Original Article

The physicochemical and microbiological quality of meat produced in a traditional slaughterhouse in Mansoura City, Egypt

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Abstract

Introduction: In several developing countries the slaughter of meat producing animals is still practiced in traditional slaughterhouses. In the Mansoura slaughterhouse, animals are subjected to various stressors and treated with cruelty, in addition to unhygienic treatment and handling of animal carcasses. Hence, this study was designed to investigate the meat quality from cattle, buffalo, and sheep carcasses processed in an old-fashioned slaughterhouse from Mansoura city, Egypt, in the context of pre-slaughter stress.

Methodology: The bleeding efficiency and the ultimate pH (pHu) of carcasses were tested, along with the effect of post-slaughter handling practice on the microbiological properties of meat.

Results: From the 351 examined animals, the ultimate pH (pHu) was less than 5.8 in 81 cases (23.1%) and higher than 6.0 in 165 cases (47%). Furthermore, 45 (12.8%), 270 (76.9%), and 36 (10.3%) of the tested carcasses were well-, moderate- and imperfectly-bled, respectively. Cultivation using the wet-dry triple swab technique sampled from the outer surfaces of cattle, buffalo, and sheep carcasses revealed that about 47.9% of the tested carcasses were contaminated, with total viable count levels exceeding 7 log₁₀ cfu/cm², and 42.7% were contaminated with *Enterobacteriaceae*, with levels > 3 log₁₀ cfu/cm². The molds and yeasts from the tested carcasses had lower counts (< 2 log₁₀ cfu/cm²).

Conclusions: Results indicated neglect in terms of sanitary measures during slaughtering and dressing of carcasses, with subsequent higher microbial contamination and impaired meat quality. Therefore, the traditional slaughtering facilities should be modernized to increase their meat producing efficiency, subsequently leading to exportation possibilities.

Key words: Preslaughter stress; ultimate pH; meat quality; viable counts; Enterobacteriaceae counts.

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Introduction

Meat is considered to be an essential component of any well-balanced, healthy diet. It is an important source of high biological value proteins, vitamins, and minerals [1]. However, its enriched nutrient composition and sufficient water activity, are conducive for the growth of both spoilage and pathogenic microorganisms. Globally, the correct implementation of standards regarding animal welfare during transport to the abattoir and slaughtering, together with proper animal handling is considered a crucial point for meat quality and safety of the resulted products [2].

The contaminated beef and mutton raw meat may carry a large diversity of important pathogenic and spoilage microorganisms (e.g. pathogenic *E. coli* strains, Salmonella spp., Shigella spp., Proteus spp., Klebsiella spp., Yersinia spp.). Meat is inevitably contaminated with bacteria during meat processing stages, such as evisceration and dressing procedures. Abattoir surfaces, knives, walls and employees themselves are considered the main causes of meat contamination at slaughtering level [3]. Also, heavily filthy cattle hides can lead to high contamination levels [4]. The microbiological monitoring of carcasses depends mainly on the determination of total viable counts (TVCs), as indicators of general hygienic conditions and Enterobacteriaceae (ECs), as indicators of faecal contamination [5]. In this regard, results of several studies conducted in other developing countries, like Ethiopia [6], and Benin [7], have revealed unsatisfactory results for TVCs and ECs in accordance

with the European Commission Regulation No 2073/2005, reflecting the poor sanitary conditions and the necessity of urgent complete renovation of old-fashioned slaughterhouses to ensure meat safety and public health.

During transport to the slaughterhouse, animals are subjected to various stressors that compromise their welfare and meat quality. After animal slaughtering, the glycogen stored in the muscle is converted into lactic acid. The resultant acidic pH improves the palatability, color, texture, microbiological quality, and shelf life of meat and meat products [8]. The rough handling immediately before slaughtering will result in the depletion of animals' glycogen reserves [9]. The subsequent high ultimate pH (pHu) values will produce the dark-cutting beef, characterized by its darker color, drier surface, and higher water-holding capacity [10]. In slaughterhouses from the southeast of Mexico, carcasses from animals exposed to stressful conditions before and during the slaughtering process had a pHu \geq 5.8 with an average of 6.3, indicating increased vulnerability towards microbial growth, a shorter shelf life and less marketability [11].

The bleeding efficiency exerts an important effect on the subsequent meat quality. The bleeding process should be conducted as quickly as possible, in a hygienic manner. In imperfectly bled carcasses, the blood is not pumped profusely out from the blood vessels during animal slaughtering [12], resulting in counteracts on the postmortem acidity due to the neutral pH of blood, considered an ideal environment for bacterial growth. Blood components, especially hemoglobin, are essential in the promotion of lipid oxidation, which may reduce meat durability [13].

In the last decade, despite the implementation of modern slaughtering procedures throughout slaughterhouses in many urban areas of Egypt, most meat-producing animals continue to be slaughtered in traditional, old abattoirs throughout most Governorates. In these slaughter units, the animals are subjected to various stressors, together with unhygienic treatments and handling of animal carcasses. Moreover, the current situation in the slaughterhouses is deplorable, but amendable, yet studies that document the negative impact of this situation on meat are scarce [14-16]. Consequently, the aim of the current study is to highlight any findings that could confirm the current situation such as the effect of the (i) pre-slaughter stress practiced in old traditional abattoirs, and (ii) postslaughter animal handling practices conducted by butchers on the final meat quality, via testing the bleeding efficiency and pH measurement, as well as to

(iii) determine the microbiological quality of slaughtered cattle, buffalo, and sheep carcasses obtained in a slaughter house from Mansoura, Egypt.

