and stressful condition (Wickham et al., 2012). The raise in such enzymes in blood result in depletion of muscle glycogen stores that contributes to high ultimate pH and relatively darker meat (Chulayo, Tada, & Muchenje, 2012).

CKUL and LDHBL didn’t showed a significant correlation with any meat quality parameters (pH24, WHC, a* and b* value). Water-holding capacity and color parameters (L*, a* and b*) were negatively correlated in the current study. Ultimate pH negatively correlated with L*, a* and b* value but positively correlated with WHC. The higher the ultimate pH, the more deterioration of color parameters and the less the water lost.

4. Conclusion and recommendation
From the study it was concluded that bulls from Arsi, Boran and Harar cattle breeds were stressed differently before loading, after unloading and at slaughter. The degree of stress expressed in stress enzymes and weight loss was higher after transport and with younger bulls. The qualities of beef based on instrumental value were different among breeds and age categories, while based on color, pH and water-holding capacity were similar. Further studies are recommended to see the breeds effect on quality of beef. The contribution of genetics or breeds and environments to the level of stress needs further investigation.

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References
Adzitey, F., & Huda, N. (2012). Effects of post-slaughter carcass handling on meat quality. Pakistan Veterinary Journal, 32(2), 161–164.
Adzitey, F., & Nurul, H. (2011). Pale soft exudative (PSE) and dark firm dry (DFD) meats: Causes and measures to reduce these incidences—a mini review. International Food Research Journal, 18, 11–20.
Agricultural Growth Program-Livestock Market Development. (2013). End market analysis for meat/live animals, leather and products, dairy products value chains: expanding livestock markets for small-holder producers. Addis Ababa, Ethiopia: USAID.
Alam, M., Hasanuzzaman, M., & Hassan, M. M. (2018). Assessment of transport stress on cattle travelling a...
long distance (~648 km), from Jessore (Indian border) to Chittagong, Bangladesh. Veterinary Record. doi:10.1136/vetrecro-2017-000248

(American Meat Science Association). (2015). Research guidelines for cookery, sensory evaluation, and instrumental tenderness, 2nd ed. Champaign, IL. Retrieved from https://tinyurl.com/5xnrxybc

Arik, E., & Karacao, S. (2017). The effect of some pre-on meat quality of bulls slaughtered in a commercial abattoir in Turkey. Indian Journal of Animal Research, 51(3), 557-563.

Birhanu, T., Birhanu, M., & Dadi, H. (2016). Growth and slaughter characteristics of Ethiopian boran breed bull. International Journal of Livestock Research, 6(3), 41–50. doi:10.5455/ijlr

Bolto, B., & Kuss, F. (2018). Influence of subcutaneous fat thickness on the carcass characteristics and meat quality of beef cattle. Journal of Animal Production, 48(1), 1–7.

Collins, C. R., & Sullivan, G. (2006) Ranking of beef muscles for tenderness. Retrieved from https://www.agnireseau.net

Chulayo, A., & Muchenje, V. (2013a). Activities of creatine kinase and lactate dehydrogenase as welfare indicators in slaughter cattle. 59th International Congress of Meat Science and Technology, 18-23rd August, 2013, Izmir, Turkey.

Chulayo, A. Y. (2015). The effects of distance, lairage duration and animal-related factors on pre-slaughter stress indicators, carcass characteristics, nanostructure and technological properties of beef (M.Sc. Thesis), University of Fort Hare, South Africa.

Chulayo, A. Y., & Muchenje, V. (2013b). The effects of pre-slaughter stress and season on the activity of plasma creatine kinase and mutton quality from different sheep breeds slaughtered at a smallholder abattoir. Asian-Australasian Journal of Animal Science, 26(12), 1762–1772. doi:10.5713/ajas.2013.13141

Chulayo, A. Y., Tada, O., & Muchenje, V. (2012). Research on pre-slaughter stress and meat quality: A review of challenges faced under practical conditions. Applied Animal Husbandry & Rural Development, 5, 1–6.

Commission International De I’ Eclairage. (1976). Colorimetry (2nd ed.). Vienna, Switzerland: CIE.

CSA (Central Statistical Authority) (2016-2017). Agricultural Sample Survey. Report on Livestock and Livestock Characteristics (Private Peasant Holdings). (2009 E.C.) Volume II.P:R.April, 2017, Statistical Bulletin 585.Addis Ababa, Ethiopia.

Davis, M. S., & Mader, T. L. (2001). Effects of water application to slaughter factors feedlot mounds during the summer. In: Proceedings of the ASAIE Sixth International Livestock Environment Symposium (pp 165–173). Louisville, KY, USA.

De la Fuente, J., Sanchez, M., Perez, C., Louzarucia, S., Viera, C., Gonzalez de Chavarri, E., & Diaz, M.T. (2010). Physiological response and carcass and meat quality of suckling lambs in relation to transport time and stocking density during transport by road. Animal, 4 (2), 250–258.

EBI (Ethiopian Biodiversity Institute). 2016. Ethiopian national strategy and plan of action for conservation and utilization of animal genetic resources. Addis Ababa, Ethiopia: Ethiopian Biodiversity Institute (EBI). Retrieved from www.ebi.gov.et

European food safety authority. (2011). Scientific opinion concerning the welfare of animals during transport: Panel on animal health and welfare. Journal of European Food Safety Authority, 9(1), 125–135.

