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Effects of Pre-Slaughter Animal Handling on Physico-Chemical and Microbiological Quality of Beef in Selected Municipal Abattoirs, Oromia Regional State, Ethiopia

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Abstract

Cattle selected for slaughter at Batu, Meki and Shashemene Municipal Abattoirs were assessed for the effect of pre-slaughter handling on physico-chemical and microbiological meat quality. A total of 300 cattle were selected randomly. Animal's behavior, handling methods, breed, and study abattoirs were considered. Carcasses were examined for physical damage (bruising, and dark, firm and dry (DFD)) while meat samples were tested for chemical properties (pH and cooking loss) and tested for microbiologic quality (load and *Salmonella* isolation). Of the total cattle, 113 (37.7%) were handled under poor welfare condition with bruises in 38% of carcass at significantly higher (91.2%) poor handled than in good handled cattle (8.8%), and in exotic breeds (62.3%) than local one (37.7%) ($P < 0.05$). Significantly high (100%) DFD meat in cattle under poor welfare than in those under good welfare 2 (1.1%) were observed. There was significant and negative correlation ($r = -0.952$; $p = 0.000$) between mean pH_{24} (6.18 ± 0.14) and mean percent cooking loss (22.51 ± 3.25) in meat from cattle subjected to poor handling condition. The overall mean log CFU/g of TPC, TCC and *S. aureus* count of meat samples were 3.21 ± 1.97, 2.14 ± 1.40 and 1.81 ± 1.11, respectively. TPC were significantly higher in meat from poor handled conditions and from exotic breeds than the counter groups while TCC and *S. aureus* count were similar in all circumstances. Of the 113 DFD meat samples from poorly handled cattle, 31% had TPCs of greater than the minimum acceptable level whereas only 5.9% meat samples from humanely handled cattle had TPCs greater than the minimum acceptable level. *Salmonella* was 14.3% in total consisting significantly higher (27.4%) in meat from poorly handled animal than the 6.4% in meat from humanely handled once ($p = 0.000$). In conclusion, meat from poorly handled cattle had DFD tendency and poor water holding capacity with high spoilage and pathogenic microbial contamination rate. It is therefore, suggested that awareness creation for stakeholders with application of regulations and legislation will have paramount to improve animal welfare and meat quality in Ethiopia.

Keywords: *Abattoirs; Animal Welfare; Cattle; Meat Quality; Microbiological Load*

Introduction

Beef animal producers take several days and efforts to raise an animal to desirable age, weight and quality. But the condition may change appreciably within few minutes to days prior to slaughter with adversely reduce weight, affect the meat quality and subsequently reduce profit [1] due to stress from poor handling conditions before slaughtering [2]. The resulted in bruises, injuries, starvation and tiredness from water and food deprivation, and loading and unloading stress [2]. Besides stress, genotype, transportation, lairage time, season of the year, environmental conditions and many other factors will affect meat quality [2,3]. The welfare of an animal can be said to have been compromised if the animal cannot cope with its environment or copes with difficulty [1,3,4]. Their response to these conditions

will have effect on their carcass and meat quality [5,6]. It implies that animals will take either longer time to produce meat, produce less meat, total meat loss or the carcass can be condemned during meat inspection [5].

In developing countries, the transports of animals are mainly by foot, or by ordinary vehicles not designed for animal transport [7]. Almost all livestock in Ethiopia are transported by people on foot [8], in rare cases during longer distances by un-designed vehicles, but usually not preferred since trekking is cheaper. Bulitta., *et al.* [9] reported 16% animal death with 7.1% due to car accidents and the rest from lack of water and food, bad condition and/or injuries during trekking from Gudar market to Addis Ababa. As a result of abuse handling of animal the reduction in quality of meat result in loss of huge amount of income e.g. losses due to PSE meat 5 dollars per carcass [10], 4.5 million dollars per year [11] and 40% of unmarketable product [12]. Meat from abusely handled animal will have insufficient level of glycogen (decreased by 70%) causes insufficient level of lactic acid, increase growth of putrid or putrescent bacteria such meat loss tenderness and resulted in the formation dry, firm and dark meat (DFD) [5,6].