Methodology

Sample collection and preparation

We selected a large traditional slaughterhouse in Mansoura city, Egypt as our investigation point. The slaughterhouse has a slaughter rate of around 700 heads of cattle and sheep per week. The slaughtering operations start after sunrise, usually between 6 a.m. and 11 a.m. The act of slaughtering is performed by butchers, while both antemortem and postmortem examinations were performed by the veterinarians. Butchers bring animals to slaughter from nearby places (within various distances) by trucks, in which treatment of animals varies until the slaughter stage. The animals were randomly selected and divided into two groups, one of them was slaughtered as usual, and the other was given a period of rest and was treated gently. Animals were hand-slaughtered in laying positions on the ground, then lifted for 5 to 6 minutes for complete bleeding, followed by skinning and evisceration at the same place of the slaughter hall.

A total of 351 food producing animals, categorized as 150 cattle, 81 buffaloes and 120 sheep, were evaluated for their quality through physical, chemical, and microbiological examinations. Besides physical (visual) examination, the bleeding efficiency of the investigated carcasses was evaluated through the malachite green chemical test, performed on meat samples of approximately 10 g, incised from the diaphragm muscle, followed by staining of the meat extract, as previously described by Collins *et al.* [17]. Additionally, about 100 g of diaphragm muscle were taken from each carcass and directed for pH measurement, after a previous chilling at 5 °C in a home refrigerator, for 24 hours, and after mixing with distilled water, as previously described by Page *et al.* [18].

The wet-dry triple swab technique was used for the microbiological examinations. In this regard, samples were collected from a limited surface area (100 cm²). For each of the investigated carcasses, three swabs were collected from the neck, shoulder and topside regions, respectively. Accordingly, the first swab was moistened with 0.1% peptone water, while the other two swabs were used dry. The loaded swabs were transferred to sterile test tubes forming a pooled sample. The tube contained 10 mL of sterile 0.1% peptone water, as an original dilution for microbiological examination [19]. All samples were directly transported, with a minimal delay in a cooling box, to the laboratory of Food

Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University and subjected to several analyses, as described below.

Examination of the bleeding efficiency of the investigated carcasses

The first step of the study, the bleeding efficiency evaluation of carcasses, consisted in the visual examination of the blood evacuated from the left ventricle. The tested carcasses were categorized according to the amount of the remaining residual blood, after incising the left ventricle of the heart. Imperfectly bled carcasses exhibited oozing of a large amount of residual blood, whereas moderately bled ones revealed a small amount of blood. The well bled carcasses showed a smaller amount of clotted blood only in the incised ventricle, without oozing of liquid blood.

Subsequently, the bleeding efficiency was also determined using the malachite green chemical test. The principle of the test consists in the formation of a green complex, as consequence of hemoglobin oxidation caused by adding the malachite green acid solution. The meat extract was prepared for this procedure by adding the collected diaphragm muscle into 14 mL of distilled water in a clean test tube. One drop of the acidic malachite green solution was added together with a drop of hydrogen peroxide 3% and 0.7 mL of the prepared meat extract in another agglutination tube. The resulted reactions were graded into three categories as follows: grade I - clear blue color representing well bleeding; grade II - cloudy green color representing moderate bleeding; and grade III - cloudy, olive-green color representing imperfect bleeding [17].

Measurement of the ultimate pH (pHu) of carcasses

The ultimate pH (pHu) level was measured in each excised diaphragm muscle, using an electric pH meter (Model: pH-206, Lutron Electronics, Australia) by immersing the pH meter electrode into the prepared meat extract, as was previously indicated [18,20].

Microbiological analyses

Sample preparation for microbiological analyses, including homogenization of swab samples in 0.1% sterile peptone water (CM0009; Oxoid, UK), and serial dilutions were performed according to the standard method [21]. Appropriate dilutions were plated on: Plate Count Agar (PCA, CM0325; Oxoid, UK), followed by incubation at 30 °C, for 2 days for determination of total viable counts (TVCs) [22]; Violet Red Bile Glucose Agar (VRBGA, CM 0485; Oxoid, UK), followed by incubation at 30 °C, for 24 hours for determination of *Enterobacteriaceae* counts (ECs) [23] and on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC; CM 0727; Oxoid, UK), followed by incubation at 25 °C, for 5-7 days, for determination of moulds (MCs) and yeasts (MCs and YCs) counts, respectively [24]. The colonies in the different countable plates were counted for all treated swab samples, beside the control plate.

Statistical analysis

The obtained data were statistically analyzed using the SPSS ver. 21.0 program. Data were expressed as the mean \pm standard error (SE). Difference between the mean of different samples was assessed by analysis of variance (ANOVA test). The strength of the relationship between variables was analyzed using Spearman's correlation coefficient (r_s).

Research ethics committee permission

The current research work was executed according to standards of Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University.

Results and Discussion

Ultimate pH levels (pHu) in diaphragm muscle

The mean levels of pHu measured in diaphragm muscles of cattle, buffalo and sheep carcasses, at 24 hours postmortem, were 6.0, 5.84, and 6.07, respectively. The obtained pHu values were categorized as lower than 5.8, between 5.8 and 6.0, and higher than 6.0. In this regard, the recorded values, according to each of the investigated species, are summarized in Table 1. Overall, the results indicate that a total of 81

Table 1. Ultimate pH (pHu) levels measured after 24 hours postmortem in diaphragm muscle of cattle, buffalo, and sheep carcasses (N = 351).