Food and Agricultural Organization. (2015). Potential driver of selection in african cattle. Trypanosomiasisby Smieto, Anamarijaet al2015. Frontiers in Genetics 6 (2015): 137. PMC. Web. 22 Aug. 2018. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4404968/

Gupta, S., Earley, B., & Crave, M. A. (2007). Effect of 12 hour road transportation on physiological, immuno logical and hematological parameters in bulbs housed at different space allowances. The Veterinary Journal, 173, 605–616. doi:10.1016/j.tvjl.2006.03.002

Juliana Giusti, J., Castan, E., Dal Pai, M., Antigoni, M. D. B., Rodrigues, S., & De Oiveira, H. N. (2013). Expression of genes related to quality of Longissimus dorsi muscle meat in Nellore (Bos indicus) and Canchim (5/8 Bos taurus × 3/8 Bos indicus) cattle. Meat Science, 94, 247–252. doi:10.1016/j.meatsci.2013.02.006

Kubakomoa, H. I., Okoli, C. I., Tizhe, A. M., & Nafaranda, D. W. (2015). Effect of environment, sex and feed management and season on some enzyme profile of pastoral Zebu cattle in Nigeria. Journal of Animal Science and Technology, 1(2), 28–42.

Lee, U. K., Anjongon Sun, M. K., Toe, S. K., & Yeon, S. P. (2008). The effects of dietary sulfur and Vitamin E supplementation on the quality of beef from the Longissimus muscle of hanwoo bulls. Asian-australasian Journal of Animal Science, 21(7), 1059–1066.

Ljungberg, D., Gebresenbet, G., & Aradom, S. (2007). Logistics chain of animal transport and abattoir Operations. Biosystems Engineering, 96(2), 267–277. doi:10.1016/j.biosystemseng.2006.11.007

Marencic, D., Ivanovic, A., Kozaciniskic, L., Popovic, M., & Cvrlja, Z. (2018). The effect of sex and age on slaughter on the physicochemical properties of baby-beef meat. Veterinari Arhiv, 88(1), 101–110. doi:10.24099/vet.arhiv.160720

Mpkamor, T., Chulayo, A. Y., & Muchenje, V. (2014). Bruising in slaughter cattle and its relationship with creatine kinase levels and beef quality as affected by animal related factors. Asian-Australasian Journal of Animal Sciences, 27(5), 717–725. doi:10.5713/ajas.2013.13483

Muchenje, V., & Chulayo, A. (2017). Activities of some stress enzymes as indicators of slaughter cattle welfare and their relationship with physico-chemical characteristics of beef. Bloemfontein: Cambridge University Press.

Mummed, Y. Y. (2015). Beef carcass quality, yield and cause of condemnation in Ethiopia (PhD Dissertation), Pretoria University, South Africa, Pretoria.

Mummed, Y. Y., & Webb, E. C. (2016). Ethiopian Beef Carcass Characteristics. African Journal of Agricultural Research, 9(51), 3766–3775.

Njisane, Y. Z., & Muchenje, V. (2016). Farm to abattoir conditions, animal factors and their subsequent effects on cattle behavioural responses and beef quality — A review. Asian-Australasian Journal of Animal Sciences, 79, 755–764. doi:10.5713/ajas.2016.0037

NMSA (National Meteorology Service Agency). (2003). Addis Ababa, Ethiopia.

O'Neill, H. A., Webb, E. C., Fynlinc, L., & Strydom, P. E. (2006). The stress responsiveness of three different beef breed types and the effect on ultimate pH and meat colour. In Proceedings 52nd international Congress of Meat Science and Technology (pp.13–18) Dublin, Ireland.

Plessis, I. D., & Hoffman, L. C. (2007). Effect of slaughter age and breed on the carcass traits and meat quality of beef steers finished on natural pastures in the arid subtopics of South Africa. South African Journal of Animal Science, 37(3), 317–325. doi:10.4314/sajas.v37i3.4084

S E, H., Huff-Lonergan, E., S M, L., W R, J., & Rankins, D. (2001). Antioxidant status affects color stability and tenderness...
of calcium chloride-injected beef. Journal Science of Animal Science, 79, 666–677. doi:10.2527/2001.793666
Sanitary & Phyto-Sanitary Standards and Livestock & Meat Marketing program. (2010). Focus on Ethiopia’s meat and live animal export. Trade Bulletin Issue, 1,1–4.
SAS. (2011). In SAS/STAT Guide to Personal Computers, Version 9.3. Statistical Analysis System Institute Inc. Cary, NC.
Strydom, P. E., Frylinck, L., & Smith, M. F. (2011). Variation in meat quality characteristics 554 between Sanga (Bos taurus africanus) and Sanga-derived cattle breeds and between 555 Sanga and Brahman (Bos indicus). Animal, 5(3), 483–491.
Torell, R., Bruce, B., & Kvasnicka. (2003). Method of determine Age of cattle. Cattle producer Library-CL712. Reno: University of Nevada.
Viljoen, H. F., DeKock, H. L., & Webb, E. C. (2002). Consumer acceptability of dark, firm and dry and normal pH beef steaks. Meat Science, 61(12), 181–185.
Whiting, R. C., & Jenkins, R. K. (1981). Determination of Water Holding Capacity of Meat. Journal of Food Science, 46, 1693–1696.
Wickham, S. L., Collins, T., Barnes, A. L., Miller, D. W., Beatty, D. T., Stockman, C., … Fleming, P. A. (2012). Qualitative behavioral assessment of transport-naive and transport-habituated sheep. Journal of Animal Science, 90, 4523–4535.
Wold, H. T., Legesse, G., & Alemu, D. (2011). Market structure and function for live animal and meat exports in some selected areas of Ethiopia. Research Report 79. Addis Ababa, Ethiopia.