According to the World Bank [13] report, Ethiopian meat production and marketing has been plagued by lack of quality and sanitation, prevalence of disease and unqualified meat production process. Although methods of animal transportation [8] and side effects of such transportation on life animal [9] were assessed in Ethiopia, effects of pre-slaughter animal handling on physico-chemical and microbiological quality of meat were not yet assessed in Ethiopia. This is, therefore, to investigate the effects of pre-slaughter animal handling on physico-chemical and microbiological quality beef meat in Batu, Meki and Shashemene towns Municipal Abattoirs of Oromia Regional State, Ethiopia.

Materials and Methods

Description of the study area

The study was carried out in three selected municipal abattoirs in central Rift Valley of southern Oromia Regional State towns namely, Meki (130 km), Batu (116 km) and Shashemene (250 Km) from Addis Ababa City, Ethiopia (Figure 1).

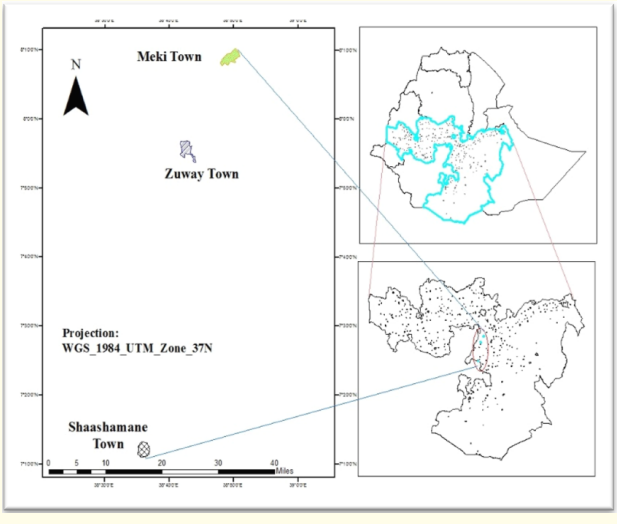

Figure 1: *Map of the study area.*

Study design

Cross sectional study comprises of ethogram and laboratory analyses were employed to assess pre-slaughter handling condition and effects on the meat quality. The meat was inspected for the physical, chemical and microbiological quality and from both exotic and local cattle breeds slaughtered at the three abattoirs.

Animal Sampling technique and assessing methods of handling

Simple random sampling was employed to select a total of 300 male cattle at abattoirs. Combination of both behavioral and physiological measures (Table 1) of the animal welfare [14-16] were used. Considering that, the wide variability of same species, breed and rearing conditions [17], each animal and its respective meat product were assessed independently.

Indicators	Example	Score methods
Clinical/pathological	Disease (fever, immobility, dysentery)	Present/Absent
	Injuries (lesions, fractures)	Present/Absent
Ethological	Abnormal behavior (fear, aggression, flight)	Expressed/Not
Physiological	Changed heart rate (tachycardia)	Present/Absent
	Changed body temperature and	Present/Absent
	Abnormal posture	Present/Absent

Table 1: *Indicators for animal welfare used to assess pre-slaughter handling condition.*

Source: Hartung., et al [14].

Meat examination for physical profile and sampling for laboratory analysis

Physical examination of meat: The presence of bruises was assessed according to the Finnish Meat Research Institute's carcass evaluation system [10]. Evaluation categories used in this system is: "none", denoting a clean and non-bruised surface and "bruised" meaning the bruise is reddish, deep and bleeding from damage can be observed on the surface. Moreover, the meat was examined for the presences of DFD and categorized into normal or DFD meat.

Meat sampling for laboratory analysis: After slaughter of respective study cattle, meat samples were aseptically collected immediately after ante mortem quality inspection using sterile gloves. From a carcass, six classical excision method from the 6^{th} to 12^{th} rib section of the *M. longissimus dorsi* (LD) muscle, *M. infraspinatus* (IS), *Triceps brachii* (TB), *Caput longum,* from the flank and the brisket portions of the carcass [18,19]. A total of 120 gram (20 gram from each portion) of meat samples were collected from each of the selected carcass and were kept into sterile polyethylene bags. Parallelly, pH at 0hr of post slaughters was measured. The samples were transferred immediately to Batu Fishery and Other Aquatic Life Research Center Laboratory using icebox at 4°C. Portion of meat samples were kept in the refrigerated at +4°C until for determination of cooking loss, microbial load and *Salmonella* isolation while the remaining kept at ambient temperature (22 - 27°C of Batu town) for pH_{24} measurement at specific times intervals.