Company	M::	M	Mean ± SE –	Number and (%) of carcasses in relation to their pHu					
Carcasses	Minimum	Maximum	$Mean \pm SE$ –	< 5.8	from 5.8 to 6	> 6			
Cattle	5.57	6.65	6.00 ± 0.25	39 (26%)	42 (28%)	69 (46%)			
Buffalo	5.51	6.18	5.84 ± 0.19	36 (44.4%)	27 (33.3%)	18 (22.2%)			
Sheep	5.67	6.48	6.07 ± 0.17	6 (5%)	36 (30%)	78 (65%)			
Overall	5.51	5.65	5.99 ± 0.23	81 (23.1%)	105 (29.9%)	165 (47%)			

(23.1%) out of 351 examined carcasses had a pHu that did not exceed 5.8, another 105 (29.9%) expressed pHu values from 5.8 to 6.0, whereas 165 (47%) possessed pHu values higher than 6.0 (Table 1). The dominant occurrence of the pHu category higher than 6.0 raised our assumption that the exposure of the slaughtered animals at the Mansoura slaughterhouse to various stressors (e.g. fatigue, long-distance transportation, mixing with unfamiliar animal groups, noisy environment inside the abattoir, weather conditions, or rough handling), without any pre-slaughter treatment, can be considered as major cause of reduced muscle glycogen concentration and occurrence of the dark cutting condition. However, further investigations are recommended to clarify this hypothesis.

Several researchers have established the pHu value of 5.8, as a demarcating limit for differentiation between good and poor-quality meat [25-27]. A higher value than this limit, together with the increasing the meat toughness, are considered to be important factors positively affecting the growth and multiplication of pathogenic microorganisms, that can contaminate the carcasses during the slaughtering process as well as meat products during processing and storage [26]. Numerous researchers have also considered a pHu value of 6.0 as beneficial unit for measuring the efficiency of pre-slaughter care, during lair aging in the abattoir. In this regard, special emphasis was put on measuring the evolution of pHu in the cattle longissimus dorsi muscle. Thus, pHu values higher than 6.0 were recorded in cattle slaughtered without an adequate resting period [27,28]. It is also worth mentioning that there were records of wide pHu variations, from 5.17 to 7.07 [29], and from 5.18 to 6.86 [30], respectively, in these muscle samples, even under the influence of several stressing and animal related factors. In the case of lambs and of the same muscle

group, a mean pHu value of 6.38 has been recorded in animals subjected to high stress levels [31].

Correlations between bleeding efficiency and pHu under the effect of pre-slaughter stress

The correlation between pHu and bleeding efficiency in all of the examined carcasses is presented in Table 2. The obtained data showed statistically significant relationships between the obtained pHu values and bleeding efficiency among all the examined carcasses. In this regard, the pHu in the examined species was, statistically speaking, significantly higher in case of imperfect bleeding compared to cases of moderate bleeding, as well as between cases of moderate bleeding compared to well-bled carcasses. The exhibited pHu values in well-bled carcasses ranged from 5.51 to 5.83, with a mean \pm SE count of 5.67 \pm 0.08. The pHu values of moderately bled carcasses ranged from 5.64 to 6.31 with a mean \pm SE of 5.99 \pm 0.16, whereas in the case of imperfectly bled carcasses the pH values ranged from 6.17 to 6.65, with a mean \pm SE of 6.39 ± 0.12 . The mean pHu levels in cattle muscles were 5.68, 6.0 and 6.4 in well-bled, moderately-bled and imperfectly bled samples, respectively. Similar levels were observed in buffalo muscles: 5.66, 5.9, and 6.2 in well-bled, moderatelybled, and imperfectly bled samples, respectively. Meanwhile, the same values but in sheep muscles were 5.69, 6.07, and 6.4 in well-bled, moderately-bled, and imperfectly bled samples (Table 2). The results of the abattoir and laboratory examinations regarding the bleeding efficiency degree revealed a defect in the slaughtering system, as most (270/351; 76.9%) of the examined carcasses were moderately bled, resulting in poor meat quality with shorter shelf life. This may be attributed to the slaughter of the animals at the abattoir

Table 2. Correlation between the ultimate pH (pHu) and bleeding efficiency in all investigated carcasses.

			Bleeding efficiency				
pHu		Well bled Moderate bled		Imperfectly bled	rs	р	
Cattle carcasses (N = 150)	17 (11.3%)	111 (74%)	22 (14.7%)			
"IIn lavala	Range	5.57-5.77	5.63-6.30	6.28-6.65	0.761	< 0.0005	
pHu levels	Mean \pm SE	$5.68 \pm 0.07^{ m C}$	6 ± 0.17 ^B	6.4 ± 0.11 ^A			
Buffaloes carcasses $(N = 81)$		21 (25.9%)	54 (66.7%)	6 (7.4%)			
TT 1 1	Range	5.51-5.84	5.71-6.14	6.16-6.18	0.656	< 0.0005	
pHu levels	Mean \pm SE	5.66 ± 0.12 ^C	$5.9\pm0.16^{\rm \ B}$	$6.2\pm0.$ 08 $^{\rm A}$			
Sheep carcasses (N = 120)	7 (5.8%)	105 (87.5%)	8 (6.7%)			
	Range	5.67-5.70	5.89-6.30	6.31-6.48	0.521	0.001	
pHu levels	Mean \pm SE	5.69 ± 0.11 ^C	6.07 ± 0.13 ^B	$6.4\pm0.09{}^{\rm A}$			
Overall carcasses	(N = 351)	45 (12.8%)	270 (76.9%)	36 (10.3%)			
pHu levels	Range	5.51-5.83	5.64-6.31	6.17-6.65	0.712	< 0.0005	
	Mean \pm SE	$5.67\pm0.08^{\rm \ C}$	$5.99 \pm 0.16^{\;\rm B}$	$6.39\pm0.12^{\rm \ A}$			

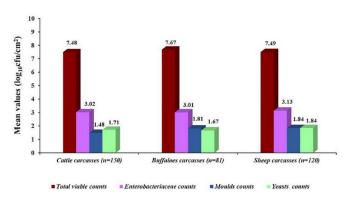
rs: Spearman's correlation coefficient; p value by One-way ANOVA test significantly different at p < 0.05; A-C: Means with the different letter in each row are significantly different at p < 0.05. after being subjected to various stressors, without lairaging or any preslaughter treatment.

These findings are largely in agreement with those obtained by Roça [32], who emphasized the importance of bleeding efficiency in obtaining a high-quality meat. Likewise, in a study conducted in Ethiopia, Kumar et al. [33] revealed a relationship between the pHu and the bleeding efficiency in 83 emergency slaughtered cattle, using the malachite green test. They reported that 13 (15.66%), 58 (69.88%) and 12 (14.46%) of the examined carcasses were categorized as grade I, grade II and grade III, representing satisfactory, moderatelyimperfect. and completely-imperfect bleeding, respectively. The reasons like starvation, fatigue and metabolic disturbances, which result in depletion of the animals' glycogen reserves and a high pHu of meat, may also lead to produce imperfect bleeding [8,12]. Although the normal postmortem biochemical changes in all types of muscle samples result in decline of the pHu, in the case of insufficiently bled meat. The blood, with neutral or mildly alkaline reactions after death, counteracts the low pHu (acid) of the muscle [26,27].

Microbiological quality of the investigated animal carcasses

The determined values of TVCs on all examined carcasses ranged from 5.23 to 8.46 (\log_{10} cfu/cm²), with a mean of 7.53; from 5.23 to 8.47, with a mean of 7.58; and from 5.34 to 8.42 with a mean of 7.49 on the neck, shoulder and topside regions, respectively. Moreover, the mean values of TVCs were 7.48, 7.67 and 7.49 in cattle, buffalo and sheep carcasses, respectively (Figure 1). The obtained high contamination level of the examined surfaces according to Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs (2005) indicates negligent

Figure 1. Mean values $(\log 10 \text{ cfu/cm}^2)$ of total viable, Enterobacteriaceae, mold, and yeasts counts of investigated swab samples taken from the outer subcutaneous surfaces of cattle, buffalo, and sheep carcasses.



sanitary measures during slaughter and dressing of carcasses, favored by the fact that the removal of hides and cleaning of viscera were carried out on the same floor. Furthermore, all the surveyed carcasses had dirty skin. In case of sheep the dirty fleece may significantly increase the microbial load up to 1000 times more, compared to carcasses coming from sheep with clean fleece [34]. Contrary to these observations, Hauge et al. [35] suggested that a higher microbial load present in hides of dirty cattle, does not influence the hygienic quality of carcasses, resulting in a similar microbial quality with those obtained from clean hides. However, the slaughtering of animals, especially cattle and buffaloes with hides that are very heavily contaminated by dirt, results in heavy bacterial contamination of their carcass [36].

Frequency distribution of TVCs on all three sites of the tested carcass surfaces showed that 100% of the examined samples had counts higher than 5 \log_{10} cfu/cm², which has been set as maximum level during the skinning process [37]. Overall, most of the investigated swab samples (504 (47.9%)) were contaminated, with levels ranging from > 7 to $8 \log_{10}$ cfu/cm^2 , and > 6 to 7 log_{10} cfu/cm^2 . TVCs were higher than 6 log₁₀ cfu/cm² in 92.7%; 92.6%, and 98.3% of the tested swab samples from cattle, buffalo, and sheep carcasses, respectively (Table 3). These values reflect a higher contamination level than $6 \log_{10} \text{ cfu/cm}^2$, as the maximum permissible limit corresponding to good quality of meat [38]. Moreover, Gracey et al. [12] considered that a value of 10⁶ cfu/cm² is unacceptable for fresh meat. In some slaughterhouses from Benin, West Africa, the mean TVCs in cattle carcasses were 6.16 log10 CFU/cm² and 100% of mean TVCs were considered to be unsatisfactory according to the EC Regulation No 2073/2005 [7]. Also, Zweifel et al. [5] reported that TVCs reflect the general contamination level of food products. Therefore, its determination can be a very useful measure for screening the hygienic conditions in abattoirs.