Laboratory examination of meat samples

Measurement of cattle meat pH: From 120 gram of meat sample, about 60g [20] of meat was taken for determination of pH value. Ten gram (10g) of the sample was taken and homogenized in 90 ml of distilled water (1:10 ratio) [21]. The spear tip electrode of a pH meter was dipped into the mixture to read the pH. For each sample, pH was determined at 0hr (immediately after slaughter in the abattoir), 6hr, 18hr and 24hr time intervals after slaughter.

Measurement of cooking loss: Thirty gram (30g) of meat samples were weighed and put back into the self-sealing air tight bags and were cooked for 35 minutes in a hot water bath at 75°C. After cooking, the samples were cooled to room temperature in a bucket containing ice. Each of the samples were reweighed after cooling to room temperature and cooking loss was calculated as the weight lost during cooking divided by fresh sample weight. The result was expressed in percentage [22].

Microbiological analysis: Again 30g of meat samples were used to analyze microbial load of meat. The methods described by the Nordic Committee on Food Analysis [23] were adopted to analyze each of the parameters considered. Five grams of beef sample and 45 ml of normal sterile saline water were homogenized in blender (Stomacher 400 UK) for 1 - 3 minutes. Peptone water (Oxoid, UK) was used for serial dilutions.

For dilution factor, 1 mL of homogenized meat sample was serially diluted in 9 ml of peptone water (ratio of 1:10) dilutions. As a final dilution, 0.1 mL of diluent was gently spread over the agar plate petri dish and left to solidify for about 30 minutes for total plate count (TPC) on standard plate count agar (Oxoid UK), total coliform count (TCC) on violet red bile agar (HiMedia, India) and total *Staphylococcus aureus* count on Baird-Parker Agar plates [24]. Duplicate of paired dilution were used. The plates were then incubated at 37°C for 48hr, 37°C for 24hr, and at 37 ± 1°C for 48 hrs in cases of TPC, TCC and Total *S. aureus* count, respectively. The dilution yielding between 30 and 300 colonies were counted for characteristic colony of the bacteria. The actual number in both plates of a dilution was counted as per the formula given by APHA [25] and was expressed using common logarithm as CFU/g of meat.

With regards to *Salmonella* isolation, Grimont and Weill [26] protocols were used. Accordingly, 25 gram of meat with 225 ml buffered peptone water was blended (Stomacher 400 UK) in sterile polyethylene plastic bag and was incubated at 37°C) for 20 hours. From pre-enrichment broth, 1 ml was the transferred to 10 ml Tetrathionate broth and 0.1 ml (100 µL) was transfer to 10ml Rappaport Vassiliadis soy peptone (RVS) broth and incubated at 37°C and 42°C overnight for 20 hrs respectively. On day three, 10 µl from each tube was taken and spread on Xylose Lysine Deoxycholate (XLD) and on Brilliant Green Agar (BGA) agar in parallel and incubated at 37°C for 24 hours. Characteristic *Salmonella* colonies from both agars were cultured on nutrient agar for biochemical identification according to Grimont and Weill [26].

Data management and analysis

Data collected during ethological observations were classified, filtered, coded and entered and summarized into Microsoft Office Excel 2013. SPSS statistical software (SPSS BIM 20) was used to calculate percentage and run regression. Correlation between percent cooking loss and ultimate pH was calculated and determined using pearson correlation coefficient (r) with the significant level set to 5% (p-value < 0.05). Microbial load were calculated and described using mean $\log_{10}$CFU/g and compared by student t-test. Microbiological limits (TPC ≤ 5 $\log_{10}$CFU/g and TCC ≤ 2 $\log_{10}$CFU/g) for acceptable level of bacterial contamination in meat were used [27] where Chi square (χ^2) test was used to assesses significance at $P < 0.05$.

Result

Physical profiles of meat

Of the 300 cattle examined for ethogram, 63.2% were handled under poor welfare conditions. Total of 114 (38%) carcasses were bruised on different body parts. Significantly higher brusing were observed in carcases from poor handled animal and from exotic breeds ($P < 0.05$). Using Kendall's tau-b correlation coefficient (τ) carcass bruise were correlated with meat quality and found significant ($P < 0.05$). Following carcass examination for presence of DFD meat (Figure 2), 113 (100%) and 2 (1.1%) of carcasses from poorly and good handled animal were identified having DFD meat, respectively (Table 2).