Enterobacteriaceae counts (ECs) ranged between 2.08 and 3.98 \log_{10} cfu/cm², with a mean of 3.0 \log_{10} cfu/cm²; from 2.21 to 3.83 \log_{10} cfu/cm², with a mean of 3.07 \log_{10} cfu/cm²; and from 2.17 to 3.85 \log_{10} cfu/cm², with a mean of 3.06 \log_{10} cfu/cm² on the neck, shoulder and topside regions, respectively (Figure 1). The mean values of ECs were 3.02, 3.01, and 3.13 \log_{10} cfu/cm² in cattle, buffalo and sheep carcasses, respectively. None of the tested carcass samples, among the three evaluated species, had ECs less than 1.5 \log_{10} cfu/cm². Of the examined cattle, buffalo and sheep carcasses, 276 (61.3%), 147 (60.5%), and 180 (50%)

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had ECs \leq 3 log cfu/cm², while 174 (38.7%), 96 (39.5%), and 180 (50%) had ECs counts > 3 log cfu/cm², respectively (Table 4). These values reflect high contamination levels of carcasses that may be attributed to faulty practices during evisceration and dressing [39], with significant influence on reducing meat quality, causing severe economic losses.

Our EC related findings are consistent with those obtained by other researchers from Spain for example, Alonso-Calleja et al. [40], found that the mean ECs were 2.09 ± 0.97 and $2.50 \pm 1.61 \log_{10} \text{ cfu/cm}^2$ on lamb carcasses obtained from two commercial abattoirs. In South Africa, Nyamakwere et al. [41] reported that the mean ECs were 1.5 and 2.9 log cfu/cm² in cattle carcasses slaughtered in two abattoirs. Blagojevic et al. [42], obtained that the mean ECs were 0.98 and 1.95 log cfu/cm² from cattle carcasses, in two abattoirs in Serbia. The distribution frequency of ECs on all three tested carcass surfaces indicated that 100% of the examined samples had ECs greater than $1.5 \log_{10} \text{ cfu/cm}^2$ (Table 4). In Ireland, McEvoy et al. [43] have categorized the mean ECs < 0.8, 0.8-1.8 and $> 1.8 \log \text{ cfu/cm}^2$ as acceptable, marginal and unacceptable levels, respectively. Also, in Switzerland, Zweifel et al. [5] categorized the mean ECs < 1, 1-2 and $> 2 \log cfu/cm^2$,

as acceptable, marginal and unacceptable, respectively. In the same study, the authors also found that the ECs of positive samples (31%) ranged from an undetectable level to 1.5 cfu/cm², and only 10 bovine samples exceeded the value of 3 log cfu/cm².

Molds counts (MCs) from all examined carcasses ranged from 0.39 to 2.77 \log_{10} cfu/cm², with a mean of 1.83 \log_{10} cfu/cm²; from 0.39 to 2.47, with a mean of $1.63 \log_{10} \text{cfu/cm}^2$; and from 0.39 $\log_{10} \text{cfu/cm}^2$ to 2.46 \log_{10} cfu/cm², with a mean of 1.62 \log_{10} cfu/cm² on the neck, shoulder, and topside regions, respectively. Yeasts counts (YCs) ranged from 0.39 log₁₀ cfu/cm² to 2.69 \log_{10} cfu/cm², with a mean of 1.78 \log_{10} cfu/cm²; from 0.39 \log_{10} cfu/cm² to 2.63 \log_{10} cfu/cm² with a mean of 1.70 \log_{10} cfu/cm²; and from 0.39 \log_{10} cfu/cm² to 2.43 \log_{10} cfu/cm² with a mean of 1.76 \log_{10} cfu/cm² on the neck, shoulder, and topside regions (Table 5). The mean MCs were 1.48 \log_{10} cfu/cm², 1.81 \log_{10} cfu/cm², and 1.84 log₁₀ cfu/cm² for cattle, buffalo, and sheep carcasses, respectively, while the mean YCs were $1.71 \log_{10} \text{cfu/cm}^2$, $1.67 \log_{10} \text{cfu/cm}^2$, and $1.84 \log_{10}$ cfu/cm² for cattle, buffalo, and sheep carcasses, respectively (Figure 1). The present findings of MCs and YCs were almost similar to those reported in India, where the obtained mean MCs and YCs from buffalo

Table 3. Frequency of distribution of total viable counts of all investigated swab samples taken from the outer subcutaneous surfaces of cattle, buffalo, and sheep carcasses.

Total viable counts (log ₁₀ cfu/cm ²)	Cattle	carcasses (N	= 450)	Buffaloe	es carcasses (N = 243)	carcasses (N	- Total		
	Necks	Shoulders	Topsides	Necks	Shoulders	Topsides	Necks	Shoulders	Topsides	Total
	(N = 150)	(N = 150)	(N = 150)	(N = 81)	(N = 81)	(N = 81)	(N = 120)	(N = 120)	(N = 120)	(N = 1053)
> 5 to 6 log ₁₀ cfu/cm ²	12 (8%)	12 (8%)	9 (6%)	6 (7.4%)	3 (3.7%)	9 (11.1%)	0 (0%)	3 (2.5%)	3 (2.5%)	57 (5.4%)
> 6 to 7 log ₁₀ cfu/cm ²	57 (38%)	54 (36%)	60 (40%)	30 (37.1%)	21 (25.9%)	27 (33.3%)	36 (30%)	33 (27.5%)	45 (37.5%)	363 (34.5%)
$> 7 \text{ to } 8 \log_{10} \text{ cfu/cm}^2$	66 (44%)	66 (44%)	60 (40%)	33 (40.7%)	39 (48.1%)	33 (40.7%	69 (57.5%)	72 (60%)	66 (55%)	504 (47.9%)
> 8 log10 cfu/cm ²	15 (10%)	18 (12%)	21 (14%)	12 (14.8%	18 (22.2%)	12 (14.8%)	15 (12.5%)	12 (10%)	6 (5%)	129 (12.2%)

Table 4. Frequency of distribution of *Enterobacteriaceae* counts of all investigated swab samples taken from the outer subcutaneous surfaces of cattle, buffalo, and sheep carcasses.

Enterobacteriaceae	Cattle carcasses (N = 450)			Buffaloes carcasses (N = 243)			Sheep	- Total		
counts (\log_{10} cfu/cm ²)	Necks	Shoulders	Topsides	Necks	Shoulders	Topsides	Necks	Shoulders	Topsides	Total
counts (log10 clu/clil)	(N = 150)	(N = 150)	(N = 150)	(N = 81)	(N = 81)	(N = 81)	(N = 120)	(N = 120)	(N = 120)	(N = 1053)
> 1.5 to 2.5 log ₁₀ cfu /cm ²	45 (30%)	9 (6%)	24 (16%)	12 (14.8%)	3 (3.7%)	3 (3.7%)	27 (22.5%)	15 (12.5%)	12 (10%)	150 (14.2%)
> 2.5 to 3 log ₁₀ cfu /cm ²	45 (30%)	93 (62%)	60 (40%)	42 (51.9%)	39 (48.1%)	48 (59.2%)	39 (32.5%)	39 (32.5%)	48 (40%)	453 (43.1%)
$> 3 \log_{10} cfu / cm^2$	60 (40%)	48 (32%)	66 (44%)	27 (33.3%)	39 (48.1%)	30 (37.1%)	54 (45%)	66 (55%)	60 (50%)	450 (42.7%)

Table 5. Logarithmic values (\log_{10} cfu/cm²) of microbial counts of the outer neck, shoulder and topside surfaces of all investigated carcasses (N = 351).

Missohial satagam		Neck (N	= 351)	S	houlder	(N = 351)	Topside (N = 351)			
Microbial category	Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE	p
Total viable counts	5.23	8.46	7.53 ± 6.68	5.23	8.47	7.58 ± 6.75	5.34	8.42	7.49 ± 6.68	0.325
Enterobacteriaceae counts	2.08	3.98	3.00 ± 2.01	2.21	3.83	3.07 ± 1.93	2.17	3.85	3.06 ± 2.02	0.042
Moulds counts	0.39	2.77	1.83 ± 0.97	0.39	2.47	1.63 ± 0.75	0.39	2.46	1.62 ± 0.67	0.042
Yeasts counts	0.39	2.69	1.78 ± 0.78	0.39	2.63	1.70 ± 0.75	0.39	2.43	1.76 ± 0.75	0.193

carcasses were 1.21, 1.36, 1.49, 1.57, and 1.70 log₁₀ cfu/cm² on the leg, rump, rib, neck, and shoulder regions, respectively [44]. In Northern Ireland, the highest MCs in cattle carcasses was 1.41 log₁₀ cfu/cm² and the mean YCs ranged from $0.46 \pm 1.12 \log_{10}$ cfu/cm² [45]. In Brazil, the mean MCs and YCs in bovine carcasses were 2.24 and 2.16 log₁₀ cfu/cm², respectively [46]. These low counts indicate that mold and yeast contamination is not a serious problem and they are not a major constituent of the spoilagemicroorganism category, as they grow at a slow rate on fresh meat when compared to bacteria. However, molds can grow within an extremely wide range of temperatures and can produce toxic metabolites, called mycotoxins, which constitute potential health hazards to humans and animals [47,48]. They can also provide an unpleasant flavor and taste to the meat, with several forms of growths, especially on frozen meat, such as black and white spots, whiskers and bluish green patches [14]. In the same regard, yeasts have been frequently considered to be insignificant for meat spoilage, due to their low initial numbers and their slow growth rates. However, they could cause severe economic losses, which affect the meat industry owing to the formation of slime, offensive odor, rancidity, and discoloration [49,50].

A statistically significant correlation was observed between TVCs and MCs (p = 0.015), as well as between MCs and YCs (p = 0.012) in cattle carcasses. Also, in case of the buffalo carcasses, there was a statistically significant positive correlation between TVCs and each of the other three counted microorganisms (ECs p =0.033; MCs p < 0.0005; YCs p = 0.002), as well as between MCs and YCs (p < 0.0005). However, in sheep carcasses, there was a statistically significant positive correlation between TVCs and both MCs (p < 0.0005), and YCs (p = 0.01), as well as between MCs and YCs (p < 0.0005). The correlation between TVCs and ECs in buffalo carcasses is approximately similar to that reported in Switzerland for cattle carcasses, and the higher TVCs were associated with higher ECs in most of the surveyed abattoirs [5].

Conclusions

The results obtained in the current study indicate negligent sanitary measures during slaughtering and dressing of carcasses. In fact, most of the glycogen stores in muscles of the animals were already consumed at the point of slaughter giving an ultimate pH > 6 in case of most carcasses, resulting in the production of imperfectly bled carcasses, with a poor meat quality materialized through its darkened color and tough

texture. In addition, the obtained high microbial contamination levels on tested carcasses have proved to be evidence of the negligent hygienic practices during the processing stages. Therefore, the hygienic standards should be strictly followed in the municipal abattoir for obtaining an appropriate quality of meat.