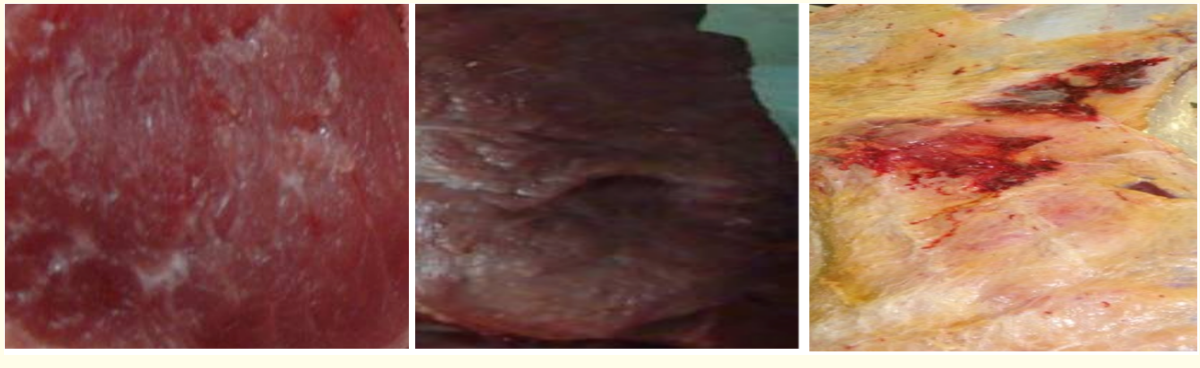

Figure 2: *Physical carcass examination A) Normal meat, B) DFD meat and C) Bruised carcasses.*

Factors		Total number of Sample Bruising No. (%)	Physical profiles		
			Meat category		
			Normal No. (%)	DFD No. (%)	
Welfare status	Poor	113 (37.7)	104 (91.23)[a]	0	113 (100)[a]
	Good	187 (62.3)	10 (8.77)[b]	185 (98.9)	2 (1.1)[b]
Abat-toirs	Batu	113 (37.7)	37 (32.46)[a]	75 (66.4)[a]	38 (33.6)a
	Meki	102 (34)	38(33.33)[a]	64 (62.7)[a]	38 (37.3)[a]
	Shashe-mane	85 (28.3)	39 (34.21)[a]	46 (54.1)[a]	39 (45.9)[a]
Breed	Local	214 (71.3)	43 (37.72)[b]	173 80.8)[a]	41 (19.2)[b]
	Exotic	86 (28.7)	71 (62.28) [a]	12 (14.9)[b]	74 (60.1)[a]
Total		300 (100)	114 (38.0)	185 (61.7)	115 (38.3)

Table 2: *Physical profiles of meat from studied cattle at abattoir.*

Note*: The same letter in the same column with respective factor is not significantly different at CI of 95% where a is significantly higher than b.*

The result of the study revealed that the proportion of DFD meat from cattle under poor welfare is statistically significant ($P < 0.05$) from that of carcass from cattle under good welfare.

Chemical profile (Meat pH and cooking loss) of meat

Mean for ultimate pH of meat against 24 hr postmortem time length of meat was shown in figure 3. Failing of pH in meat from un-stressed and local breeds' animal was found raped than in those from stressed and exotic breeds, respectively.

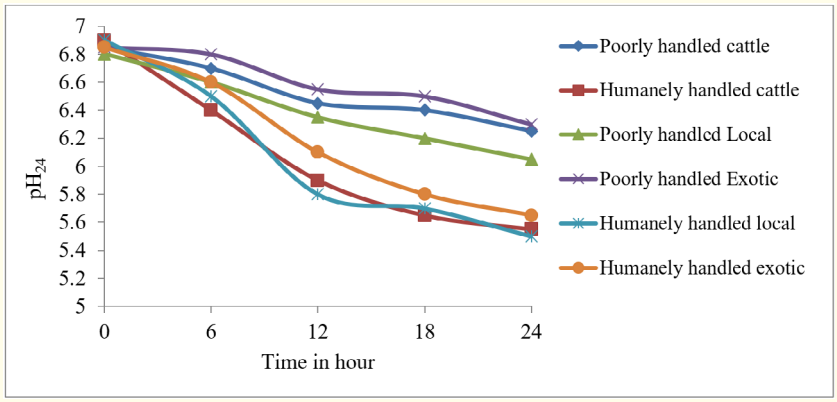

Figure 3: *Mean of ultimate pH of meat against time postmortem.*

The percentage cooking loss of meat samples from cattle subjected to poor handling was ranged from 19 to 35 with a mean of 25.99 ± 3.71 (Table 3). The mean pH_{24} of meat from the poorly-handled cattle = (6.18 ± 0.14) were significantly higher than the 5.59 ± 0.10 of meat from good handled. It was also higher in meat from exotic breeds than the counter groups ($P < 0.05$).

Factors		Total number of Sample pH Mean ± SD)	Processing/perceptible meat quality	
			Cooking loss Mean ± SD)	
Welfare status	Poor	113	6.18 ± 0.14[a]	25.99 ± 3.70[b]
	Good	187	5.59 ± 0.10[b]	36.31 ± 1.77[a]
Abattoirs	Batu	113	5.76 ± 0.25[b]	33.21 ± 5.53[a]
	Meki	102	5.81 ± 0.31[ab]	32.68 ± 5.45[ab]
	Shashemane	85	5.89 ± 0.34[a]	31.07 ± 5.93[b]
Breed	Local	214	5.70 ± 0.24[b]	34.41 ± 4.35[a]
	Exotic	86	6.09 ± 0.26[a]	27.48 ± 3.59[b]
	Total	300	5.81 ± 0.30	32.42 ± 5.67

Table 3: *Mean of pH_{24} and cooking loss of meat (n = 300 samples).*

Note*: TPC: Total Plate Count; TCC: Total Coliform Count; Staph: Staphylococcus; Mean = Mean ± SD; The same letter in the same column with respective factor is not significantly different at CI of 95% where a is significantly higher than b.*

As shown in figure 4, there was a strong negative and significant relationship (r = -0.952, n = 300, p = 0.000) between pH and percentage cooking loss. The percentage cooking loss increased as ultimate pH decreased and indicate that pH explains 90.6% of the variation in percentage cooking loss.

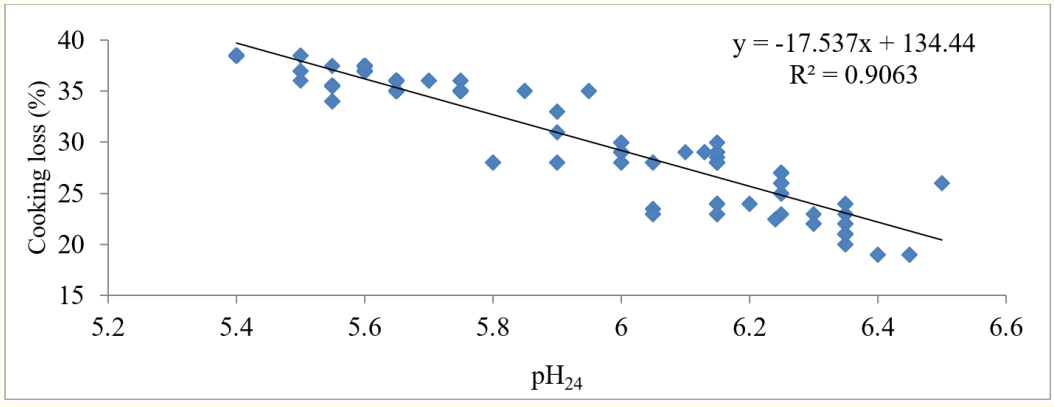

Figure 4: *Linear correlation of percent cooking loss and ultimate meat pH24 of studied meat.*

Bacteriological quality of meat

Overall log. CFU/g mean of TPC, TCC and *S. aureus* count of meat samples was 3.20 ± 1.97, 2.14 ± 1.40 and 1.81 ± 1.11 were observed, respectively.