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Authors' Contributions

Mahmoud Mahros: Interpreted Results, Editing, Revision Manuscript. Hend Elshebrawy: Methodology, Analysis Results, Writing Manuscript. Samir Abd-Elghany: Conceptualization, Methodology, Writing Manuscript. Mohammed Elgazzar: Designed the study, Revision Manuscript. Kálmán Imre: Funding, Revision, and editing Manuscript. Adriana Morar: Resources, Software, Editing Manuscript. Viorel Herman: Data analysis, Data curation. Khalid Sallam: Data Validation, Revision Manuscript.

References

- 1. Pereira PM, Vicente AF (2013) Meat nutritional composition and nutritive role in the human diet. Meat Sci 93: 586-592.
- Schwartzkopf-Genswein KS, Faucitano L, Dadgar S, Shand P, González LA, Crowe TG (2012) Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. Meat Sci 92: 227-243.
- Rao DN, Ramesh BS (1992) The microbiology of sheep carcasses processed in a modern Indian abattoir. Meat Sci 32: 425-436.
- Ridell J, Korkeala H (1993) Special treatment during slaughtering in Finland of cattle carrying an excessive load of dung; meat hygienic aspects. Meat Sci 35: 223-228.
- Zweifel C, Baltzer D, Stephan R (2005) Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance with EU Decision 2001/471/EC. Meat Sci 69: 559-566.
- 6. Teshome G, Assefa Z, Keba A (2020) Assessment of microbial quality status of raw beef around Addis Ababa city, Ethiopia. Afr J Food Sci 14: 209-214.
- Ahouandjnou H, Baba-Moussa F, Bonou J, Dougnon V, Adéoti Z, Yedji R, Baba-Moussa L (2015) Evaluation of the microbiological quality of cattle carcasses in some slaughterhouses at Benin, West Africa. Int J Sci Rep 1: 228-234.
- Matarneh SK, England EM, Scheffler TL, Gerrard DE (2017) The conversion of muscle to meat. In Lawrie's Meat Science, 159-185. Woodhead Publishing. Series in Food Science, Technology and Nutrition.

- 9. Xing T, Gao F, Tume RK, Zhou G, Xu X (2019) Stress effects on meat quality: A mechanistic perspective. Compr Rev Food Sci Food Safety 18: 380-401.
- Apple JK, Kegley EB, Galloway DL, Wistuba TJ, Rakes LK (2005) Duration of restraint and isolation stress as a model to study the dark-cutting condition in cattle. J Anim Sci 83: 1202-1214.
- Carrasco-García AA, Pardío-Sedas VT, León-Banda GG, Ahuja-Aguirre C, Paredes-Ramos P, Hernández-Cruz BC, Murillo VV (2020) Effect of stress during slaughter on carcass characteristics and meat quality in tropical beef cattle. Asian-Australas J Anim Sci 33: 1656-1665.
- 12. Gracey JF, Collins DS, Huey RJ (1999) Meat Hygiene, 10th Ed. Harcourt Brace and Company limited, London, UK.
- Maqsood S, Benjakul S (2011) Effect of bleeding on lipid oxidation and quality changes of Asian seabass (Lates calcarifer) muscle during iced storage. Food Chem 124: 459-467.
- Sallam KI, Abd-Elghany SM, Hussein MA, Imre K, Morar A, Morshdy AE, Sayed-Ahmed MZ (2020) Microbial decontamination of beef carcass surfaces by lactic acid, acetic acid, and trisodium phosphate sprays. BioMed Res Int 2020: 2324358.
- 15. Elsharawy NT, Mahran HA (2018) Determination of hygienic condition of municipal slaughterhouse and its microbial effect on the meat quality. Alex J Vet Sci 59: 11-18.
- 16. Frimpong S, Gebresenbet G, Bobobee E, Aklaku ED, Hamdu I (2014) Effect of transportation and pre-slaughter handling on welfare and meat quality of cattle: case study of Kumasi abattoir, Ghana. Vet Sci 1: 174-191.
- Collins DS, Huey RJ, Gracey JF (2015) Gracey's meat hygiene, 11th Ed. Wiley Blackwell, Chichester, West Sussex, UK
- Page JK, Wulf DM, Schwotzer TR (2001) A survey of beef muscle colour and pH. J Anim Sci 79: 678-687.
- Bell RG (1997) Distribution and sources of microbial contamination on beef carcasses. J Appl Microbiol 82: 292-300.
- Devatkal S, Mendiratta SK, Kondaiah N, Sharma MC, Anjaneyulu ASR (2004) Physicochemical, functional and microbiological quality of buffalo liver. Meat Sci 68: 79-86.
- ISO 6887-2: 2003. Microbiology of food and animal feeding stuffs – preparation of test samples, initial suspension and decimal dilutions for microbiological examination part 2: specific rules for the preparation of meat and meat products. International Organization for Standardization: Geneva, Switzerland.
- 22. ISO 4833-2: 2013 (R 2019). Microbiology of the Food Chain – Horizontal Method for the Enumeration of Microorganisms e Part 2: Colony Count at 30 Degrees – by the Surface Plating Technique. International Organization for Standardization: Geneva, Switzerland.
- ISO 21528-2: 2017. Microbiology of the food chain horizontal method for the detection and enumeration of Enterobacteriaceae part 2: colony-count technique. International Organization for Standardization: Geneva, Switzerland.
- 24. ISO 21527-1: 2008 (R 2018). Microbiology of food and animal feeding stuffs horizontal method for the enumeration of yeasts and molds Part 1: colony count technique in products with water activity greater than 0,95. International Organization for Standardization, Geneva, Switzerland.