An indecent samples *t*-test was for TPC showed higher in meat from poor handled cattle, and from exotic breeds than the counter groups (P < 0.05). However, TCC and *S. aureus* count in meat were similar among studied variables (Table 4). A total of 14.3% carcasses were positive for *Salmonella* with significantly higher in meat from poor handled cattle (72.1%) than in meat from good handled animal (27.9%) (p < 0.05) but similar among abattoirs (p > 0.05).

Factors		Total No. of Sample TPC Mean ± SD)	Microbiological quality			
			TCC Mean ± SD	*S. aureus* count Mean ± SD	*Salmonella* No. (%)	
Animal handling condition	Poor	113	3.95 ± 1.87[a]	2.31 ± 1.47[a]	1.95 ± 1.13[a]	31 (27.4)[a]
	Good	187	2.75 ± 1.89[b]	2.04 ± 1.35[a]	1.73 ± 1.09[a]	12 (6.4)[b]
Abattoirs	Batu	113	3.04 ± 2.04[a]	2.05 ± 1.32[a]	1.78 ± 1.12[a]	15 (13.3)[a]
	Meki	102	3.39 ± 1.96[a]	2.39 ± 1.45[a]	1.83 ± 1.14[a]	12 (11.8)[a]
	Shashemane	85	3.20 ± 1.89[a]	1.96 ± 1.41[a]	1.81 ± 1.07[a]	16 (18.8)[a]
Breed	Local	214	2.90 ± 1.97[b]	2.11 ± 1.40[a]	1.75 ± 1.11[a]	22 (10.3)[b]
	Exotic	86	3.96 ± 1.76[a]	2.22 ± 1.40[a]	1.95 ± 1.10[a]	21 (24.4)[a]
Total		300	3.20 ± 1.97	2.14 ± 1.40	1.81 ± 1.11	43 (14.3%)

Table 4: *Microbiological quality of meat with respect to animal handling condition, abattoir location and animal breeds.*

Note*: TPC: Total Plate Count; TCC: Total Coliform Count; Mean = Mean ± SD; the same letter in the same column with respective factor is not significantly different at CI of 95% where a is significantly higher than b.*

Of the total meat samples, 15.3% and 58.7% were found unacceptable using TPC and TCC, respectively. Relatively meat from poorly handled cattle has TPC and TCC of greater than the minimum acceptable level than meat from good handled cattle (Table 5).

Animal Welfare status	No. of carcasses examined	TPC range No. (%)		TCC range No. (%)	
		Acceptable	Unacceptable	Acceptable	Unacceptable
Good	187	176 (94.1)	11 (5.9)	83 (44.4)	104 (55.6)
Poor	113	78 (69.0)	35 (31.0)	41 (36.3)	72 (63.7)
Total	300	254 (84.7)	46 (15.3)	124 (41.3)	176 (58.7)

Table 5: *Number of carcass samples in relation to acceptable threshold for TPC and TCC.*

Discussion

Carcass bruising

Findings in this study revealed that about 38% of sampled carcass showed bruises. As carcass bruise increase the quality of meat drop in terms of pH, tenderness and microbial quality. This findings showed, 91.2% of carcasses from stressed cattle were bruised which is higher than the 66.9% reports of Mallia., *et al.* [28] and 15.5% reports of Costa., *et al* [29]. Such difference could be due to difference in degrees of animal exposed to diverse conditions. For instance, cattle in this study were transported a long distance under unfair handling and transportation condition [8,9] along which with high frequencies and risk of exposure to various mechanical damages resulting in bruises. McCausland and Miller [30] also reported 47% bruises in cattle attributed to be a non-specific time. Grandin [31] also reported almost double the bruising rate over the bodies of those cattle handled roughly during weighing and loading than in those walked quietly on to the scales and trucks. In addition to these, poor animal handling at lairage and during sticking could account for the high occurrence of bruises in the present finding. McCausland and Miller [30] reported at least 43% of all bruises in cattle incurred after arrival at the abattoir, to which most of them just before stunning.

Almost 38% of slaughtered cattle showed DFD meat indicating slaughtering of stressed cattle. This could be due to chronic or long-time exposure of animal to stress such long hours of transportation, fighting among animal before sticking, food and water deprivation and overcrowding in the lairage can cause DFD carcasses. A DFD meat is unattractive and more likely to face discrimination by consumers [32,33]. Carcasses from all cattle under poor welfare were developed DFD, but only 2 (1.1%) in those handled under good conditions. The present finding was similar with 100% DFD meat reported in abused cattle [34]. Gregory [35] reported that stress depleted glycogen in the muscles which leads to high pH_{24} values after slaughter, the meat subsequently becomes DFD prone to spoilage and poor keeping quality [28].