- 25. Mach N, Bach A, Velarde A, Devant M (2008) Association between animal, transportation, slaughterhouse practices, and meat pH in beef. Meat Sci 78: 232-238.
- Watanabe A, Daly CC, Devine CE (1996) The effects of the ultimate pH of meat on tenderness changes during ageing. Meat Sci 42: 67-78.
- Mounier L, Dubroeucq H, Andanson S, Veissier I (2006) Variations in meat pH of beef bulls in relation to conditions of transfer to slaughter and previous history of the animals. J Anim Sci 84: 1567–1576.
- Warriss PD, Kestin SC, Brown SN, Wilkins LJ (1984) The time required for recovery from mixing stress in young bulls and the prevention of dark cutting beef. Meat Sci 10: 53-68.
- 29. Sanz MC, Verde MT, Sáez T, Sañudo C (1996) Effect of breed on the muscle glycogen content and dark cutting incidence in stressed young bulls. Meat Sci 43: 37-42.
- Page JK, Wulf DM, Schwotzer TR (2001) A survey of beef muscle color and pH. J Anim Sci 79: 678-687.
- Devine CE, Graafhuis AE, Muir PD, Chrystall BB (1993) The effect of growth rate and ultimate pH on meat quality of lambs. Meat Sci 35: 63-77.
- 32. Roça RO (2002) Humane slaughter of bovine. First virtual global conference on organic beef cattle production. September, 02 to October, 15 2002: 1-14.
- Kumar A, Kebede E, Kassaye E (2010) Evaluation of quality of beef produced and sold in parts of Tigray Region of Ethiopia. Trop Anim Health Prod 42: 445-449.
- Hadley PJ, Holder JS, Hinton MH (1997) Effects of fleece soiling and skinning method on the microbiology of sheep carcases. Vet Rec 140: 570-574.
- 35. Hauge SJ, Nesbakken T, Moen B, Røtterud OJ, Dommersnes S, Nesteng O, Østensvik Ø, Alvseike O (2015) The significance of clean and dirty animals for bacterial dynamics along the beef chain. Int J Food Microbiol 214: 70–76.
- Yousafzai HA, Rind R, Khan MA, Abro SH, Korejo NA, Ejaz M, Memon S (2019) Microbiological contamination of cattle and buffalo meat in Peshawar. Pakistan J Anim Health Prod 7: 11-16.
- European Community Commission Regulation no. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Off J Eur Union L 338: 1-26. Available: https://eurlex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:32005 R2073&from=EN. Accessed 9 February 2021.
- 38. International Commission on Microbiological Specifications for Foods (1986) Microorganisms in foods 2. Sampling for microbiological analysis: principles and specific applications. International Commission on Microbiological Specifications for Foods, 2nd ed. University of Toronto Press, Toronto, Ontario.
- Sierra ML, Sheridan JJ, McGuire L (1997) Microbial quality of lamb carcasses during processing and the acridine orange direct count technique (a modified DEFT) for rapid enumeration of total viable counts. Int J Food Microbiol 36: 61-67.
- Alonso-Calleja C, Guerrero-Ramos E, Capita R (2017) Hygienic status assessment of two lamb slaughterhouses in Spain. J Food Prot 80: 1152-1158.
- Nyamakwere F, Muchenje V, Mushonga B, Makepe M, Mutero G (2016) Assessment of *Salmonella*, *Escherichia coli*, *Enterobacteriaceae* and aerobic colony counts contamination levels during the beef slaughter process. J Food Saf 36: 548-556.

- 42. Blagojevic B, Antic D, Ducic M, Buncic S (2011) Ratio between carcass-and skin-microflora as an abattoir process hygiene indicator. Food Control 22: 186-190.
- 43. McEvoy JM, Sheridan JJ, Blair IS, McDowell DA (2004) Microbial contamination on beef in relation to hygiene assessment based on criteria used in EU Decision 2001/471/EC. Int J Food Microbiol 92: 217-225.
- 44. Yashoda K, Sachindra NM, Sakhare PZ, Rao DN (2000) Microbiological quality of hygienically processed buffalo carcasses. Food Control 11: 217-224.
- 45. Murray KA, Gilmour A, Madden RH (2001) Microbiological quality of chilled beef carcasses in Northern Ireland: a baseline survey. J Food Prot 64: 498-502.
- 46. Barros MDAF, Nero LA, Monteiro AA, Beloti V (2007) Identification of main contamination points by hygiene indicator microorganisms in beef processing plants. Food Sci Technol 27: 856-862.
- 47. Aziz NH, Youssef YA (1991) Occurrence of aflatoxins and aflatoxin-producing moulds in fresh and processed meat in Egypt. Food Addit Contam 8: 321-331.

- 48. Imre K, Herman V, Morar A (2020) Scientific achievements in the study of the occurrence and antimicrobial susceptibility profile of major foodborne pathogenic bacteria in foods and food processing environment in Romania: review of the last decade. BioMed Res Int 2020: 5134764.
- 49. Odeyemi OA, Alegbeleye OO, Strateva M, Stratev D (2020) Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. Compr Rev Food Sci Food Safety 19: 311-331.

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