Meat pH and cooking loss

The present findings showed higher pH in meat from poor handled cattle than in those handled under good conditions. The present mean pH of 6.18 for meat from poor welfare animal was failed within the 5.9 - 6.5 pH of DFD meat and 5.59 for meat from good handling conditions within the 5.4 - 5.8 pH of normal meat [10]. Ljungberg., *et al.* [36] and Aradom., *et al.* [37] stated that DFD meat from stressed cattle has a shorter shelf life and cooking loss. The present findings also showed strong negative and significant relationship (r = -0.952) between pH and percentage cooking loss demonstrating that lower cook losses are associated with higher muscle pH and better protein functionality described bay Grandin [38]. In a study on turkeys by Owens and Sams [39], also reported negatively correlated function of ultimate pH with cooking loss (r = -0.52) but much lower than this finding due to either species related factor or stress conditions of animal. DFD meat has a very high water-binding capacity with a dry or sticky texture [40], thus, suited for spoilage microbial growth prone to short shelf life [35,41]. In connection with this, higher log CFU/g of TPC (3.95 ± 1.87) and TCC (2.31 ± 1.47) in meat from poor handling conditions than in good handling conditions were observed. The level of TPC in this study is in accordance with previous studies [42-45], but lower than Hiko., *et al.* [46] report in beef mortadella. Meat from abusely handled cattle is mostly characterized by having highest pH, which favor microbial growth.

Meat bacteriological quality

Observing higher in TPC in meat from poor handled cattle and from exotic breeds could have a connection with high DFD and bruised meat from those stressed animal which favor microbial growth. A total of 14.3% carcasses were positive for *Salmonella* with significantly higher in meat from poor handled cattle (72.1%) than in meat from good handled cattle (27.9%) ($p < 0.05$) but similar among abattoirs ($p > 0.05$). Following the unacceptable numbers (TPC > 10^5 CFU/g) of the meat spoilage [27], significantly higher unacceptable meat by TPC (31.0%) from poor handled cattle than (5.9%) from good handled cattle. This could be associated with presence of bruise and DFD meat from in poor handled cattle. On the other hand, a total of 58% meat samples were unacceptable by TCC to which it is higher than TPC in meat from cattle under both handling conditions. This could be due to carcasses contamination from gastrointestinal content stressed cattle during eviscerating or from already contaminated abattoir environment.

With regards to *S. aureus* count, the findings were lower than TPC and TCC. It was low and similar in meat from cattle handled under both conditions. These could be die to the fact that *S. aureus* was frequently associated with human carrier conditions which act a sources of carcasses contamination [47-49].

The present 14.3% *Salmonella* isolation in total meat samples were lower than the 26.3% from the abattoir line but higher than the 5.3% from the processing plant line reported by Hiko., *et al* [50]. The presence *Salmonella* at significantly higher (27.4%) in poorly handled cattle than 6.6% in humanely handled cattle could be attributed from factors like animal get stressed for shading of *Salmonella* to which a risk for carcasses contamination or from already contaminated abattoir environment. Unlike between animal handling conditions, prevalence of *Salmonella* was similar among studied abattoir and between studied animal breeds. This could be due to carcasses contamination from stressed animal and/or from already contaminated abattoir environments. *Salmonella* was also frequently isolated from abattoir environment [50-56] indicating from which meat can be contaminated.

Conclusion

The effects of unfair animal handling on physico-chemical and microbiological quality of beef were predominant. Of considered study factors i.e. animal handling conditions, the abattoirs and animal breeds, the effect of poor handling conditions at pre-slaughter stage resulted in higher bruise, DFD meat, TPC and *Salmonella* prevalence. Regardless other factors, under studied circumstances, poor animal handling at pre-slaughter and exotic animal at tropical abattoir condition which prone the animal to stress condition reflated on meat with high physical damage, slow failing of pH, and cooking loss which intern prone to microbial contamination and multiplication. It is therefore, awareness creation for stakeholders with application of regulations and legislation could have paramount to improve animal welfare and meat quality in Ethiopia.

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